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The Phenotype of Huntington's Disease Like 2



David Graham Anderson

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy

Johannesburg, 2019

Declaration

I, David Graham Anderson, declare that this thesis is my own work. Where others have contributed and assisted, they have been duly acknowledged. This thesis is submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at this, or any other university.



David Graham Anderson

Signed on the 20th of November 2019

Dedication

I dedicate this thesis to my best friend, Nathan, whom I am also lucky enough to call husband. My reasons for starting this journey were very different from my reasons for finishing it, but, because of you, I did not lose reason.

“Avec toi, je suis moi”

Presentations arising from this research project

Conference presentations

1. **Anderson, D. G**., Margolis, R. & Krause, A. (Abstract and oral presentation). Eye Movements in Huntington Disease Like 2. The 21st International Parkinson and Movement Disorder Society Conference, Vancouver, Canada, 4-8 June 2017. (*Movement Disorders,* 1(32), pp. 1–1079).

2. Krause, A., **Anderson D.G. &** Baine, F. (Oral presentation). Huntington disease in South Africa – where are we? 17th Biennial Congress of the Southern African Society for Human Genetics, Durban, South Africa, 13-16 August 2017.

3. **Anderson, D.G.,** Haagensen, M., Ferreira-Correia, A., Pierson, R., Carr, J., Krause, A. & Margolis, R. (Abstract). E02 Selective thalamic volume loss in Huntington's disease like 2: A novel MRI finding compared to Huntington's disease. 17th Biannual Meeting of the European Huntington's Disease Association, Vienna, Austria, 14-16 September 2018 (*Journal of Neurology, Neurosurgery and Psychiatry*, 89(1)).

4. Krause, A., **Anderson, D.G.**, Levesley, J., Baine, F.K., Ciosi, M., Monckton, D.G. & Margolis, R. Huntington's Disease in South Africa. (Abstract) 68th Annual Meeting of the American Society of Human Genetics, Emerging Genetic and Phenotypic Differences, San Diego, USA, 16-20 October 2018.

Peer-reviewed publications arising from this research project

The findings arising from this research have been written as four separate manuscripts and submitted to international peer-reviewed scientific journals. All four manuscripts have been accepted and published. These manuscripts are presented in the four chapters of Section 2. The original publications presented in this thesis are listed below. This is followed by a description of the student's contribution to these publications. The co-author agreement for the use of each publication within this thesis can be found in Appendix 3. This has been signed by each co-author. A typeset version of each manuscript can be found in Appendix 4.

1. **Anderson, D.G.**, Walker, R.H., Connor, M., Carr, J., Margolis, R.L. and Krause, A. (2017). A systematic review of the Huntington disease like 2 phenotype. *Journal of Huntington's Disease*, 6(1), pp. 37-46. http://doi.org/10.3233/JHD-160232.

2. **Anderson, D.G.,** Carmona, S., Naidoo, K., Coetzer, T.L., Carr, J., Rudnicki, D.D., Walker, R.H., Margolis, R.L. and Krause, A. (2017). Absence of Acanthocytosis in Huntington's Disease Like 2: A Prospective Comparison with Huntington's Disease. *Tremor and Other Hyperkinetic Movements*, 7:512. http://doi.org/10.7916/D81J9PDX.

3. **Anderson, D.G.**, Haagensen, M., Ferreira-Correia, A., Pierson, R., Carr, J., Krause, A. and Margolis, R.L. (2019). Emerging differences between Huntington's disease-like 2 and Huntington's disease: A comparison using MRI brain volumetry. *NeuroImage*: *Clinical*, 1(21), pp.101666. https://doi.org/10.1016/j.nicl.2019.101666.

4. **Anderson, D.G.**, Ferreira-Correia, A., Rodrigues, F.B., Aziz, N.A., Carr, J., Wild, E.J., Krause, A. and Margolis, R.L. (2019). Comparison of the Huntington's disease-like 2 and Huntington's disease clinical phenotypes. *Movement Disorders Clinical Practice*. 6(4), pp. 302–311. doi: 10.1002/mdc3.12742

PhD student's contribution to each publication and the overall study

1. Overall study design and the conceptualisation of the project.
2. Design and the conceptualisation of each sub-study.
3. Project coordination that involved overseeing data collection and designing standard operating procedures.
4. Each participant was interviewed, examined and video-recorded by the student.
5. Data cleaning, statistical analyses in consultation with statistician and interpretation of results were performed by the student.
6. The writing, revision, submission and coordination between authors of each manuscript.
7. Co-authors provided methodological advice, statistical and technical assistance and critically reviewed each of the manuscripts.

Abstract

Background

Huntington's disease like 2 (HDL2) is described as the disease with the greatest resemblance to Huntington's disease (HD). Both disorders clinically comprise a movement disorder, dementia and psychiatric symptoms. HDL2 has been reported exclusively in people of African ancestry, with the highest frequency in South Africa. Anecdotally, HDL2 has been associated with acanthocytes on blood smears, less oculomotor dysfunction and greater parkinsonism, potentially differentiating it from HD.

Aim

To describe systematically the HDL2 phenotype and compare it to that of HD.

Method

A systematic review of published HDL2 clinical descriptions was conducted to establish the extant HDL2 phenotype. Subsequently, a blinded cross-sectional observational study compared African ancestry subjects. HDL2 (n=15) study participants were compared to subjects with HD (n=13); these two groups were concurrently compared to an unaffected control group. Data collected included a standardised history, general and neurological examination results, blood samples and MRI scans. Motor rating scales were used to assess the movement disorder of affected subjects which was video-recorded. The data were analysed with respect to clinical phenotypes, quantitative radiological volumes and erythrocyte features.

Results

The literature generally described the HDL2 phenotype as similar to HD, however, HDL2 subjects were reported to have less oculomotor dysfunction, dystonia, dysarthria and greater parkinsonism. Only four HDL2 cases had reported acanthocytes. The current study found no acanthocytes in HDL2 or any other

participants. The MRI intracerebral volumes of subjects with HDL2 and HD showed similar cortical and subcortical atrophy but the thalamic volumes in subjects with HDL2 were smaller than those of the HD groups. Blinded raters could not distinguish between HDL2 and HD after analysing individual subjects. The two affected groups had similar overall Unified Huntington's Disease Rating Scale (UHDRS) motor scores, with worse scores for some of the motor and cognitive components in the HDL2 group.

Conclusion

This research suggests that HDL2 and HD cannot be differentiated based on the presence of acanthocytes or by examining individual patients. The study shows that there is a predominant clinical similarity between HDL2 and HD, however, there are additional findings that suggest HDL2 has clinical and radiological features that are more severe. This finding may have implications for the pathogenic mechanisms of both diseases. Furthermore, this research has found potential clinical and neuroimaging biomarkers for HDL2 that may give reference for future research into the pathogenesis and treatment of HDL2.

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List of abbreviations

ADL	Activities of daily living
ALS	Amyotrophic Lateral Sclerosis
ASO	Antisense Oligonucleotides
BDNF	Brain Derived Neurotrophic Factor
Biomarkers	Biological Markers
BRAINS	Brain Research: Analysis of Images, Networks and Systems
C9orf72	Chromosome 9 Open Reading Frame 72
COHORT	Cooperative HD Observation Research Trial
DBS	Deep Brain Stimulation
DGMC	Donald Gordon Medical Centre
DNA	Deoxyribonucleic acid
DRPLA	Dentatorubral-Pallidoluysian Atrophy
ENROLL HD	A Prospective Registry Study in a Global Huntington's Disease Cohort
FTLD	Frontotemporal Lobar Dementia
FISH	Fluorescent *In Situ* Hybridization
fMRI	Functional Magnetic Resonance Imaging
GABA	γ-aminobutyric acid
HD	Huntington's Disease
HDL2	Huntington's Disease Like 2
HEAT	**H**untington elongation factor 3 (**E**F3), protein phosphatase 2**A** (PP2A) and the yeast kinase **T**OR1
HIV	Human Immunodeficiency Virus
HTT	Huntingtin
JPH3	Junctophilin 3
MoCA	Montreal Cognitive Assessment
MRS	Magnetic Resonance Spectroscopy
MRI	Magnetic Resonance Imaging
NHLS	National Health Laboratory Services
NHLS(WITS)	National Health Laboratory Services at the University of the Witwatersrand
NMDA	N-methyl D-aspartate glutamate

Nfl	Neurofilament light chain
PET	Positron Emission Tomography
PhD	Doctor of Philosophy
polyQ	Polymorphic Polyglutamine Chain
PREDICT HD	Neurobiological Predictors of Huntington's Disease Study
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PHAROS	Prospective Huntington At Risk Observational Study
RNA	Ribonucleic Acid
RAN	Repeat-associated Non-ATG Translation Proteins
REDCap	Research Electronic Data Capture
PKAN	Pantothenate Kinase-Associated Neurodegeneration
SARA	Scale for Assessment and Rating of Ataxia
SCA	Spinocerebellar Ataxia
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
SNP	Single Nucleotide Polymorphism
TRACK HD	Multinational prospective observational study of Huntington's disease (HD) that examines clinical and biological findings of disease progression in individuals with premanifest HD (preHD) and early-stage HD.
TMS	Total Motor Score
UCT	University of Cape Town
UHDRS	Unified Huntington's Disease Rating Scale
UMRS	Unified Myoclonus Rating Scales
UPDRS	Unified Parkinson's Disease Rating Scale
WITS	University of the Witwatersrand

Units and symbols

The International System of Units (SI) has been used throughout this thesis.

%	percentage
>	greater than
<	smaller than
≥	greater than or equal to
±	plus/minus
+	plus
-	minus
kg	kilogram
g	gram
µg	microgram
pg	picogram
l	litre
dl	decilitre
fl	femtolitre
m	metre
cm	centimetre
n	number
y	year
T	Tesla

Genetic nomenclature

Nucleotide notation

A	Adenine
C	Cytosine
G	Guanine
T	Thymine

The symbols for genes are italicised (e.g. *JPH3*), whereas the symbols for proteins are not italicised (e.g. JPH3).

Preface

The first time I met a patient with Huntington's disease (HD) was in 2003. I was in my first year as a training neurology registrar in Johannesburg at the University of the Witwatersrand. The Division of Human Genetics at the University had just reported the highest number of Huntington's disease like 2 (HDL2) patients in the world, a disease that had only been described a few years before. I was fortunate enough to be assigned as the neurologist on the HD multidisciplinary team for the rest of my tenure as a neurology fellow and assisted in reviewing HDL2 patient files for an ongoing study. Subsequently, as a consultant, I have continued as a member of the HD multidisciplinary team. Although rewarding, being part of this team also exposed me to the full devastation of both HD and HDL2.

In 2006, I completed my Fellowship of the College of South Africa of Neurology and initiated a Masters degree (MMed) in neurology, studying chronic inflammatory demyelinating polyradicular neuropathy and its association with HIV. I completed this degree in 2008. My interest in movement disorders led me to join the Functional Neurosurgery unit at the Milpark Hospital where Deep Brain Stimulation (DBS) and epilepsy monitoring and surgery are performed in Johannesburg. In 2007 I joined the University of the Witwatersrand Donald Gordon Medical Centre as a joint consultant at the University and in private practice. Between 2006 and 2010, I spent time in movement disorder units in Europe and the USA and completed a fellowship in electrophysiology at the Cleveland Clinic where I also visited the movement disorder unit.

Throughout this time, I remained deeply involved with the University of the Witwatersrand Division of Human Genetics and I have continued to treat and assess HD and HDL2 patients for predictive and diagnostic testing in both a private and State setting. My relationship with this Department has afforded me a role in a multidisciplinary team, addressing other complex neurogenetic cases. Early on, we identified the need for a specialised service for our HD patients. In 2009, with the help of Professor Krause and her Department, we established a Huntington's Disease Clinic to service the population around Johannesburg. In the South African

black population one HDL2 diagnosis is made for every two HD diagnoses. Therefore, it was not surprising that the clinic was servicing many HDL2 patients.

Professor Russel Margolis from Baltimore described the phenotype of the index pedigree of HDL2 as similar to HD but with absent or fewer eye signs and more parkinsonism. Virtually at the same time, in 2003, Professor Ruth Walker from New York reported acanthocytes in four HDL2 cases. I read with keen interest how Professor Margolis, Professor Dobrila Rudnicki and others were unravelling some of the molecular mysteries of HDL2. Still others were describing retrospective cases from Europe, the USA and South America. However, the recent reports did not describe significant differences in the HDL2 phenotype compared to those established from the original cases. Increasingly, I was noting that the cases in our clinic were indistinguishable from the HD cases upon examination; all had typical HD ocular motor features, and none were found to have acanthocytes on blood smear. This raised the question of whether the HDL2 phenotype had features that could distinguish it from HD.

Subsequently, between 2014 and 2015, there were three factors that lead to the conceptualisation of this PhD. Firstly, Professor Krause, in collaboration with other laboratories, used haplotype studies to confirm that all HDL2 cases likely had common African ancestry. This meant that the cases I was seeing in the clinic and those reported in the literature likely had common ancestry. However, I was not observing the same clinical features as those reported in the literature. Secondly, I met with Professor Margolis to discuss my findings. He pointed out that the reason for my observations may be that I had seen a larger sample of HDL2 cases than previously described and therefore was observing the full spectrum of the disease phenotype. Therefore, Professor Margolis suggested I collect novel cases and study their clinical phenotype. Lastly, in 2015, Professor Sarah Tabrizi announced the first human trials of antisense oligonucleotides (ASO) in HD. With these trials, the hope for a possible treatment for many of our patients became a reality and with each passing year, that hope has grown. However, this hope has excluded the HDL2 patients in our clinic. What led to this landmark point in HD has been the meticulous study of the HD phenotype, researching the progression of the disease and the search for biomarkers in studies such as PREDICT HD and TRACK HD, in

conjunction with the study of the HD pathophysiology. Therefore, if similar therapeutic interventions were ever to be realised for HDL2 patients, a better understanding of the clinical phenotype would be needed.

Pursuing a PhD has always been an aspiration of mine. The disparity in the body of HDL2 knowledge between its pathophysiology and its phenotype, coupled with the unique opportunity that the Johannesburg HD clinic population provides, has allowed me to study the HDL2 phenotype.

My hope is that this research will bring attention to patients with HDL2 and inform the pathophysiological research on HDL2 that has outpaced the understanding of its clinical phenotype. Furthermore, the results generated from my research may add to the global body of literature on the subject of HDL2. I would also like to see these findings as the beginning of future research and international collaboration for HD and the HD phenocopies in South Africa.

This doctoral thesis is presented in the 'integrated' format, in accordance with the University of the Witwatersrand's Faculty of Health Sciences guidelines and includes four manuscripts. The manuscripts have all been published. The thesis is divided into three sections which are further sub-divided into chapters.

- *Section I: Background and Study Context*
  This section provides an overview of Huntington's disease like 2 and Huntington's disease with reference to the literature. It then sets out the aims and objectives of the study. Subsequently, the overall methods applied to achieve the objectives of this study are described.
    - Chapter 1: Literature Review
    - Chapter 2: Study Context and Methods

- *Section II: Published Manuscripts*
  This section consists of four peer-reviewed publications that report on the findings of this study:
    - Chapter 3: A Systematic Review of Huntington's Disease Like 2 Phenotype.

- Chapter 4: Absence of Acanthocytosis in Huntington’s Disease Like 2: A Prospective Comparison with Huntington’s Disease.
- Chapter 5: Emerging differences between Huntington's disease-like 2 and Huntington's disease: A comparison using MRI brain volumetry.
- Chapter 6: Comparison of the Huntington’s disease like 2 and Huntington’s disease clinical phenotypes.

- *Section III: Integrated Discussion*

  This section discusses and consolidates the findings from the four manuscripts and ends with an overall conclusion.

SECTION I

Section I consists of Chapters 1 and 2. This section gives an overview of Huntington's disease like 2 and Huntington's disease with reference to the literature and then sets out the aims and objectives of the study. Subsequently, the methods applied to achieve the objectives of the study are described.

- Chapter 1: Literature Review
    - This chapter gives an overview of Huntington's disease like 2 (HDL2) and Huntington's disease (HD).

- Chapter 2: Study Context and Methods
    - This chapter describes the overall methods applied to achieve the objectives of this study.

CHAPTER 1

Literature Review

The main focus of this study is to describe the Huntington's disease like 2 (HDL2) phenotype and its relationship to Huntington's disease (HD). This chapter provides an overview of the subject literature to date. This is done to define important terms, establish the framework of the study and give the study context. The discussion framework for this thesis is how multiple genotypes can result in similar phenotypes. A brief history of HDL2 is then outlined, followed by a review of the prevalence of HD and HDL2. As this is a South African study of patients with African ancestry, particular attention has been given to the social circumstances and geographic distribution of the study participants. The genetic, pathogenic mechanisms and histopathological features of HDL2 and HD are then broadly discussed and compared. The phenomenology as it pertains to movement disorders and their classification is considered and the phenotypes of HD and HDL2 are reviewed. This is followed by a brief overview of the clinical rating scales and some biomarkers in HD to establish their use in the study. The motivation behind the study is then discussed and aspects from the literature review are highlighted with regard to the HDL2 phenotype. These are compared to HD in the context of translating genotype to phenotype. Finally, the overarching aim and the specific objectives of the study are described.

1 Literature Review

1.1 Introduction

Huntington's Disease (HD) is an autosomal dominant neurodegenerative disorder comprising of a movement disorder with prominent cognitive and psychiatric features (Ross *et al.,* 2014). Approximately 1% of cases with a HD phenotype tested for the causative mutation are found to have another cause and are referred to as HD phenocopies (Wild *et al.,* 2008). Huntington's disease like 2 (HDL2) is one of the most common HD phenocopies and has been noted due to its striking clinical resemblance to HD and unique demographic features (Margolis *et al.,* 2001).

In 2001, the first pedigree with HDL2 was described by Margolis *et al.* (2001). This African American family consisted of eight patients who were thought to have HD until testing for the expanded triplet repeat causing HD became available. The family tested negative for the *huntingtin (HTT)* gene mutation which causes HD. Subsequently, an expansion mutation in exon 2A of *junctophilin-3 (JPH3)* on chromosome 16q24.3 was found to cause HDL2 (Holmes *et al.,* 2001). A CAG repeat expansion in exon 1 of the *HTT* gene on chromosome 4p16.3 causes HD whereas HDL2 is caused by (CTG/CAG) repeat expansion.

The description of the HDL2 phenotype and its comparison to HD is limited and is based on the descriptions of the index pedigree and a few retrospective cases (Margolis *et al.,* 2001; Walker *et al.,* 2002; Walker, Rasmussen, *et al.,* 2003; Walker, Jankovic *et al.,* 2003; Bardien *et al.,* 2007; Rodrigues *et al.,* 2008; Rodrigues *et al.,* 2011; Schneider and Bird, 2016). These descriptions report HDL2 as the disease with the greatest clinical resemblance to HD (Walker, Jankovic *et al.,* 2003), even though the mutations in the two diseases are on different chromosomal loci and encode proteins with no clear similarities (Holmes *et al.,* 2001). Both diseases are devastating neurodegenerative disorders that begin insidiously, usually in the third or fourth decade of life, and progress gradually, causing cognitive and behavioural disability with hyper- and hypo-kinetic movement disorders which result in death within 10-20 years (Margolis *et al.,* 2001). Unlike HD, HDL2 has been exclusively

described in patients of African ancestry (Krause *et al.,* 2015) and some of the initial cases were found to have acanthocytes on blood smear (Walker *et al.,* 2002). The highest number of HDL2 cases reported in the world is from the Division of Human Genetics National Health Laboratory Service (NHLS) and the University of the Witwatersrand (WITS) in Johannesburg, South Africa which was the setting for this study.

Most novel cases of HDL2 have been discovered by screening for the CAG/CTG expansion in the *JPH3* gene in stored genetic samples that were sent for HD laboratory testing but were negative for the *HTT* mutation or by examining series of subjects with a HD phenocopy (Stevanin *et al.,* 2002; Krause *et al.,* 2002; Magazi *et al.,* 2008; Castilhos *et al.,* 2013; Mariani *et al.,* 2016; Anderson, Walker, *et al.,* 2017). The description of the HDL2 phenotype has then been limited to the description of these cases, often from retrospective records.

Research into the molecular and pathophysiological mechanisms of HDL2 has outpaced the study of its phenotype (Rudnicki *et al.,* 2007; Wilburn *et al.,* 2011; Seixas *et al.,* 2012). Without fully understanding the phenotype of HDL2, the molecular mechanisms cannot be related to the disease and future therapeutic interventions may never be fully realised. The scope of this thesis is therefore to evaluate clinical aspects of the phenotype of HDL2 and how these clinical features are related to those of HD. Specific attention is paid to the motor features, magnetic resonance imaging (MRI) findings and the presence of acanthocytes.

1.2 Background

1.2.1 Definitions

Some definitions are ambiguous or controversial in the literature. These are clarified in the following sections as used in this thesis.

1.2.1.1 Genotype

The entire genetic constitution of an individual organism (Mahner and Kary, 1997).

1.2.1.2 Phenotype

A collection of traits possessed by a cell or organism that results from the interaction of the genotype and the environment (Mahner and Kary, 1997).

1.2.1.3 Phenocopy and genocopy

The definitions of phenocopy and genocopy have evolved over time. The term 'phenocopy' was used by Goldschmidt in 1938 to describe diseases caused by environmental factors which would normally be attributed to genetic causes. Later, in 1942, Schmalhausen used the term 'genocopy' as the opposite of phenocopy to describe a genetic disease which was typically produced by an environmental effect (Gage *et al.,* 2016). With time, the term 'genocopy' came to describe a single phenotype that was caused by multiple mutations (Zori and Williams, 1991). However, with reference to the field of HD, the term 'HD phenocopy' has come to represent all syndromes that mimic Huntington's disease clinically, including genetic ones (Wild *et al.,* 2008). Therefore, the designate 'phenocopy' will be used in this study as the preferred term to refer to conditions with similar phenotypes but different genetic aetiologies, as this is used in the majority of the HDL2 and HD literature.

There are now several genetic diseases that have been reported with an HD phenotype without the HD mutation (Wild and Tabrizi, 2007). These include:

- HDL1, described in a Swedish family (CHR) and caused by an expanded octapeptide repeat in the prion protein gene on chromosome 20p12 (Moore *et al.,* 2001).
- HDL2, reported to be clinically and pathologically nearly indistinguishable from HD, and occurring exclusively in patients with African ancestry, which is caused by a CTG/CAG expansion mutation as described in 1.3.2.1 (Holmes *et al.,* 2001) and is the focus of this thesis.

- HDL3, described in a Saudi Arabian family, with a possible locus on chromosome 4p15.3 (Kambouris *et al.,* 2000).
- Spinocerebellar ataxia (SCA) 17/(HDL4), reported in Japan and Europe, and caused by a CAA/CAG repeat expansion in the TATA binding protein gene on chromosome 6 (Stevanin and Brice, 2008).
- Neuroacanthocytosis syndromes, which include chorea acanthocytosis, McLeod syndrome, and pantothenate kinase-associated neurodegeneration (PKAN), which may also present with HD features (Jung *et al.,* 2011).
- Chromosome 9 Open Reading Frame 72 (C9orf72) mutation is a GGGGCC (G4C2) repeat expansion and is the most common cause of inherited amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD) (Cooper-Knock *et al.,* 2014). In a study from University College London, the C9orf72 mutation was found to be the most common cause of HD phenocopies (Moss *et al.,* 2014). A report on subjects with HD phenotypes and African ancestry from South Africa was published by Baine *et al.* (2016). Specimens of subjects who tested negative for HDL2 or HD were screened for other mutations. There were no reported cases with C9orf72 and a single case of SCA2. SCA2 is not a common HD phenocopy (Orr and Zoghbi, 2007).
- Dentatorubral-Pallidoluysian Atrophy (DRPLA) is a CAG triplet repeat disorder of the atrophin-1 gene and is most prevalent in Japan. Ataxia and myoclonus form part of the phenotype with HD features (Koide *et al.,* 1994).

1.2.2 Correlating genotype to phenotype

A cardinal question that has been asked for decades in the field of genetics is how different genes can result in similar phenotypes (Waddington, 1942). The explanation of how a genotype translates into a phenotype is complex and involves understanding the impact of extrinsic environmental factors and intrinsic factors such as genetic variation, the effects of different genes on the expression of other genes as well as subcellular functions. In the last decade, these genetic components and molecular mechanisms have been explored and developed into a unifying reference tool of gene products and cellular functions, referred to as Gene Ontology (Dutkowski *et al.,* 2012). Gene Ontology has helped to describe many genes and

their functions and has provided insight into a number of diseases, such as in the field of oncology, for example (Yu *et al.,* 2016). In order for molecular and genetic research to result in similar insights into movement disorders, well-defined disease outcome measures which correlate with disease phenotypes are required (Wild and Tabrizi, 2017). Objective and sensitive measures of a disease phenotype are crucial in monitoring therapeutic interventions (Weir *et al.,* 2011). TRACK HD (Tabrizi *et al.,* 2013) and PREDICT HD (Paulsen *et al.,* 2014) are two seminal studies that established important outcome measures in HD. Both of these longitudinal studies used phenotypic features of HD as outcome measures.

The question of how different aetiologies, both genetic and nongenetic, can result in a similar phenotype is encountered extensively in the field of movement disorders (Crossman, 2000). Disruption of common motor neural networks, which encompass the cerebral cortex, basal ganglia, thalamus and cerebellum, are reported to contribute to the shared clinical symptoms in movement disorders. Neurosurgical interventions target common sites for different diseases in support of shared networks being at least in part responsible for similar abnormal movements (Montgomery and Gale, 2008, Wijemanne and Jankovic, 2015). However, in certain movement disorder phenocopies, some of the shared phenotypic features cannot be explained by common motor networks alone and there may be other pathogenic similarities (Yu *et al.,* 2016). The psychiatric and cognitive symptoms common to the HD phenocopies are a prime example (Wild *et al.,* 2008).

Genotype-phenotype correlations between HDL2 and HD, with their molecular and phenotypic similarities, may provide potential therapeutic targets for both diseases (Yu *et al.,* 2016). However, as is shown in Chapter 3, the description of the HDL2 phenotype is based on studies with a small number of subjects which have not been systematically reported. Therefore, a clearer understanding of the HDL2 phenotype is needed. This study aims to clarify some aspects of the phenotype and phenomenology of HDL2 and thereby contribute to the wider effort to understand the pathophysiology of HD and HDL2 in order to ultimately treat these diseases.

Models and frameworks for the relationships that affect the genotype-phenotype translation have been reported (Orgogozo *et al.,* 2015). These models include the

impact of extrinsic, environmental factors and intrinsic factors such as genetic variation and the effects of different genes on each other's expression. The contributions of these factors to the genotype-phenotype correlation are complex. Therefore, for the purposes of this study, a simplified line representing a map, genotype-phenotype correlation is referred to in Figure 1.1. This illustration provides a reference and framework in the subsequent study chapters and the possible points of convergence of HDL2 and HD.

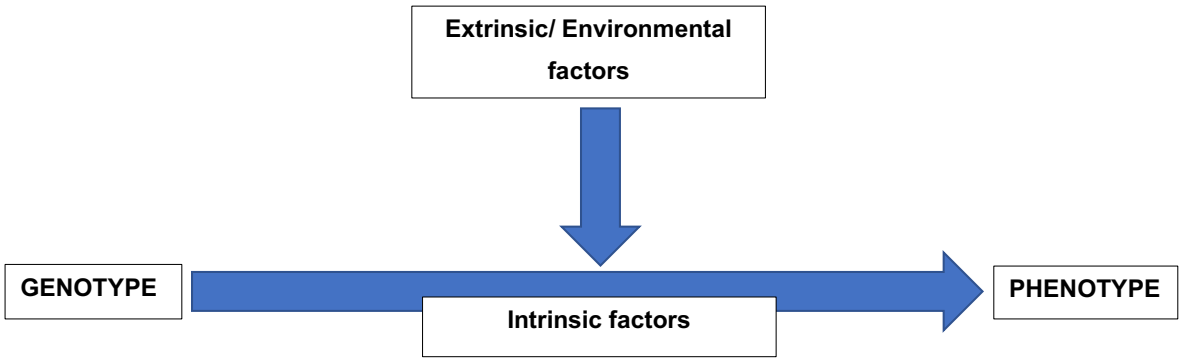

Figure 1.1 Genotype-phenotype correlation

1.2.3 A brief history of Huntington's disease like 2

The CAG repeat expansion on chromosome 4 which causes HD was first reported by MacDonald in 1993 (Macdonald *et al.*,1993). Inevitably, this led to the discovery of HD phenocopies where cases tested negative for the HD mutation but had similar inheritance patterns and clinical features (Mencacci and Carecchio, 2016). The HDL2 index family, pedigree 'W', was an example. This was an African American family from the south eastern United States of America (USA) with eight effected individuals. Most members of the family had been followed in the Baltimore Huntington's Disease Clinic for many years as a family believed to have HD but tested negative for the HD mutation genetic testing became available (Margolis *et al.,* 2001). This led to the discovery that the disease in this family was caused by a CTG/CAG expansion of the *JPH3* gene, which was given the designation of HDL2 (Holmes *et al.,* 2001). A year later, three members from another African American family with an autosomal dominant chorea-acanthocytosis were described (Walker *et*

al., 2002). In 2003, these three cases were found to have the HDL2 mutation. They were reported with one other unrelated case from Mexico with HDL2 and acanthocytes (Walker, Rasmussen *et al.,* 2003). When the erythrocyte cell membranes were analysed, these three cases were found to have abnormal band 3 proteins. Band 3 proteins are involved in maintaining red cell volume and their osmotic homeostasis. These membrane proteins have been implicated in the pathogenesis of several haematological diseases. Alterations in band 3 proteins are associated with neurological diseases such as neuroacanthocytosis but have not been demonstrated to cause any of the neurological symptoms (Kay, 2004).

Since these four subjects with acanthocytes have been described, HDL2 has been designated as both an HD phenocopy and as a neuroacanthocytosis syndrome in neurological and hematological reviews and texts (Danek and Walker, 2005; Walker *et al.,* 2007; Siegl *et al.,* 2013; Adjobo-Hermans *et al.,* 2015).

With the availability of testing for the CTG/CAG expansion which causes HDL2, laboratories have reported an increasing number of HDL2 cases in samples that had tested negative for HD. Consequently, HDL2 has been found to be the most common HD phenocopy in parts of Europe, the USA and South America (Margolis *et al.,* 2004; Stevanin *et al.,* 2002; Mariani *et al.,* 2016; Walker *et al.,* 2018). In 2002, Krause *et al.* first reported that South Africa had the highest number of cases of HDL2 in the world (Krause *et al.,* 2002). Analysis of multiple HDL2 genetic samples from around the world has now led to the discovery of a shared common haplotype and that HDL2 likely has African origins (Krause *et al.,* 2015).

1.2.4 The prevalence of Huntington's disease like 2 and Huntington's disease

In the western world, the prevalence of Huntington's disease is reported to be 4–10/100 000 in the general population (Ross *et al.,* 2014). However, the prevalence of HD varies widely by geographical region. This is in part due to the fact that some prevalence studies were performed before HD genetic testing was available and different diagnostic criteria and methods were applied in different studies (Rawlins *et*

al., 2016). The epidemiology of HD in Africa is poorly researched, with only 25% of cases reported having a confirmed genetic diagnosis (Lekoubou *et al.,* 2014).

1.2.4.1 The prevalence of Huntington's disease in Southern Africa

In South Africa, the reported prevalence of HD is reflective of the country's political history and social inequalities (Baine *et al.,* 2016). The first description of HD from South Africa was in 1962 and relied on a clinician survey which reflected racial attitudes in the country at the time (Klintworth, 1962). The findings from this report suggested that HD was more common in South Africa's white population than in the rest of the world due to a higher proportion of HD in the Afrikaner population. The study proposed that this was due to a founder effect from the 17th century. Individuals of black or mixed ancestry were mentioned briefly as having been diagnosed with HD, without specific numbers ascribed to these population groups. At that time, there was less access to specialist health care for patients of these ethnic backgrounds, making the findings from this report unreliable. In 1977 Hayden et al. reported a minimum HD prevalence in the Cape Coloured population of 3.56/100 000 (Hayden and Beighton, 1977). This study was based on a retrospective record review and the authors suggested that HD was present in this population due to European ancestry.

In 1980, a prevalence study of HD in South Africa was published (Hayden *et al.,* 1980). The prevalence of HD was described as very low in the black population, 0.01/100 000, compared to 2.22/100 000 and 2.17/100 000 in the white and mixed ancestry populations respectively (Figure 1.2). This study had some limitations resulting from the time when it was carried out. Firstly, it was a hospital-based survey in South Africa during the apartheid era, when the black population did not have access to as many clinical services as the white population (McLaren *et al.,* 2014), and therefore black patients with an HD phenotype may have been underreported. Secondly, enrolment was based on clinical diagnosis as genetic testing was not yet available, which could have resulted in under or overreporting of HD cases. In contrast to the low rate of HD described in the South African black population, the prevalence of HD in the region of Zimbabwe adjacent to Mozambique, Manicaland,

was estimated at 1/100 000 (Figure 1.2) (Scrimgeour and Pfumojena, 1992). Several large black families with HD have been described from this region (Samuels and Gelfand, 1978; Scrimgeour and Pfumojena, 1992). Figure 1.2 (Rawlins *et al.,* 2016) shows the estimated HD prevalence in South Africa from 1976 and the subsequent findings in the white, black and coloured populations, as reported by Hayden *et al.* (1980). Furthermore, it illustrates the HD prevalence in the black Zimbabwean population from Manicaland, as described by Scrimgeour *et al.* (Scrimgeour and Pfumojena, 1992, Rawlins *et al.,* 2016).

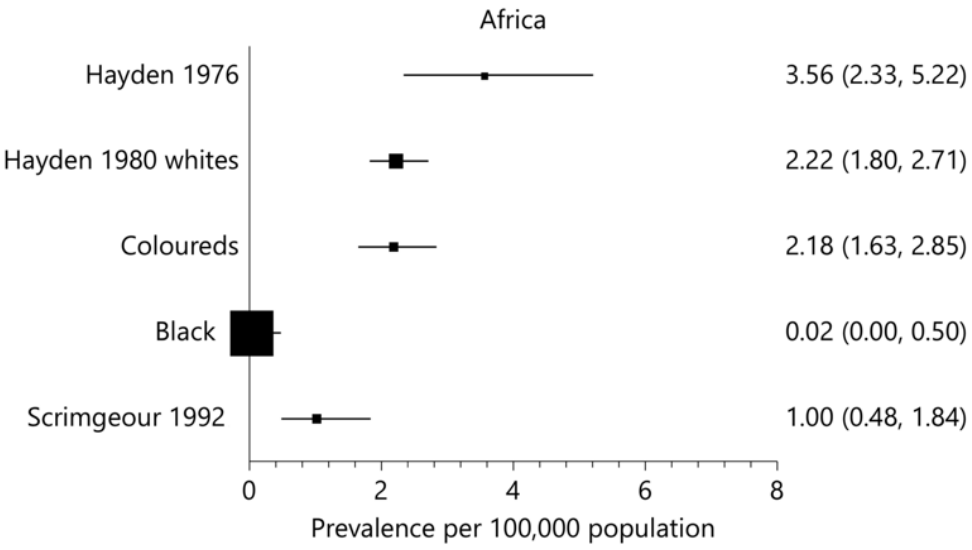

Figure 1.2 Prevalence of HD in Africa.

Subsequently Baine *et al.* (2016) published HD and HDL2 minimum frequency estimations using laboratory testing records for HD and HDL2 from the State National Health Laboratory Service (NHLS) in South Africa. The University of the Witwatersrand (WITS) and the University of Cape Town (UCT) are the two NHLS laboratories which provide HD and HDL2 testing in South Africa. The frequency of the two diseases was combined and showed significantly higher rates than previously described (black 0.25/100 000, mixed ancestry 2.10/100 000 and white 5.10 /100 000). Notably, most of the cases from this study were from urban areas of South Africa. The black people contribute more than 90% of the total rural population (Statistics SA, 2017), and are less likely to access healthcare due to distance to these facilities noting that this population the large (McLaren *et al.,* 2014). Furthermore, apartheid legacies have resulted in inadequate healthcare facilities and

specialist availability in rural areas (McLaren *et al.,* 2014). Huntington's disease is therefore less likely to be diagnosed through the NHLS in the black population. The study by Baine *et al.* (2016) therefore had similar limitations to that of Hayden *et al*. (1980) in terms of the underreporting of cases from rural areas. Furthermore, a retrospective study in South Africa found HD genetic counseling and testing to be underutilised by the black population, suggesting that this was due either to a lack of awareness of HD or to a low prevalence of HD in this population (Sizer *et al.,* 2012). Patients who had accessed genetic counselling would have been included in the study and a lack of access by one population group would have skewed the data.

The epidemiological HD research in Africa or in black populations is scant and many of these studies have methodological limitations (Lekoubou *et al.,* 2014). Overall, the HD prevalence in the black population is considered low, based on these studies. The website of the Huntington's Outreach Project for Education at Stanford (HOPES) (Liou, 2010) states that prevalence in the black population is very low (< 6/100 000) but acknowledges the poor epidemiological evidence for this, basing these data on Hayden *et al.* (1980). In North America the prevalence of HD has been documented as being similar in black and white populations (black= 6.37 [95% CI 4.87–8.18] per 100 000 and whites= 4.79 [95% CI 4.06–5.60] per 100 000). This higher prevalence of HD among black Americans from the United States compared to other black populations has been attributed to the mixed-race ancestry of African Americans. (Rawlins *et al.,* 2016).

There may be some support from genetic studies for a lower prevalence of HD in the black population. Genome wide studies have demonstrated specific haplotypes associated with a higher prevalence of HD in certain populations. Baine *et al.* (2013) have demonstrated the absence of these high-risk variant haplotypes in South African black subjects when compared to Caucasians from the same population. Some of these high-risk haplotypes are associated with higher rates of mutations and are less common in black and Asian populations. Lower risk haplotypes may account for the lower prevalence of HD seen in these population groups (Kay *et al.,* 2018).

In HD, the abnormal repeat length accounts for up to 73% of the variation of age of onset of disease (Langbehn *et al.,* 2004), with the remaining effect attributed to genes other than *HTT* and environmental factors (Wexler *et al.,* 2003; Bates *et al.,* 2015). The number of CAG triplet repeats that are found when tested have been divided up into four groups and these groupings clinical relevance with respect to disease manifestation. The four groups are: normal (≤ 26), intermediate (27–35), reduced or incomplete penetrance (36–39), and full penetrance (≥ 40) with almost all subjects developing HD symptoms with 40 repeats or greater. The CAG repeat shows instability with a higher rate of mutation with increasing length; therefore, a child may manifest the disease earlier the parent. This occurs through paternal meiotic transmission in HD and is referred to as anticipation (Ross and Tabrizi, 2011). The prevalence of HD has been noted to be higher in populations with a longer average number of CAG repeat lengths. The average length of the normal CAG repeat length is lower in the black population than in the Caucasian population, as shown in Figure 1.3 (Bates et al., 2015), and may contribute to the lower prevalence of HD in black populations (Bates *et al.,* 2015).

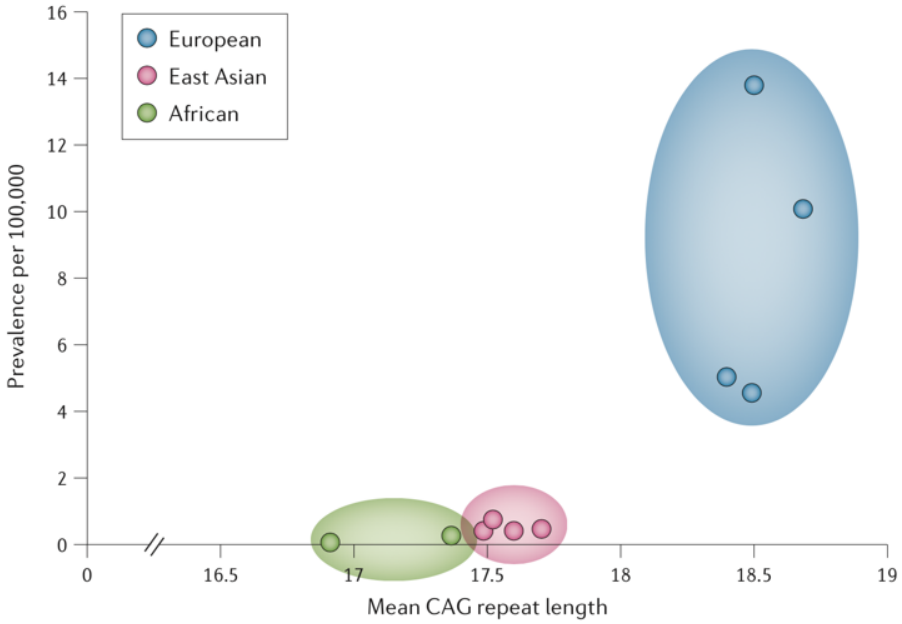

Figure 1.3 Ethnic differences in the prevalence of Huntington's disease correlate with average CAG repeat length in each population. (Bates *et al.,* 2015).

Each of the smaller circles in Figure 1.3 represent studies that document HD prevalence in that region.

1.2.4.2 The prevalence of Huntington's disease like 2 in Southern Africa

The general prevalence of HDL2 is unknown, although as previously noted, in several regions and countries around the world, HDL2 is the most frequently reported genetic HD phenocopy, with South Africa having the highest number of cases to date (Krause *et al.,* 2015, Anderson *et al.,* 2017). An analysis of blood samples from individuals with an HD-like phenotype referred for testing to NHLS (WITS) for the HD mutation (Krause *et al.,* 2015) found that 15% of black South African patients and no white patients had an expansion in the *JPH3* locus, while 62% of white patients and 36% of black patients had an expansion in the *HTT* locus. This meant that for every two black patients diagnosed with HD there was approximately one patient diagnosed with HDL2.

1.3 The genetics and pathophysiology of Huntington's disease like 2 and Huntington's disease

The broad aim of this thesis is to compare the clinical phenotypes of HD and HDL2. Important genetic and pathophysiological differences between HDL2 and HD are highlighted below. The purpose of this section is to highlight differences and similarities between the two diseases that have relevance to the published papers from this study and the discussion in the following chapters. In keeping with the theme of genotype translating to phenotype, the sequence of the topics commences with genetics, and is then followed by the pathogenesis and pathology of HD and then HDL2. In the subsequent section, the reported phenotypes of both diseases are briefly addressed.

1.3.1 Huntington's disease

1.3.1.1 Genetics of Huntington's disease

Huntington's disease is an autosomal, dominant inherited disorder. An abnormal CAG repeat expansion in exon 1 of the *HTT* gene on chromosome 4p16.3 causes HD (MacDonald, 1993). Normally, the CAG codon is repeated 9 to 35 times (Kremer *et al.,* 1994). The age of disease onset is inversely proportional to the length of the triplet repeat (Ross and Tabrizi, 2011). If the CAG expansion is greater than 75 repeats, a juvenile onset is seen with a variable phenotype that is often more parkinsonian. Seizures may also be seen which have not been reported in adult onset HD (Andresen *et al.,* 2006). In HD, the CAG repeat shows anticipation, which is defined as a higher rate of mutation instability with increasing repeat length. This occurs more commonly during spermatogenesis. Therefore, an unaffected father is more likely to have offspring that manifest with HD if his triplet repeat length is in the intermediate range (Telenius *et al.,* 1994).

Huntingtin (HTT) is an intracellular protein found predominantly in the cytoplasm and consists of 3,144 amino acids (Figure 1.4) (Saudou and Humbert, 2016). The amino acids form multiple tandem repeat structures composed of two alpha helices linked by a short loop (Li *et al.,* 2006). These hairpin-like structures are called 'HEAT repeats' (Huntingtin, elongation factor 3 (EF3), protein phosphatase 2A (PP2A), and the yeast kinase TOR1). Huntingtin has been implicated in diverse roles such as vesicle transport, gene transcription regulation and RNA trafficking. The ability of HTT to function across multiple cellar domains has been attributed to its acting as a scaffold protein and a co-ordinator of multiple protein interactions (Li and Li, 2004) although all of it's functions are still not completely understood (Saudou and Humbert, 2016). Huntingtin is ubiquitously expressed throughout the body and is preserved phylogenetically across many species, emphasising its importance (van der Burg *et al.,* 2009).

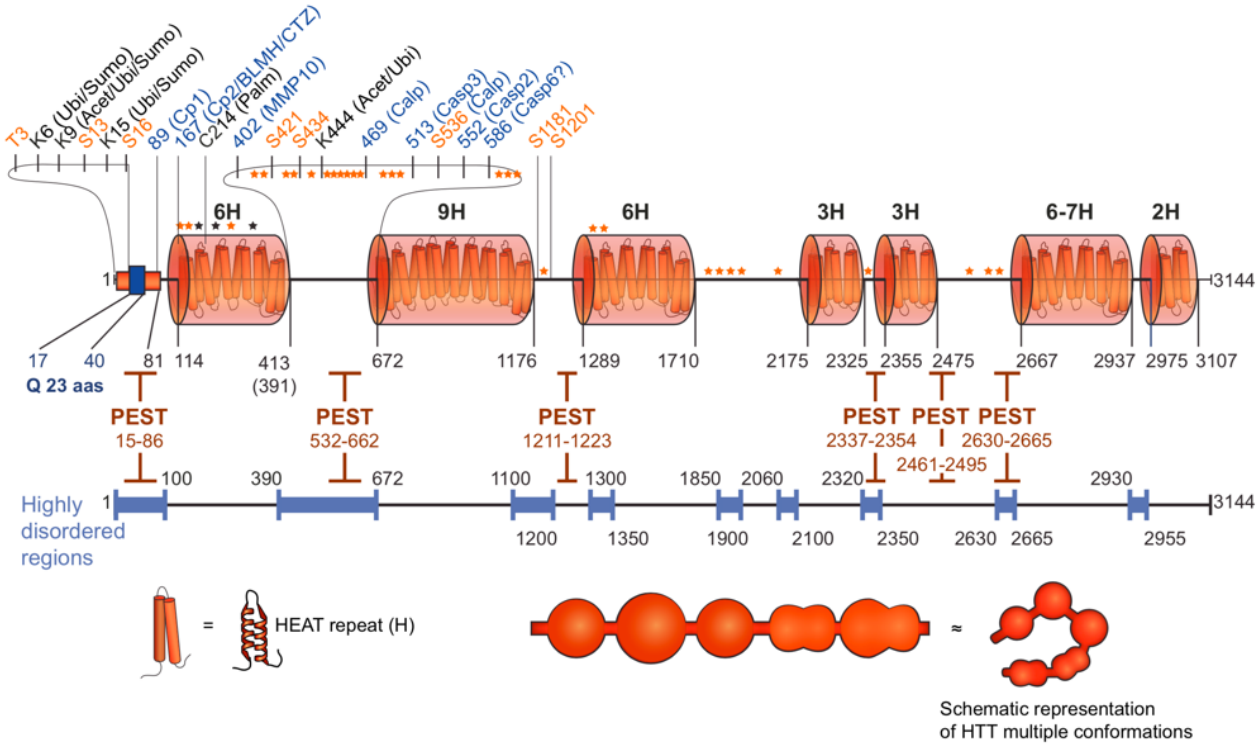

Figure 1.4 Human huntingtin protein structure.

Illustration of the human huntingtin protein structure (Saudou and Humbert, 2016): Amino acid (aa) positions in orange: phosphorylation sites. Amino acid positions in black: sites of indicated modifications. Amino acid positions in blue: cleavage sites. Orange and black stars indicate, respectively, phosphorylation and acetylation sites. Numbers arranged linearly on the 2D structure correspond to the limits of the indicated domains (HEAT, PEST and highly disordered regions). H indicates the number of predicted HEAT repeats organised in larger domains and PEST regions are proteolysis-sensitive domains.

Recently, the post-translational modification of expanded HTT protein has been shown to be an important regulator in the pathogenesis of HD. Specific post-translational modifications resulted in the disruption of normal mitochondrial function whereas others were protective thus providing possible therapeutic targets (Arbez *et al.,* 2017).

1.3.1.2 Pathogenesis of Huntington's disease

CAG repeats in HTT code for a polyglutamine (polyQ) stretch found at the N-terminal region of the protein. HD arises predominantly from the gain of a toxic function due to abnormal mutant HTT (Li and Li, 2004). Multiple pathogenic mechanisms attributed to mutant HTT are illustrated in Figure 1.5, and include:

- Mutant HTT protein forming abnormal ß-sheet structures (Li and Li, 2004);
- Cellular mechanisms for disposing of the mutant HTT are abnormal (Yanai *et al.,* 2006);
- Toxic N-terminal fragments are produced when the abnormal protein is cleaved (Shao and Diamond, 2007);
- The toxic effects of HTT are further magnified by post-translational modifications, aggregation and reduced clearance of proteins (Wild and Tabrizi, 2017); and
- Huntingtin (HTT) toxicity results in interference with gene transcription of brain-derived neurotrophic factor (BDNF) and cellular metabolic pathways through mitochondrial mechanisms (Ross and Tabrizi, 2011).

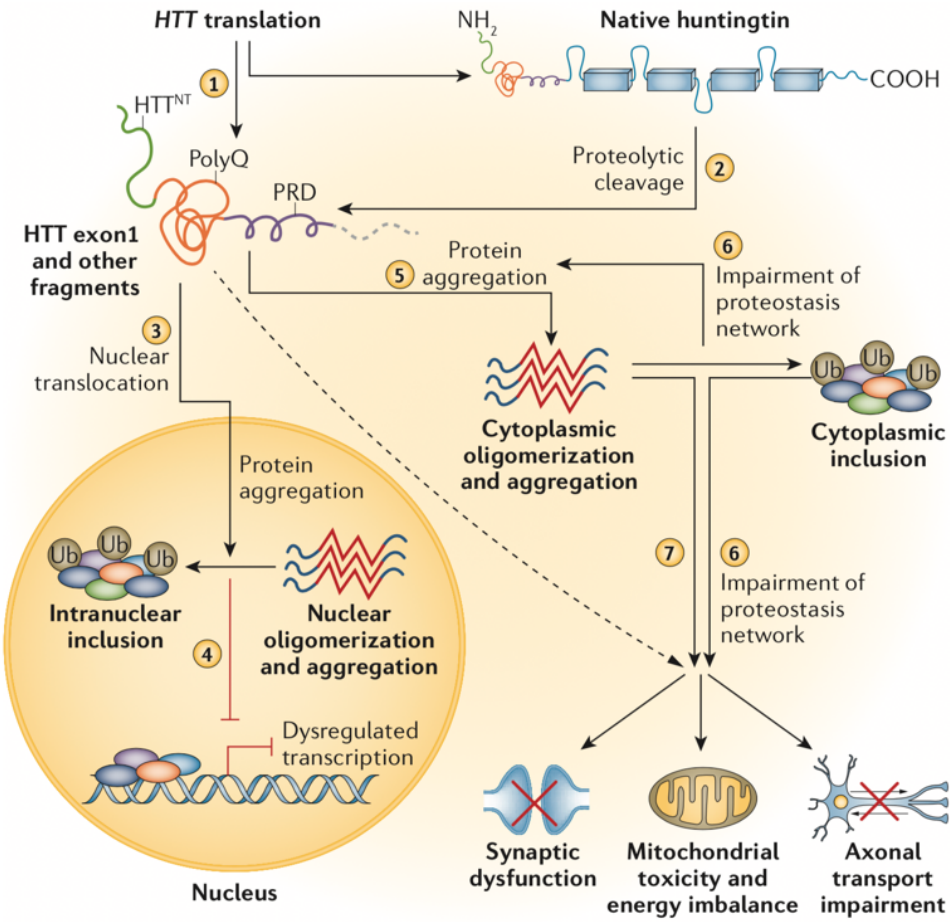

Figure 1.5 Pathogenetic cellular mechanisms in Huntington's disease (Bates *et al.,* 2015)

Illustration of some of the potential pathogenetic cellular mechanisms in Huntington's disease (Ross and Tabrizi, 2011): (1) *HTT* is translated to produce the HTT protein as well as an amino-terminal HTT exon1 fragment (the result of aberrant splicing). The length of the polyQ tract in these proteins depends on the extent of somatic instability. (2) Full-length native HTT is cleaved through proteolysis to generate additional protein fragments. (3) Protein fragments enter the nucleus. (4) Fragments are retained in the nucleus through self-association, oligomerisation and aggregation. This leads to the formation of inclusions, a process that causes transcriptional dysregulation through the sequestration of other proteins and through other incompletely defined mechanisms. (5) HTT fragments oligomerise and aggregate in the cytoplasm. (6) The aggregation of HTT is exacerbated through the disease-related impairment of the proteostasis network, which also leads to global cellular impairments. (7) The aberrant forms of HTT result in additional global cellular impairments, including synaptic dysfunction, mitochondrial toxicity and a decreased rate of axonal transport. HTT = Huntingtin, PRD = proline-rich domain, Ub = ubiquitin.

The pathogenic mechanisms in HD have predominantly been attributed to the HTT polyQ expansion, however, novel research is demonstrating that other processes

may contribute to the disease processes. Firstly, there may be an RNA toxic gain-of-function (Martí, 2016) whereby expanded CAG repeats within exon 1 of the HTT messenger RNA induce toxicity. It has been proposed that other types of diseases with CAG expansion (such as some of the SCAs) may share pathogenic aspects of RNA toxicity with HD (Martí, 2016). Secondly, the repeat-associated non-ATG (RAN) translation proteins form proteins similar to the polyQ chains but with a different amino acid repeat sequence (Bañez-Coronel *et al.,* 2015). In HD, RAN proteins are found in areas of the brain with the greatest neuronal loss such as the caudate nucleus. Other triplet repeat expansion diseases have been found to have RAN proteins such as Fragile X syndrome, C9orf72mutations and several of the SCAs (Todd *et al.,* 2013).

1.3.1.3 Pathology of Huntington's disease

Atrophy of the caudate and putamen is the hallmark of the gross neuropathology of HD. Although there is cell loss in the cerebral grey and white matter and in specific thalamic and hypothalamic nuclei, atrophy in these structures is less severe compared to the striatum (Vonsattel *et al.,* 1998). Histologically, in the striatum, large interneurons are selectively spared but there is up to 95% loss of γ-aminobutyric acid GABAergic medium spiny projection neurons. In the cerebral cortex, neuronal loss is greatest in layers III, V, and VI (Vonsattel, 2007). Thalamic degeneration is now a well-established, if not prominent, feature of HD. The intralaminar centromedian-parafascicular complex of the thalamus is anatomically associated with the striatum and linked to the associative, limbic and oculomotor circuits that are thought to be related to cognitive, emotional and ocular motor features seen in HD (Rüb *et al.,* 2015). Thalamic atrophy contributes significantly to early executive decline in HD (Kassubek *et al.,* 2004).

Intranuclear nuclear and neutropil inclusions, consisting predominantly although not exclusively of mutant HTT protein aggregates, are found in neurons of the striatum and cortex (Vonsattel, 2007). The dissemination of aggregates does not correlate well with the distribution of cell death and it has been proposed that inclusions are a

'coping response' to the toxic effects of HTT, however, this is controversial (Arrasate *et al.,* 2004; Arrasate and Finkbeiner, 2012).

1.3.2 Huntington's disease like 2

1.3.2.1 Genetics of Huntington's disease like 2

As noted, HDL2 is an autosomal, dominantly inherited disorder caused by a variable CTG/CAG expansion mutation in exon 2A of the *JPH3* gene on chromosome 16q24.3 which codes for the JPH3 protein (Holmes *et al.,* 2001). Holmes *et al.* (2001) suggest that within the *JPH3* locus, on exon 2A, there are multiple acceptor sites resulting in alternative-splicing of variable triplet repeats coding for polyleucine and polyalanine chains (Figure 1.6). This is different from HD where only a polyQ chains is produced. Normal allele sizes range from 6 to 28 triplets and complete penetrance of the disease is seen with greater than 40 triplet repeats measured in blood (Margolis *et al.,* 2004). The longest CTG/CAG repeat size reported in HDL2 to date is 59 triplets (Bardien *et al.,* 2007). Anticipation has been reported in only one HDL2 case with a paternal inheritance (Greenstein *et al.,* 2007), therefore the extent of anticipation remains unknown.

While multiple haplotypes have been described in HD (Kay *et al.,* 2018), in HDL2 only a single haplotype has been reported (Krause *et al.,* 2015). Haplotype analysis has suggested that HDL2 cases share three single nucleotide polymorphisms (SNP) and therefore there is likely a single founder origin of the mutation (Krause *et al.,* 2015). This finding likely explains the association that HDL2 has to date with African ancestry. Reviewing the distribution of the cases of HDL2 from around the world, the majority of which share a common haplotype, suggests that the mutation originated in sub-Saharan Africa at least 2,000 years ago (Krause *et al.,* 2015).

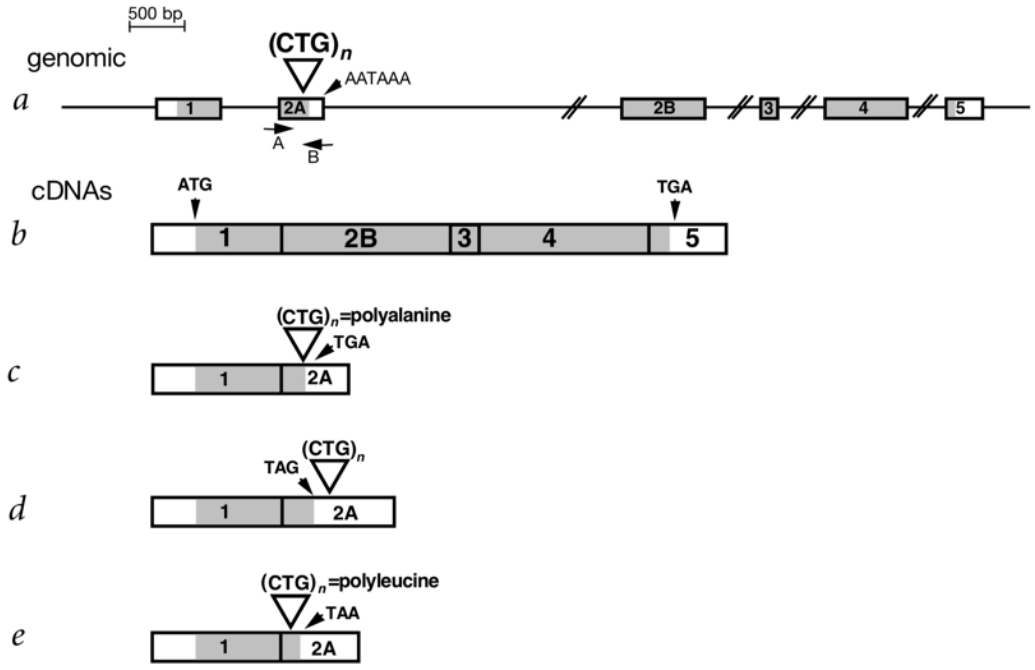

Figure 1.6 Localisation of the HDL2 CTG/CAG repeat to the *JPH3* locus.

Localisation of the HDL2 CTG/CAG repeat to the *JPH3* locus. Alternate transcripts, identified by RT–PCR of normal brain, consist of JPH3 exon 1 spliced to the repeat-containing exon (2A) with three different splice acceptor positions leading to different reading frames. Depending on the splice acceptor site, the repeat is in a 3' untranslated region in frame to encode polyalanine or in frame to encode polyleucine. Shading indicates open reading frame.

The junctophilin (JPH) family of proteins (Nishi *et al.,* 2000) is crucial for the maintenance of cell ultrastructure and for regulating calcium signalling proteins in excitable tissues. Furthermore, studies have shown there is phylogenetic preservation of the JPH proteins across multiple species, highlighting the the importance of the gene (Nishi *et al.,* 2003). There are four JPH proteins (JPH1-4) with JPH1 and 2 being expressed in skeletal and cardiac muscle respectively (Nishi *et al.,* 2000). The JPH3 and 4 proteins are expressed primarily in the neurons although there is some expression of JPH3 in the testis. Both JPH3 and 4 potentially have a role in coupling membrane neurotransmitter receptors and intracellular calcium channels (Nishi *et al.,* 2003). Figure 1.7 illustrates the ultrastructure and relationship of JPH3 and 4 to the endoplasmic reticulum and the cell membrane. JPH3 and 4 may be involved with the positioning of the endoplasmic reticulum in relation to the cell membrane. Furthermore these transmembrane proteins are

crucial for the normal functioning of voltage gated channels, N-methyl D-aspartate glutamate (NMDA) and ryanodine receptors (Landstrom *et al.,* 2014).

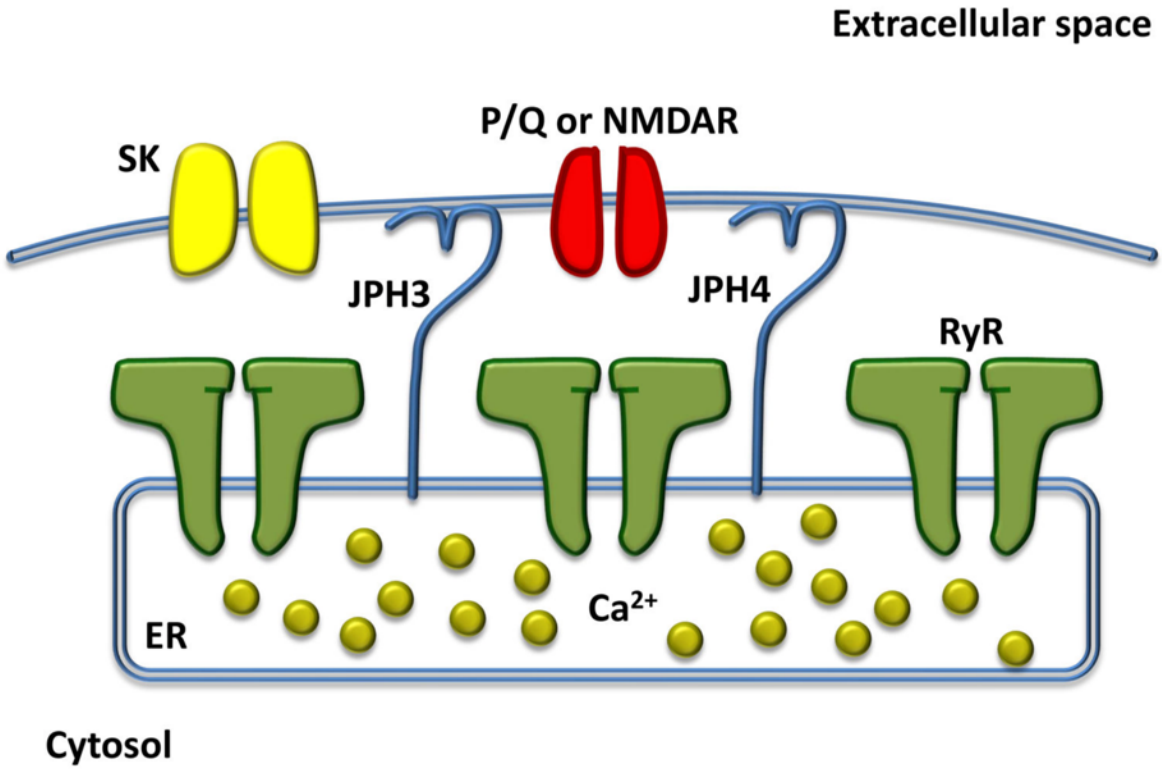

Figure 1.7 JPH3/4 and neuronal ultrastructure.

Illustration of JPH3/4 and neuronal ultrastructure (Landstrom *et al.,* 2014). The two JPH3 and 4 isoforms (light blue). This is critical for effective Ca2+ signalling from the P/Q-type channel (red), RyR (green) and SK channels (yellow). Abbreviations: ER, endoplasmic reticulum; JPH3, junctophilin-3; JPH4, junctophilin-4; NMDAR, N-methyl D-aspartate glutamate receptor; P/Q, P/Q-type voltage gated channel; RyR, ryanodine receptor; SK, small-conductance Ca2+-activated potassium channel.

1.3.2.2 Pathogenesis of Huntington's disease like 2

The pathogenesis of HDL2 remains largely unresolved (Orr and Zoghbi, 2007, Krench *et al.,* 2016). Over the last two decades, since the *JPH3* trinucleotide repeat expansion was first described, there have been several possible pathogenic mechanisms suggested in HDL2. These include:

- RNA toxicity: In 2007, RNA foci were reported in HDL2 *post mortem* brain specimens using fluorescent *in situ* hybridisation (FISH) analysis (Rudnicki *et*

al., 2007). The RNA foci appeared to be formed by *JPH3* transcripts containing the CTG/CAG repeat expansions. These RNA molecules were found to be toxic in cell models.

- Loss of function: JPH3 proteins usually secure the plasma membrane to the endoplasmic reticulum and therefore allow for signalling between intracellular ion channels and the cell surface. The hypothesis that the *JPH3* triplet expansion repeat may reduce messenger RNA expression, thus resulting in partial loss of the protein function, has been supported by two sources. Firstly, Nishi *et al.* (2003) developed *JPH3* knockout mice which developed a motor disorder but showed no intra cellular aggregates or neurodegeneration histologically. Secondly, the amount of JPH3 protein was found to be reduced in HDL2 brain specimens as compared to controls, supporting the theory that the loss of *JPH3* expression could possibly contribute to the pathogenesis of HDL2 (Seixas *et al.,* 2012).

- Gain of function: PolyQ tracts have been found to cause neurotoxicity in HD (Li and Li, 2004) as described above and in other triplet repeat diseases (Orr, 2012). In HDL2, polyQ tracts from the antisense strand of the HDL2 locus, or polyleucine or polyalanine tracts from variant splicing of the gene, have likewise been thought to contribute to the pathogenesis of HDL2 (Wilburn *et al.,* 2011). Wilburn *et al.* (2011) developed a mouse model that contained an expanded human *JPH3* CTG/CAG repeat and demonstrated polyQ-containing intranuclear aggregates in regions of the mouse brain that were similar to those described in human HDL2. This suggests that polyQ tracts are pathogenic in HDL2. However, Seixas *et al.* (2012) have found that polyglutamine is not expressed in the HDL2 brain, or is in low quantities, and therefore expanded polyQ tracts are unlikely to make a significant contribution to the HDL2 pathogenesis (Seixas *et al.,* 2012).

The implication of these findings is that multiple processes may contribute to the pathogenesis of HDL2. Some of these mechanisms are distinctly different from those in HD such as the loss of function of *JPH3*. This therefore brings into question how

different genotypes produce similar phenotypes. It is likely that there is pathogenetic overlap between HDL2 and HD that is yet to be described (Rudnicki *et al.,* 2008; Seixas *et al.,* 2012).

1.3.2.3 Pathology of Huntington's disease like 2

Reports of the pathological findings in HDL2 are rare and limited to eight specimens (Margolis *et al.,* 2001; Greenstein *et al.,* 2007; Rudnicki *et al.,* 2008). Furthermore, it is not reported at what stage of the disease these specimens were obtained. Neurodegeneration is greatest in the striatum and is directed at striatal medium spiny neurons and a subgroup of cortical pyramidal neurons, with the findings generally described as similar to HD (Greenstein *et al.,* 2007). Similarities in pathology have been highlighted by a study which systematically compared the brains of five HDL2 patients with age matched HD specimens (Rudnicki *et al.,* 2008). Prominent findings in both diseases included cortical atrophy with disproportionally greater striatal atrophy. Nuclear protein aggregates were common in HDL2 and HD, and the ultrastructural features of HDL2 and HD aggregates were similar. The similarity between HD and HDL2 is striking, and confirms that HDL2 is the condition that most resembles HD histopathologically (Rudnicki *et al.,* 2008). However, the distribution of intracellular protein aggregates was not identical in HD and HDL2. Both nuclear and neuropil aggregates were found in HD whereas aggregates were detected only in the nuclei of HDL2 specimens (Rudnicki *et al.,* 2008). Aggregates were most common in the amygdala, followed by the entorhinal and the motor cortex in both HD and HDL2, but were only found in the pons and medulla in HD samples. In HDL2, aggregates were more frequent in the upper cortical layers II and III compared to deep cortical layers, whereas in HD they were concentrated in layers III, V, and VI (Rudnicki *et al.,* 2008). Despite extensive striatal degeneration in both diseases, few striatal aggregates were found in either disease. Aggregates were typically present in the thalami of HD brain specimens but were detected in a single thalamus from a subject with HDL2. The thalamus has otherwise been reported as normal in HDL2 (Rudnicki *et al.,* 2008). However, pathological studies in HDL2 and the small number of subjects in these reports limits the interpretation of these findings. The final

characterisation of the aggregates in HDL2 is important as it may give further clues to the pathogenic overlap between HDL2 and HD.

1.4 Phenomenology and phenotypes

Phenomenology is the tool that clinicians have used to classify study the movement disorders resulting in an understanding of their pathobiology and redefining conditions like dystonia (Albanese *et al.,* 2013). The description of a movement disorder's phenomenology contributes to the knowledge of a disease's phenotype (Fahn 2011).

Movement abnormalities can be classified into two broad categories – hypokinetic and hyperkinetic syndromes. The hypokinesias are defined by the loss of spontaneous and voluntary movements resulting in the clinical features of akinesia – slowness (bradykinesia) and rigidity. Parkinson's disease is considered the classic hypokinetic disease. By contrast, hyperkinesias are increased movements which are generally uncontrollable and include chorea, tremor, dystonia, tics and myoclonus (Fahn, 2011). Diagnosing movement disorders is often impossible without an evaluation of the phenomenology of the disease even in the era of neuroimaging and genetic testing (Fahn, 2011).

1.4.1 Huntington's disease phenotype

In 1872, George Huntington first described the disease that now bears his name (Huntington, 1872). Huntington's description of the inheritance pattern and clinical features of the disease was remarkable for the time, although accounts of chorea preceded his report (Wexler, 2013). Many of the features he described, including tongue protrusion, motor persistence and chorea, remain part of the assessment of the disorder today (Huntington Study Group, 1996). HD comprises the three cardinal features of a movement disorder, cognitive dysfunction and psychiatric symptoms. However, there is heterogeneity to the HD phenotype (Bates *et al.,* 2015).

1.4.1.1 Movement disorder

The motor disorder of HD has two broad components (Bates *et al.,* 2015). The first is that of hyperkinetic features, which consist predominantly of chorea and dystonia. Chorea is the most prominent of these and often presents early in typical adult onset form and gives HD its characteristic appearance. Choreiform movements are involuntary, irregular and unpredictable; they may begin very subtly, in the extremities of patient, and then progress to violent movements of the whole body. However, the severity of the chorea does not necessarily correlate with disease progression as it may plateau or reduce in the later stages of the condition (Rosenblatt *et al.,* 2011). Dystonia is a sustained muscle contraction which is involuntary and often intermittent. In HD, dystonia is seen in 95% of patients once the disease has manifested and often affects the axial muscles. Unlike chorea, dystonia progresses with disease duration in HD (Louis *et al.,* 1999).

The second component of the HD movement disorder is the hypokinetic features. These consist of bradykinesia and rigidity. The hypokinetic features of HD become more prominent as the disease progresses and correlate with disability (Rosenblatt *et al.,* 2006). These hypokinetic features of HD are sometimes referred to as parkinsonism. Parkinsonism refers to tremor, rigidity, bradykinesia and gait disturbances (Jankovic, 2008). Tremor is not a common feature of HD (Dorsey, 2013). Therefore, it is more accurate to refer to the individual components of bradykinesia and rigidity when describing the clinical features of HD. The term ‘parkinsonism’, however, often features in the HD and HDL2 literature.

When subjects present with HD before the age of 20 years, the disease is referred to as the Westphal variant or juvenile variant of HD. There is variability in this presentation. The abnormal CAG expansion in these subjects is usually greater than 55 repeats in length (van Dijk, 1985). The early onset HD phenotype differs from the classic HD form in that often, there are greater hypokinetic features which may been associated with seizures (Cloud *et al.,* 2012). Subjects can present with behavioral changes and rarely have chorea. Juvenile HD is seen in less than 5% of all HD cases (Quarrell *et al.,* 2012).

With disease progression, the gait of HD patients deteriorates, usually in the late stage. This is due to worsening rigidity and dystonia, with resultant falls. Patients therefore require greater care at this stage of the disease (Rosenblatt *et al.,* 2011). Dysarthria and dysphagia are common features of HD and aspiration pneumonia is a common contributor to mortality in the disease (Heemskerk and Roos, 2010). Research on HD and dysphagia remains underdeveloped.

1.4.1.2 Cognitive disorder

Huntington's disease has a subcortical dementia associated with the preferential pathology in the striatal and subcortical structures (Wahlin and Byrne, 2012). Therefore, poor attention, planning and cognitive slowing predominate in HD, which help to differentiate it from Alzheimer's dementia, where amnesia is the primary cognitive dysfunction (Aretouli and Brandt, 2010). HD dementia may present many years before the motor features (Paulsen *et al.,* 2008; Tabrizi *et al.,* 2013) and may be a significant source of disability. Language is relatively spared until the later stages of the disease although the motor disorder can disrupt the motor components of speech (Aretouli and Brandt, 2010).

1.4.1.3 Psychiatric disorder

The psychiatric symptoms of HD can present inconsistently, unlike the motor and cognitive features, which generally progress with the duration of the disease. The psychiatric features can be just as disabling as either the cognitive or motor components of the disease. These features, sometimes referred to as the behavioral component of HD, include depression, irritability, aggression and apathy. Psychotic features are less common. Depression is reported in more than half of subjects with HD (Thompson *et al.,* 2012) and can result in suicide. Suicide is the most common cause of death in HD in the early stages of the disease and occurs five to ten times more commonly in HD than in the general population (Walker, 2007). Depression is important to assess in HD as there is evidence that it can respond to treatment (Frank, 2013). Apathy is a significant source of disability in HD and has been found to progress with the motor features of the disease (Thompson *et al.,* 2012). In the

TRACK HD study, apathy was found in subjects 36 months before the onset of the motor symptoms (Tabrizi *et al.,* 2013).

1.4.1.4 Natural history of Huntington's disease

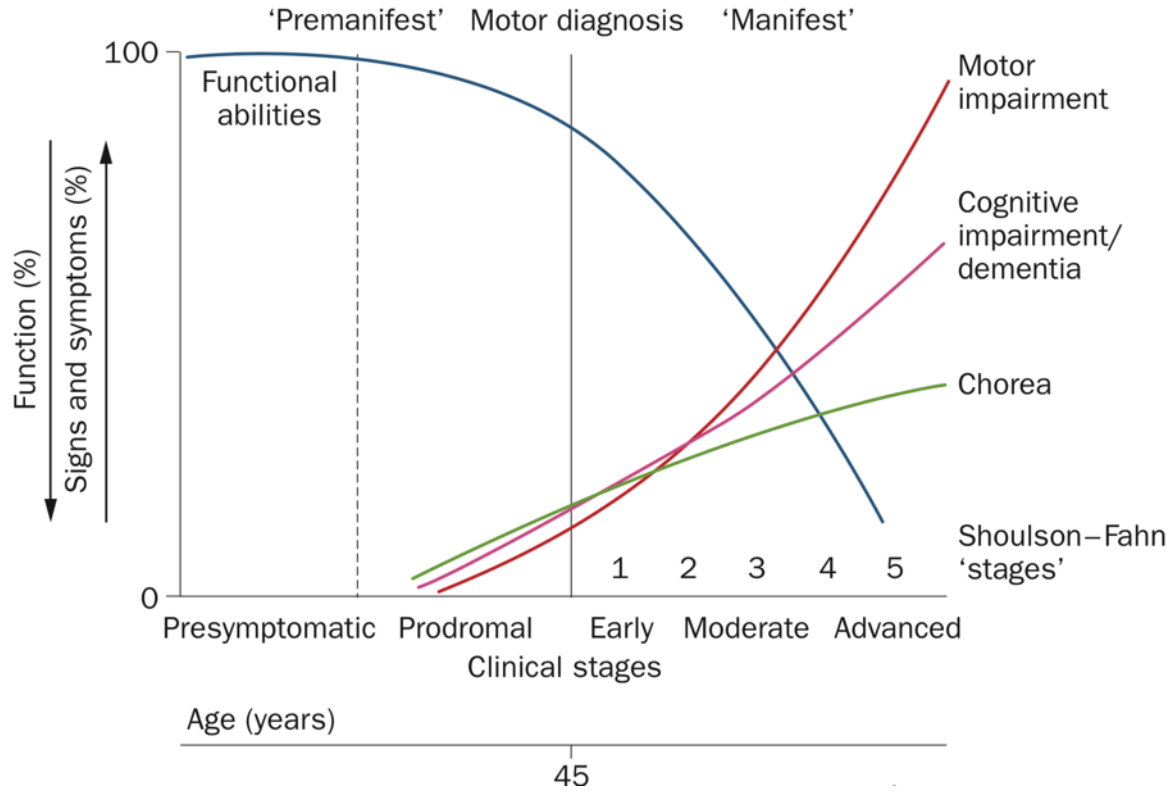

Figure 1.8 The natural history of Huntington's disease (Ross *et al.,* 2014)

Defining the onset of disease in HD is crucial in describing the natural history of HD. This can be challenging due to the phenotypic heterogeneity of the disorder. Currently, the most consistent and widely agreed clinical feature used to mark the onset of the disease is the initial manifestation of abnormal motor signs (Ross *et al.,* 2014) (Figure1.8). Specifically, HD is defined as 'manifesting' once a subject scores greater than four points on the motor component of the Unified Huntington's Disease Rating Scale (UHDRS) (Klempír *et al.,* 2006).

The natural history of HD has been divided into various periods. 'Premanifest' is considered the period before motor disorder onset and there is a wide variability in the clinical features of this stage. Different terms define the early or premanifest stages of HD. These include the 'presymptomatic' period where there are no motor signs or symptoms of HD. This is followed by the 'prodromal' stage, where subtle

cognitive decline and psychiatric symptoms such as apathy (Tabrizi *et al.,* 2013). 'Manifest' HD is characterised by the evidence of motor abnormalities (Figure 1.8). Longitudinal reports have shown cognitive and psychiatric symptoms may be detected in subjects before the motor symptoms manifest (Paulsen *et al.,* 2008; Tabrizi *et al.,* 2013). PREDICT HD was a study that monitored clinical, MRI and cognitive changes in premanifest and manifest HD subjects. This was a matched control study which compared premanifest and early-stage HD subjects with unaffected matched control subjects. It measured multiple features, including clinical MRI and cognitive function. As shown in the PREDICT HD study, changes in MRI can predate the motor features in HD by 15 to 20 years (Paulsen *et al.,* 2008). However, the onset of the motor symptoms remains the clinical feature that defines disease onset in HD because this is considered the most consistent sign, given the considerable variability of the HD non-motor signs (Ross *et al.,* 2014).

Initially, it was suggested that the manifest period of HD be divided into five clinical stages (Shoulson and Fahn, 1979). These stages were based on descriptive characterisations which did not have any practical implications for treatment or prognosis of the disease. Ross *et al*. (2014) have therefore proposed simplifying the stages of manifest HD into three, based on a patient's function:

1. Early stage: this is where patients function well in most areas and can often continue to work. Patients are usually completely independent.
2. Moderate stage: patients are no longer able to work but still perform activities of daily living (ADL). Patients are no longer completely independent and complex functions are impossible.
3. Late stage: patients cannot perform ADLs without assistance. Patients are completely dependent on others.

The mean age of onset in HD is 45 years (Bates *et al.,* 2015). Once the disease has manifested, greater functional disability ensues, with dementia, weight loss, gait dysfunction, dysarthria and dysphagia (Heemskerk and Roos, 2010, Bates *et al.,* 2015). Motor impersistence and greater hypokinesia are associated with greater need for assistance for ADL from carers (Roos, 2010). Inevitably, death follows within approximately 15-20 years from the onset of the disease (Ross *et al.,* 2014) (Figure 1.8).

Decades of research have defined the HD phenotype and genotype. Longitudinal studies of the natural history of HD (Young *et al.,* 1986; Penney *et al.,* 1990) and correlations of the HD phenotype and genotype resulted in the discovery of the *HTT* gene (MacDonald, 1993). Other studies such as the Prospective Huntington At Risk Observational Study (PHAROS) (Huntington Study Group PHAROS Investigators, 2006) and the Cooperative HD Observation Research Trial (COHORT) (Huntington Study Group COHORT Investigators, 2012) have established important HD clinical features. The TRACK HD and PREDICT HD studies have collected neuroimaging, clinical and neuropsychological data on HD which have contributed to the establishment of biomarkers in HD. Importantly, at the Johns Hopkins University HD Clinic, families with HD have been systematically studied for more than 30 years, with some individuals having data collected from the premanifest stage up to autopsy, which has further contributed to the understanding of the disease (Rosenblatt *et al.,* 2011). These findings have shown that not only is there a strong inverse correlation between the age of disease onset and the length of the CAG repeat expansion (Langbehn *et al.,* 2004) but longer CAG expansions have been found to be associated with faster disease progression and poorer prognosis of the disease (Rosenblatt *et al.,* 2011). Furthermore, these longitudinal studies have shown significant variation in the age of disease onset for a given abnormal CAG expansion. The abnormal repeat length accounts for up to 73% of the variation of age of onset of disease (Langbehn *et al.,* 2004). The remaining variation has been attributed to genes other than HTT and environmental factors (Wexler *et al*., 2003). A recent example of external factors affecting the onset of HD has been perinatal insults, which are associated with an earlier age of onset (Barkhuizen *et al.,* 2018). This research has now pathed the way for future therapeutic interventions in HD (Wild and Tabrizi, 2017).

1.4.2 Huntington's disease like 2 phenotype

A formal review of the literature on the HDL2 phenotype is presented in Chapter 3 of this thesis. It will show that, to date, reports of the HDL2 phenotype are anecdotal. Therefore, the clinical features of HDL2 require systematic study. Phenotypically,

HDL2 is similar to HD because cases from the literature have either been identified from screening large cohorts sent for HD genetic testing or from subjects with an HD phenotype. The initial description of HDL2 is such an example. Margolis *et al.* (2001) described a family that was very similar to HD phenotypically. The initial symptoms were weight loss and decreased coordination, which led to dystonia and chorea. The disease then progressed to include rigidity and bradykinesia and, in some subjects, psychiatric symptoms were reported. Dementia was present in all cases and progressed with the motor symptoms. Similar to the prognosis in HD, Margolis *et al*. (2005) described these subjects as dying within approximately 15 years of the onset of symptoms.

1.5 Instruments, rating scales and biomarkers

To compare the phenotypic differences between HDL2 and HD, validated rating scales, biomarkers and established instruments that have been used in evaluating HD and other movement disorders were considered. Consideration was given to the cost of each investigation, the time each would take and which of the instruments were valid in the context of this research. Specifically, the UHDRS was the clinical scale used for the current study. The MRI protocol that was established for the PREDICT HD (Paulsen *et al.,* 2008) study was used for the neuroimaging component of the current research.

A biological marker (biomarker) can be defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention” (Ross, 2003). The development of biomarkers to measure the staging and progression of neurodegenerative diseases has become essential to measure and assess the efficacy of therapeutic interventions (Ross *et al.,* 2014). The development of biomarkers for HD has included neuroimaging and objectively measurable clinical feature specific to the disease (Tabrizi *et al.,* 2013). Biofluid-based biomarkers are measurable biochemical compounds found in the blood or cerebrospinal fluid (CSF); these have been the focus of more recent research (Tabrizi *et al.,* 2009; Paulsen *et al.,* 2008; Ross *et al.,* 2014; Byrne *et al.,* 2018). TRACK HD (Tabrizi *et al.,* 2013)

evaluated biomarkers for clinical trials and facilitated the development of several therapeutic studies which include the promising ASO trial in HD that begins phase III in 2019 (Wild and Tabrizi, 2017).

1.5.1 Clinical rating scales and biomarkers

Clinical rating scales have the advantage that they can be used in most locations, are inexpensive and can be video-recorded and therefore re-evaluated (Klempír *et al.,* 2006). Furthermore, clinical rating scales can be used to examine multiple constituents of a disease phenotype, including the cognitive and functional components, and can measure the spectrum of the movement disorder. The disadvantage of using rating scales is the outcome variability between raters who cannot usually differentiate between therapeutic interventions that have a disease-modifying effect or a symptomatic effect (Henley *et al.,* 2005).

Clinical biomarkers are clinical features of a disease that need to be reliably and objectively assessed (Ross *et al.,* 2014). Therefore, clinical rating scales such as the UHDRS are not ideal due to their inability to detect some of the complexities of the clinical features of HD. Moreover, rating scales are susceptible to bias and intra-rater and inter-rater variability (Weir *et al.,* 2011). TRACK HD employed quantitative motor measures to overcome these limitations and these tools have now been used more extensively (Tabrizi *et al.,* 2013). The clinical biomarkers used in TRACK HD are referred to as the Q (quantitative) battery. These are computerised quantitative motor measurements and assess tongue force, grip force, speeded and self-paced tapping (Reilmann *et al.,* 2013).

1.5.1.1 The Unified Huntington Disease Rating Scale (UHDRS)

The Unified Huntington's Disease Rating Scale (UHDRS) was developed by the Huntington Study Group (1996) as a research tool to monitor and assess the clinical features of patients with HD. Four domains of clinical performance and capacity are assessed by the UHDRS: motor function, cognitive function, functional capacity and behavioural abnormalities. The first part of the UHDRS, importantly with regards to

this study, measures the motor features of the disease. The Total Motor Score (TMS) consists of multiple sub-scores that measure motor features in different domains. The sub-score ranges from 0-4, with higher scores indicating greater disability. The TMS UHDRS total score ranges from 0–124. The UHDRS assesses both the hypokinetic features, rigidity, bradykinesia and the hyperkinetic features of HD, namely, chorea and dystonia as well as other clinical components such as gait and oculomotor function (Huntington Study Group, 1996). The behavioural component is predominantly an assessment of the HD psychiatric features. The UHDRS has informed HD research, including the development of biomarkers, over the last two decades (Franciosi *et al.,* 2013). The UHDRS remains the only quantitative motor rating method recommended by the International Parkinson and Movement Disorder Society (Mestre *et al.,* 2018). The justification for using the UHDRS in this study is discussed in the subsequent chapters.

1.5.2 Neuroimaging biomarkers

As noted above, the hallmark of HD pathology is striatal atrophy. Changes on MRI show linear atrophy of the caudate and putamen that can precede disease onset by 5 to 20 years (Paulsen *et al.,* 2008). However, there may be compensatory mechanisms that reduce the effect on the HD patients' cognitive and motor function early in the disease, making clinical measures unreliable (Papoutsi *et al.,* 2014). Therefore, MRI striatal atrophy is a useful and sensitive biomarker in all stages of HD (McColgan and Tabrizi, 2017).

In HD, neurodegeneration and atrophy are likely preceded by neuronal dysfunction (Weir *et al.,* 2011). Therefore, imaging studies which measure metabolic brain function such as functional MRI (fMRI), positron emission tomography (PET) and magnetic resonance spectroscopy (MRS) could, in theory, detect physiological changes disturbances prior to standard MRI changes. However, these methods require longitudinal validation and are costly (Weir *et al.,* 2011).

1.5.3 Biofluid biomarkers

Useful blood and CSF HD biomarkers have emerged over the past decade (McColgan and Tabrizi, 2017). Some of these are specific to HD such as HTT protein measurements (Ross *et al.,* 2014). Neurofilament light chain (Nfl) protein is not specific to HD but has emerged as a potential HD biomarker (Byrne *et al.,* 2017). This is an intracellular structural neuronal protein that is released into the CSF with neuronal damage and can be detected in blood samples. Nfl serum levels correlate with disease progression and MRI changes in HD. Furthermore, Nfl plasma concentrations can differentiate between pre-manifest HD and manifest HD (Byrne *et al.,* 2018). The presence of Nfl in HD blood samples was reported three years after this study was initiated. Nfl provides an interesting avenue to compare HDL2 to HD as it may show further correlation between the two diseases.

1.6 Summary of the literature review

The literature review has highlighted some important aspects about HDL2 and HD – two conditions which were indistinguishable until genetic studies found them to be two separate disorders. HDL2 has been exclusively described in patients with African ancestry and is being reported as the most common HD phenocopy in countries with high African populations. The similarities between HD and HDL2 extend to pathological and radiological findings, although these data remain limited. Finally, the use of systematic rating scales and other biomarkers has led to a better understanding of the HD phenotype and the incorporation of these markers into therapeutic trials.

1.7 Gaps in the literature and motivation for the study

The HDL2 literature can be divided into two parts. The first part is the description of the HDL2 clinical phenotype. This consists of the original description of HDL2 (Margolis *et al.,* 2001) and subsequent case reviews and case series. These are discussed in Chapter 3 – the HDL2 clinical phenotype systematic review. In brief,

this part of the HDL2 literature has remained undeveloped and requires systematic study to provide a framework for the HDL2 genotype-phenotype correlation.

There are two distinct motor phenotypes that have been proposed (Margolis *et al.,* 2001): a bradykinetic form, where oculomotor function is preserved in HDL2 compared to HD, and a second form with more classic HD clinical signs. Furthermore, HDL2 has been described with acanthocytes in four cases (Walker *et al.,* 2002; Walker, Rasmussen *et al.,* 2003; Walker, Jankovic *et al.,* 2003). Although this haematological finding has not been subsequently verified, HDL2 has been classified as a neuroacanthocytosis syndrome in several reviews and texts (Jung *et al.,* 2011; Adjobo-Hermans *et al.,* 2015; Hoffman *et al.,* 2013). The relationship between HDL2 and acanthocytes will be discussed in Chapter 4. The pathological and radiological reports of HDL2 cases are limited but suggest that HDL2 and HD are strikingly similar (Greenstein *et al.,* 2007; Rudnicki *et al.,* 2008). The suggestion has been made that further studies are needed to find the pathogenic confluences that cause the clinical and pathological similarities between HDL2 and HD (Seixas *et al.,* 2012). In summary, the HDL2 clinical phenotype as described in the literature has remained unverified and limited to date, primarily due to the rarity of the disease.

The second part of the literature has seen advancements in the understanding of the molecular and pathogenic mechanisms of HDL2 (Rudnicki *et al.,* 2007; Wilburn *et al.,* 2011; Seixas *et al.,* 2012). Although this research remains far from complete, more information about the clinical HDL2 phenotype may give the pathogenic and molecular research context. The finding of two diseases that are similar in so many ways but different in others, provides a unique opportunity to study their genotype-phenotype correlations and to explore the pathogenesis of movement disorders, triplet codon repeat disorders and neurogenerative disorders in general, and to ultimately discover novel therapies for both HDL2 and HD. This brings the purpose of this study into focus. By substantiating clinical similarities between HDL2 and HD, this study can provide a clinical and phenotypic reference for future research of the pathogenic confluences that exist between these diseases. Figure 1.9 illustrates how the framework of the thesis has evolved in light of this introduction.

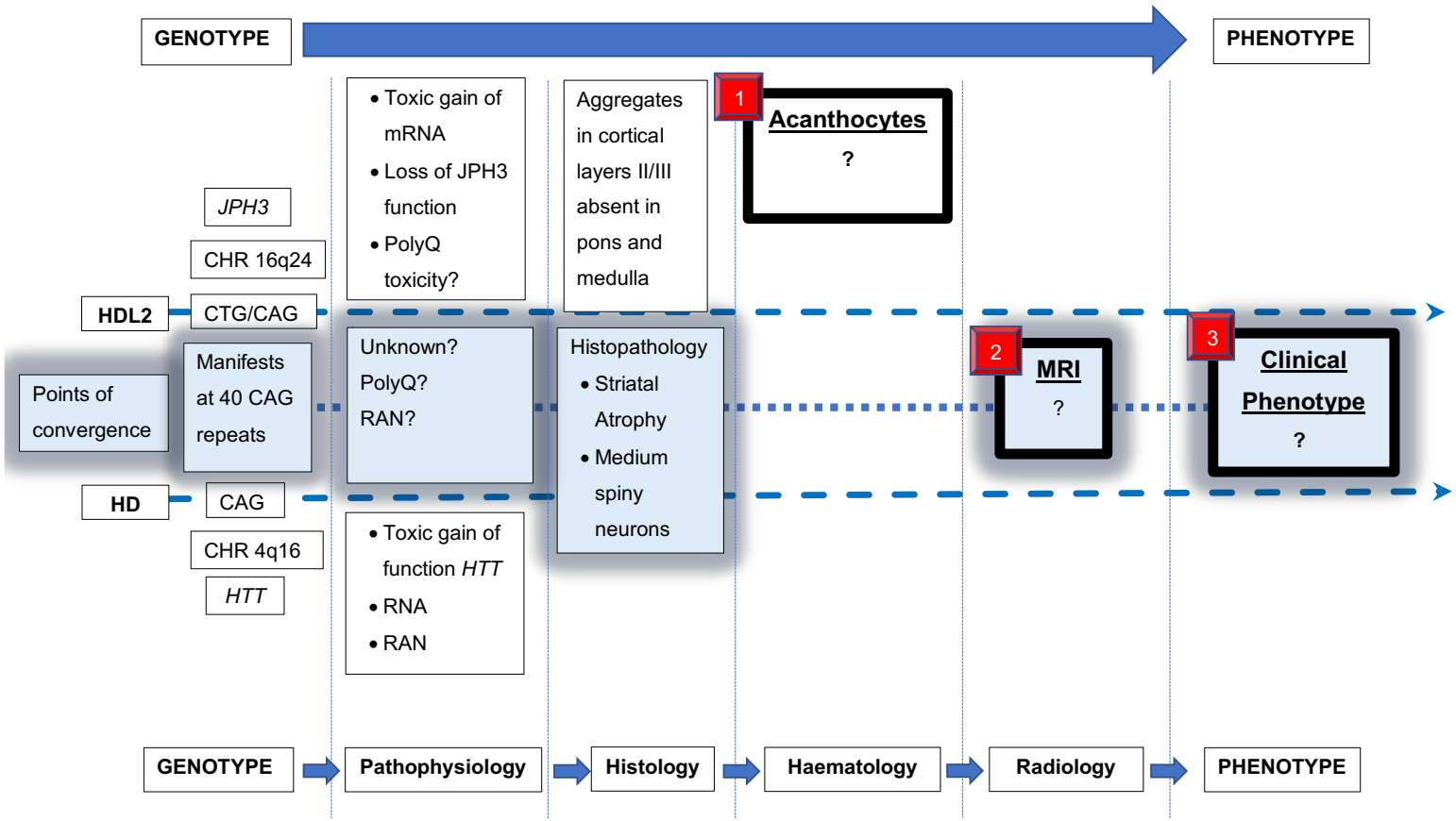

Figure 1.9 Genotype-phenotype correlation

Framework for the thesis illustrating the genotype to phenotype correlation, from left to right, for HDL2 and HD. The dashed horizontal line between HDL2 and HD represent points of possible convergence, which are shaded. These points of possible convergence span multiple fields. Clinical features that are unverified or unsubstantiated and which will be studied in this thesis are highlighted and labelled: 1 – Haematology, 2 - Radiology and 3 - Clinical Phenotype.

1.8 Study aims and objectives

The overarching aim of this study is to define the clinical phenotype of HDL2 and compare it to that of HD.

The specific objectives are:

1. To review the literature and determine the current description of the HDL2 phenotype.
2. To determine whether acanthocytes occur in HDL2 subjects.
3. To use structural MRI to quantitatively compare regional brain volumes of HDL2 and HD subjects.
4. To systematically study the clinical phenotype of HDL2 and compare it to that of HD, with particular attention paid to the movement disorder.

CHAPTER 2

Study Context and Methods

The aim of this chapter is to describe the overall research design and methodology in the context of the study setting. A detailed methodology for each of the published papers is described in the subsequent chapters of Section II. This chapter initially discusses the research design used to achieve the study aims and some of the advantages the unique setting provided. The study participants are then described, including ethnicity, inclusion and exclusion criteria and the difficulties in obtaining affected subjects. Data collection and the specific database application used to store and capture information for this research is then discussed. A detailed description of the subject assessments and the study team is then provided and illustrated. The methodology of the overall research is then discussed. This is followed by a description of the method used for genetic testing at The Division of Human Genetics at the National Health Laboratory Service (NHLS) for HDL2 and HD. The methodology of each published paper is then summarised. This includes the time frames and number of subjects who participated in each part of the research, in conjunction with a description of the instruments used to achieve each of the study objectives. The use of the Unified Huntington's Disease Rating Scale (UHDRS) as the primary investigation tool is then discussed. How the study methodology and instruments relate to the thesis framework of translating genotype to phenotype is then illustrated. Finally, the ethical foundation for this study is provided.

2 Study Context and Methods

2.1 Introduction

Diverse fields across the spectrum of science are needed in order to understand a disease such as HDL2 and correlate its genotype to its phenotype. These scientific fields start with the disciplines of molecular genetics and molecular pathobiology and extend to the clinical fields at the other end of the spectrum, with the field of systems neurobiology in between (Yu *et al.,* 2016). This study focused on the clinical phenotype of HDL2 at one end of this genotype-phenotype correlation. Expertise, tools and methods were required from multiple disciplines including neurology, radiology, psychology, psychiatry, genetics and clinical haematology to achieve this study.

2.2 Research design and strategy

This was a blinded observational study of the HDL2 phenotype, comparing it to that of HD.

This study aimed to clarify aspects of the HDL2 phenotype by focusing on the presence or absence of acanthocytes, comparative MRI features and the clinical phenotype of HDL2, with a particular interest in the movement disorder. Therefore, this study relied on aspects of both cross-sectional design and case control design (Mann, 2003). Data were collected on the same day on each subject and compared across groups. In this study, all available subjects with a confirmed diagnosis of HDL2 or HD were assessed. An accompanying unaffected family member or friend with African ancestry was evaluated to establish normal controls for the haematological and radiological components of the study. The data collected included a standardised history, general and neurological examinations. The movement disorder was assessed using validated rating scales. These were video recorded. Blood samples were then taken, and MRI scans were performed. Case controlled studies have an intrinsic associated rater bias (Mann, 2003). To overcome

this, the raters were blind to the participants diagnoses. All HDL2 cases have to date had African ancestry (Krause *et al.,* 2015). Therefore, to avoid further potential unblinding, all study participants were of African ancestry.

2.3 Study setting

The study was centred in the city of Johannesburg. This setting had advantages which facilitated this research, including a high reported frequency of HDL2 cases and the well-established diagnostic laboratory where testing for HDL2 and HD is performed (Krause *et al.,* 2015). Johannesburg is the largest city in South Africa and is situated in the Gauteng province (Figure 2.1). Gauteng has a population of 14 million people, is predominantly urban and accounts for most of the South African economy (Statistics SA, 2017). The Division of Human Genetics at the National Health Laboratory Service (NHLS), which is associated with the University of the Witwatersrand (WITS), is located in Johannesburg. The NHLS (WITS), a state health laboratory, performs the main genetic diagnostic services for the region and has diagnosed the highest number of HDL2 cases in the world to date (Krause *et al.,* 2015).

Samples for HD and HDL2 genetic testing are sent to NHLS (WITS) from secondary, tertiary and academic hospitals as well as from private health care practitioners in the northern and eastern provinces of South Africa (Krause and Greenberg, 2008; Krause *et al.,* 2015). These provinces include Gauteng, Mpumalanga, Limpopo, KwaZulu Natal, Free State, the Northwest and Northern Cape. Samples for HD and HDL2 testing are also sent to this laboratory from neighbouring countries such as Namibia (Krause and Greenberg, 2008). In 2002, Krause *et al.* (2002) reported that from the samples tested at NHLS (WITS), 21% of black subjects with an HD phenotype had HDL2. Since this finding, NHLS (WITS) has routinely tested for both diseases, increasing the number of HDL2 cases diagnosed.

Johannesburg provides a further advantage insofar as an HD multidisciplinary clinic was established in 2009 by NHLS (WITS) at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) to specifically address the needs of subjects with HDL2

and HD. This clinic is the only HD clinic in the region and patients come from as far as 400km away to attend the clinic. This clinic provided the setting to compare the two diseases. The Johannesburg HD clinic therefore provided the environment to recruit, examine and test the subjects systemically for the study.



Figure 2.1 Map of South Africa showing Johannesburg in the Gauteng province and the other provinces.

2.4 Study subjects

The NHLS (WITS) database was searched for all subjects with HDL2 or HD and with African ancestry. Study participants were identified as subjects of African ancestry and included subjects with black and mixed ancestry as defined by the NHLS. This definition has been previously published as follows:

> Black individuals are those whose gene pool is derived from one or more of the indigenous Bantu-speaking African populations. Mixed ancestry individuals are those whose gene pool is derived from one or more of the indigenous African populations (San, Khoikhoi or Bantu speaking), European immigrants from western Europe and/or slaves and indentured labourers from Madagascar, the Malaysian archipelago and India (Krause *et al.,* 2015,).

Once the database was established, the laboratory results were checked regularly by an unblinded investigator who was not assessing study participants. By the end of 2017, when data collection concluded for the study, there were 55 HDL2 and 105 HD individuals with confirmed diagnoses in the database. Unaffected controls with African ancestry, who were the affected subjects' friends or genetically unrelated family, were enrolled for the radiology and haematology components of the study.

Subject contact numbers were often not available with their record. Where possible, the referring doctor or hospital department was contacted to obtain the information. If this information was obtained, the subjects and their families were then contacted telephonically or by email and invited by a genetic counsellor to enrol in the study.

Many subjects were lost to follow-up after their DNA sample had been sent to the laboratory. There are two reasons for this:

1. It is likely that many potential study participants had died since the samples were submitted for genetic testing. Samples dated back to 1994.
2. Contact details had changed.

In addition, some patients were unable to travel the distance to the study site or were unwilling to participate. The final number of affected participants in this study was 13 subjects with HD and 15 subjects with HDL2. Inclusion and exclusion criteria are outlined in Table 2.1.

Table 2.1 Inclusion and exclusion criteria for study participants

Affected participants (HDL2 or HD)	
Inclusion criteria	**Exclusion criteria**
Genetic testing confirmed at NHLS (WITS)	Genetic testing performed at laboratory other than NHLS (WITS)
≥ 18 years of age	≤ 18 years of age
Subject or carer able to give informed consent	Subject or carer unable to give informed consent
African ancestry	
	History of head injury or stroke
Unaffected controls	
Inclusion criteria	**Exclusion criteria**
Not at risk for developing HDL2 or HD	Directly related to the affected participant and therefore at risk for disease
≥ 18 years of age	≤ 18 years of age
Able and willing to give informed consent	Unable or unwilling to give informed consent
African ancestry	
	History of head injury or stroke

2.5 Data collection and storage

2.5.1 Data collection

Data collection for the overall study took place from 1 February 2015 to 20 December 2017. The haematology and radiology components of the study ended earlier due to specific limitations on resources. The haematology component of the study was limited to two years as this component of the study was time consuming for the haematologists who were participating in this research project. The radiology component of the study was limited by the allocated number of funded MRIs.

A genetic counsellor from NHLS (WITS) assisted with the recruitment of the study participants and the collection of demographic data. Subjects and accompanying, unaffected friends/family members were counselled; a study information sheet was given to each participant and informed consent was obtained prior to their participation (Appendix 1). The affected participants received a second information sheet (Appendix 1) and signed a separate informed consent for the recording, and storing of videos for the UHDRS and other motor rating scales (Appendix 1). If the participant was unable to provide consent, their carer was asked to consent. The clinical team remained blinded to the subjects diagnoses until the assessments were completed.

2.5.2 Database application

Research Electronic Data Capture (REDCap) was used to collect and manage the data for this study. This data system is supported by the University of the Witwatersrand. REDCap is a secure, web-based system and allows for data tracking, auditing, exporting and importing (Harris *et al.,* 2009). All of the data collection sheets that were created and completed for this study can be found in Appendix 2.

2.6 Participant assessments

A collaborative team was established for the study which included health professionals with expertise in neurology, radiology, psychology, genetics and clinical haematology.

2.6.1 Role of each member of the team on the day of assessment

- Dr. D.G. Anderson: neurologist and PhD candidate, principal investigator, study coordinator. Established and maintained the REDCap database, completed all history-taking, neurological evaluations including the UHDRS scales (except the cognitive component) and other motor rating scales, videography and phlebotomy.

- Ms. A. Ferreira-Correia: a neuropsychologist who performed the neuropsychological evaluations, including the UHDRS component. Ms. Ferreira-Correia has used some of these data to compare the neuropsychological aspects of HDL2 and HD for her own PhD.
- Prof. A. Krause: 1st supervisor and Head of the Division of Genetics at NHLS (WITS). Oversaw genetic testing and is the main supervisor of this PhD.
- Ms. M. Gomes: a genetic counsellor at NHLS (WITS). Contacted and counseled subjects, obtained informed consent, recorded genetic and demographic data.
- Dr. M. Haagensen: a radiologist at WDGMC. Performed and analysed the MRI scans for this study.
- Prof. S. Carmona: a clinical haematologist at CMJAH oversaw the prompt methodical preparation and subsequent analysis of the subjects' blood samples. This included immediate analysis of each sample for acanthocytes by microscopy as has been previously described (Storch et al., 2005) and assessment of band 3 band three proteins. The band 3 protein of the erythrocyte membrane is an intrinsic membrane protein. The membrane proteins and glycoproteins were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), using a discontinuous buffer system as previously described by Coetzer and Palek (1986). Protein concentrations were measured by the Bio Rad protein assay. The amount of spectrin to band 3 was then quantitated by densitometry of the stained gels at 540 nm and integration of the surface area under the spectrin and band 3 peaks.

The HDL2 and HD participants and unaffected controls were each assessed in one day over a 5-6-hour period with regular breaks, as required. Lunch and money for transport was provided on the day of the examination for each participant. Only the genetic counsellor was not blinded to the subjects diagnoses as she was not assessing the subjects and needed to search the NHLS data base and contact the subjects. The assessment sequence for the participants is shown in Figure 2.2.

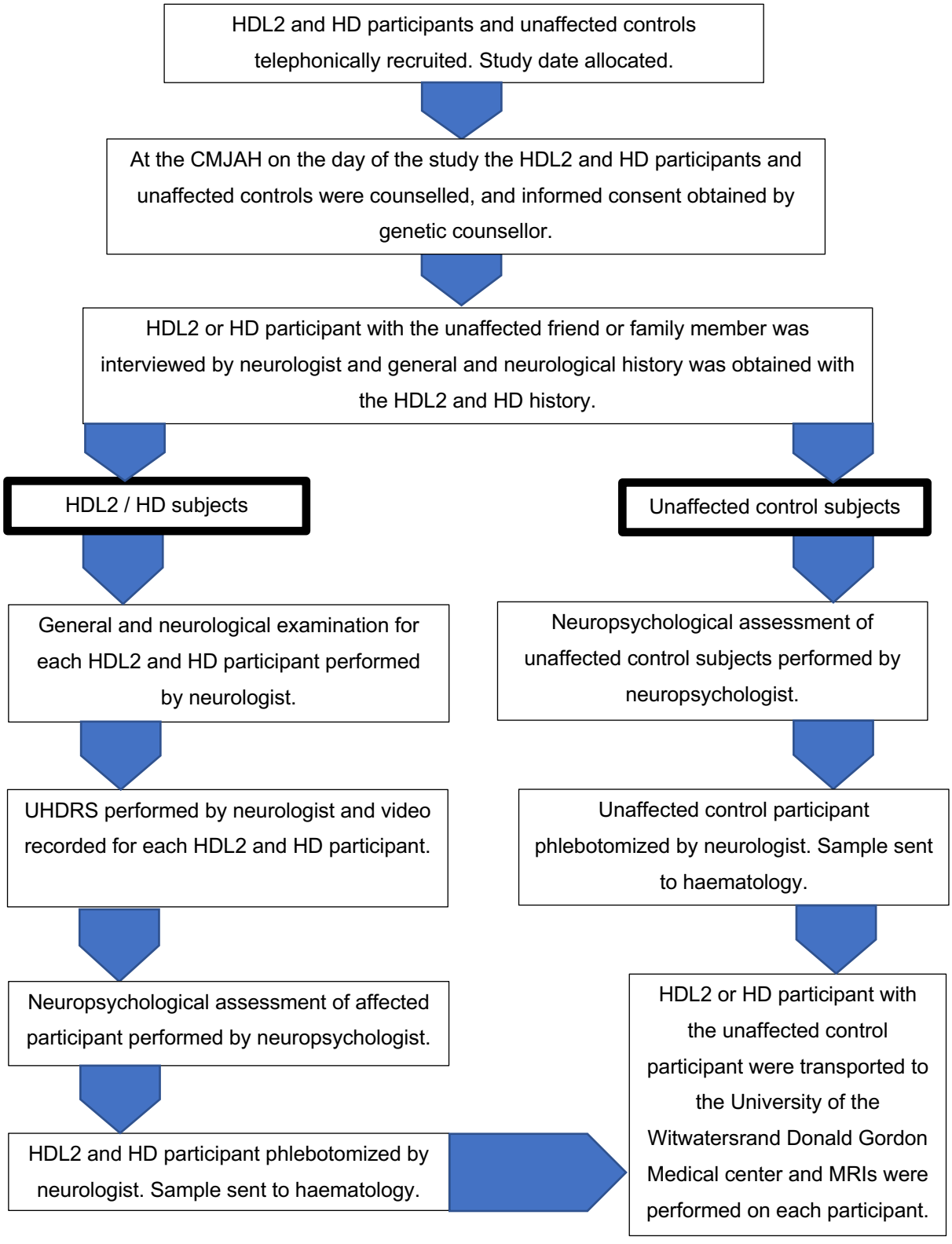

Figure 2.2 Sequence of subject assessments on the day

2.7 Methodology

All subjects with an HDL2 or HD diagnosis who participated in the study had a history taken, were examined and rating scales were performed. The motor rating scales were video recorded. However, not all of the subjects underwent the entire battery of investigations described in the methodology section of this chapter. There were several reasons for this. The haematology component of the study was labour-intensive. Therefore, this component was terminated in May 2017. The MRI scans were donated to this project by the DGMC Radiology Department. Therefore, the study was limited to 35 MRI scans. Not all subjects could complete the cognitive assessment of the UHDRS due to specific limitations such as English proficiency or being unable to participate due to advanced disease. This is described further in Chapter 6.

The published papers in Section II describe the specific samples used from the overall data set and the methodology that was applied in each publication to investigate the objectives and the aims of the study. This includes the statistical analysis for each of the publications.

2.7.1 Genetic testing for Huntington's disease like 2 and Huntington's disease

All HDL2 and HD subjects who were enrolled in the study were genetically diagnosed at the NHLS (WITS) laboratory. The method used by this laboratory for the genetic testing of HDL2 and HD blood specimens has been previously described (Krause *et al.,* 2015). Specifically, the HU4 (HEX labeled) and HDC2 primers *HTT* primers were used to label and amplify the *HTT* CAG expansion (Yu *et al*., 2008) and the *JPH3* primers L237-1 (FAM labeled) and L237-2 were used for the *JPH3* CTG expansion (Holmes *et al*., 2001). NHLS (WITS) routinely tests for both HDL2 and HD triplet repeat expansions in a multiplex reaction when blood samples are sent for testing for either disease due to the high frequency of HDL2 reported in South Africa (Krause and Greenberg, 2008).

2.7.2 Methodology and instruments for each study objective

The methodology used to address each of the study objectives is summarised below. The instruments employed to achieve these objectives are then discussed.

2.7.2.1 Objective 1: To review the literature and determine the current description of the Huntington's disease like 2 phenotype

An extensive search of the HDL2 literature was undertaken. Literature pertaining to clinical descriptions of the HDL2 phenotype was included and reported in a systematic review of the HDL2 phenotype.

2.7.2.2 Instrument used in the review of the clinical Huntington's disease like 2 phenotype in Chapter 3

In brief, an online search pertaining to the clinical phenotype of HDL2 was carried out across multiple databases, in accordance with PRISMA guidelines. The relevant literature from this search was then analysed. The PRISMA guidelines were used as they are widely considered to be the gold standard in conducting systematic reviews (Moher *et al.,* 2009). This study is described in Chapter 3.

2.7.2.3 Objective 2: To determine the frequency of acanthocytes in Huntington's disease like 2 subjects

A blinded case-controlled study was undertaken to evaluate the presence of acanthocytes in HDL2 subjects. Blood samples were obtained by the neurologist on the day of the examination and immediately transported to the haematology laboratory. The samples of 12 HDL2 subjects were compared with 13 HD cases and 21 unaffected control subjects using this method from February 2015 to May 2017.

2.7.2.4 Instruments used to assess the subjects for acanthocytes in Chapter 4 - Haematology

Acanthocytes are described morphologically as spiny erythrocytes, believed to be caused by primary alterations in the red blood cell membrane (Stevenson and Hardie, 2001). In contrast, echinocytes are erythrocytes that, although morphologically similar to acanthocytes, are associated with secondary causes from metabolic or physical stress, including inadequate sample processing (Stevenson and Hardie, 2001). Echinocytes mimic acanthocytes under light microscopy and can create a high false positive rate of acanthocytes (Storch *et al.,* 2005). The method described by Storch *et al.* (2005) for detecting acanthocytes, accepted as the gold standard in the field (Hoffman *et al.,* 2013), was used in this study, as discussed in Chapter 4.

2.7.2.5 Objective 3: To use structural MRI to quantitatively compare regional brain volumes of Huntington's disease like 2 and Huntington's disease subjects

MRI brain volumetry was performed in 9 HDL2, 11 HD and 9 normal control subjects. MRIs were initially screened for clinically significant findings and were then assessed by a blinded radiologist for structural abnormalities. Subsequently, the quantitative MRI volumetric assessment was performed with semi-automated software. The published paper from this study is described in Chapter 5.

2.7.2.6 Instruments used to assess subjects using MRI in Chapter 5 – Radiology

One of the aims of this study was to quantitatively compare brain structure in HD and HDL2 subjects using MRI. To achieve this goal, a standardised and validated method was required to measure brain volumes. PREDICT HD (Paulsen *et al.,* 2008) and TRACK HD (Tabrizi *et al.,* 2013), the two largest HD studies including analysis of structural MRIs, used BRAINS, image analysis software that measures the volumes of subcortical structures as well as grey and white matter volumes of the

hemispheres (Pierson *et al.,* 2011). PREDICT HD applied the BRAINS analysis system to images obtained on 1.5T scanners. TRACK HD used 3T scanners and employed four different image analysis techniques, one of which was BRAINS (Tabrizi *et al.,* 2013). Therefore, BRAINS was used to analyse the 1.5T MRI scans in the current study as it has been extensively used in HD research on 1.5T MRI scans. This is further discussed in Chapter 5.

2.7.2.7 Objective 4: To define systematically the clinical phenotype of Huntington's disease like 2 and compare it to that of Huntington's disease, with particular attention paid to the movement disorder

A blinded cross-sectional design was used to compare the clinical phenotypes of 15 HDL2 and 13 HD subjects. Participants of mixed or African descent underwent an assessment including the Unified Huntington's Disease Rating Scale (UHDRS). The neurocognitive profile of each subject was assessed by a neuropsychologist using the UHDRS cognitive tests. The UHDRS motor component was video-recorded and further evaluated by three blinded experts and the interrater reliability calculated. This study is described in Chapter 6.

2.7.2.8 Instruments used to assess subjects clinical features in Chapter 6 - Clinical phenotype

Careful consideration was given to the choice of instruments used to measure the phenotypic features of HDL2. The data sheets that were used, including all the rating scales, are provided in Appendix 2.
The following were performed and recorded:

1. A standardised history was taken from each subject and their accompanying friend or family member.
2. Age of disease onset, as defined by the manifestation of motor symptoms of the disease.
3. The initial symptoms.
4. General and neurological examinations.
5. Rating scales were performed and video-recorded

a. The UHDRS (Huntington Study Group, 1996).
b. The Scale for Assessment and Rating of Ataxia (SARA) (Weyer *et al.,* 2007).
c. The 'Timed Get Up And Go' test (Podsiadlo and Richardson, 2015).
d. The Unified Myoclonus Rating Scale (UMRS) (Frucht *et al.,* 2002) was included if subjects presented with myoclonus.

The HDL2 literature suggests that HDL2 is the HD phenocopy that has the greatest resemblance to HD clinically. To systematically compare the two conditions, a validated instrument that measures the HD clinical phenotype was considered ideal. Therefore, to achieve the aim set out in Chapter 6, to clarify the clinical phenotype of HDL2, the UHDRS was chosen as the primary instrument. Clear limitations with this approach are that features that are not found in HD might not be detected in the HDL2 subjects. Therefore, additional rating scales were included to try and capture the full spectrum of the HDL2 movement disorder that had been suggested in anecdotal reports.

It is important to note that although the UHDRS is widely used in HD research, it has limitations (Winder, Roos *et al.,* 2018; Winder, Achterberg *et al.,* 2018; Mestre *et al.,* 2018). The UHDRS is unable to differentiate HD stages well, especially early in the disease (Franciosi *et al.,* 2013). Initial interrater agreement of the motor assessment of the UHDRS was found to be adequate (Hogarth *et al.,* 2005). Recently, with wider use of the scale and the assessment of the UHDRS motor component by video-recording, the interrater reliability of the scale has been found to be lower on some of the sub-scores, with dystonia having particularly poor interrater agreement (Winder, Roos *et al.,* 2018).

There had been reports in the literature of greater parkinsonian like features in HDL2 (Margolis *et al.,* 2001; Schneider *et al.,* 2012). Therefore, the UPDRS, which has been established for use in Parkinson's disease, was considered to measure the hypokinetic aspects of HDL2 in this study. However, because there is considerable overlap between the UPDRS and UHDRS and as the UHDRS measures many hypokinetic features, adding the UPDRS was considered redundant and thus was not included.

The UHDRS does not measure ataxia however cerebellar clinical features such as dysdiadochokinesia and action tremor have been described in HDL2 subjects (Bardien *et al.,* 2007, Santos *et al.,* 2008,). Therefore, the SARA test was included in the assessments (Weyer *et al.,* 2007). The SARA test was performed on all of the affected subjects and generally, the results indicated high scores associated with significant ataxia. However, in the absence of nystagmus and other phenomenology that typifies ataxia and the lack of cerebellar atrophy on MRIs of the study subjects, the high SARA scores were attributed to the participants' hyperkinetic movement disorder. The phenotypic features shared between ataxic syndromes and hyperkinesia have been attributed to the interaction between the cerebellar and basal ganglia circuits (Lawerman *et al.,* 2015). The SARA test was designed to monitor the progression of cerebellar ataxia and therefore cannot differentiate between diseases with similar phenotypic features. It was important to have a measure of ataxia available if any of the subjects had cerebellar signs, however, it did not prove to be useful in this study and the data were excluded from the final analysis.

Myoclonus had been reported in four cases of HDL2 (Bardien *et al.,* 2007; Schneider *et al.,* 2012) and is further described in Chapter 3. The UHDRS does not measure myoclonus, therefore the UMRS was added to the study protocol. None of the HDL2 subjects showed features of myoclonus although three of the HD subjects did. As this study focused on the HDL2 phenotype, the findings from the UMRS scale have not been not presented.

The measure of gait in the UHDRS is relatively subjective (Huntington Study Group, 1996). The 'timed get up and go test' measures the time taken to walk over a specified distance. It is therefore a more objective way of quantifying mobility and disability as well as monitoring change in gait. This rating scale was therefore added to the study protocol (Podsiadlo and Richardson, 2015).

Despite the shortcomings of the UHDRS described above this clinical rating scale remains the only quantitative motor rating method recommended by the International Parkinson and Movement Disorder Society (Mestre *et al.,* 2018). This

recommendation supported the use of the UHDRS in this study as a standardised measure of features in HDL2, a condition reported to resemble HD.

2.7.3 Summary of the methodology

This chapter has described the methods and tools that were applied in this study to assess the HDL2 phenotype and compare the clinical features of HDL2 to those of HD. Figure 2.3 illustrates how the methods and instruments, described in this chapter address the aims of the study and focuses on some of the clinical aspects of the HDL2 genotype-phenotype correlation.

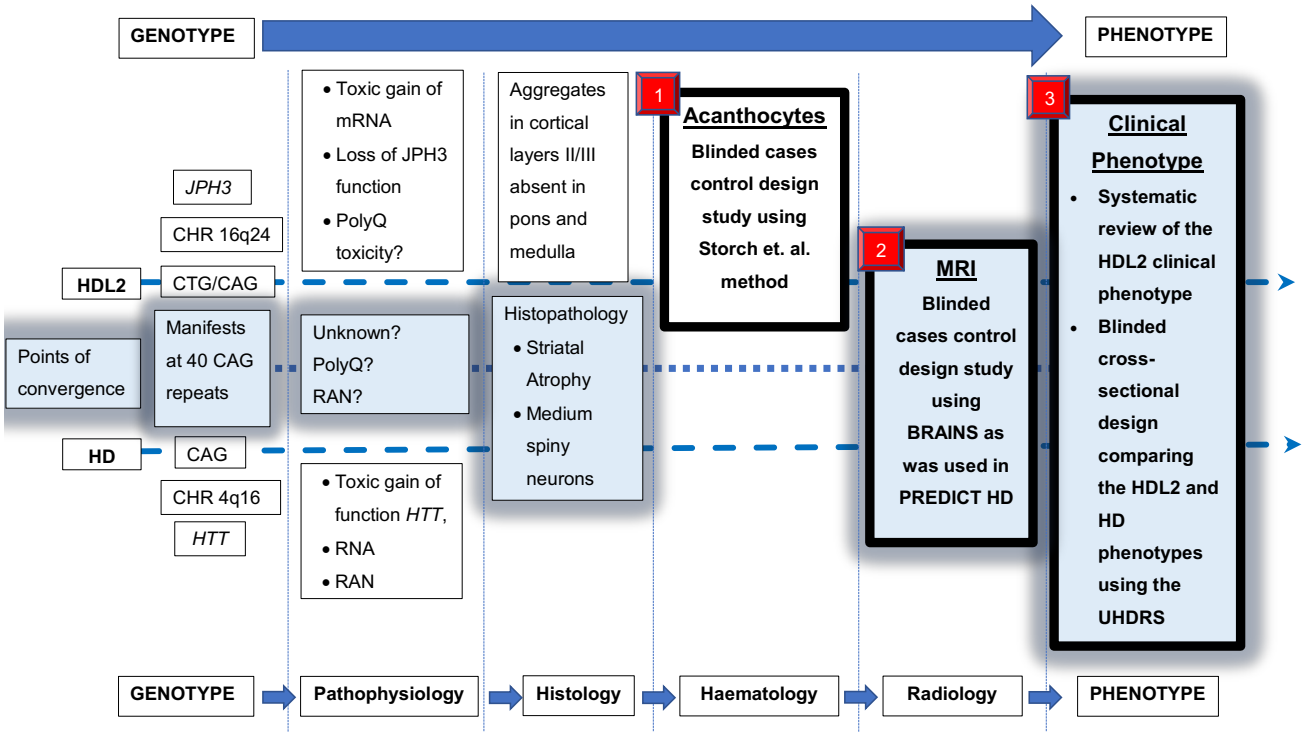

Figure 2.3 Framework illustrating the study methodologies and instruments

Framework for the thesis illustrating the genotype to phenotype correlation, from left to right, for HDL2 and HD. The dashed horizontal line between HDL2 and HD represents points of possible convergence, which are shaded. The bold frames highlight the methodologies and tools that address the aims and objectives of the study. 1 – Haematology, 2 – Radiology, 3 - Clinical Phenotype.

2.8 Ethical considerations

This study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (Ethics certificate number: M140872, Appendix 3). Permission for the use of the UHDRS and the use of images or publications from this thesis have been obtained from the respective copyright holders.

SECTION II

Section II presents the four published papers produced from this study in their final accepted form in Chapter 3, Chapter 4, Chapter 5 and Chapter 6 respectively. The Typeset versions of each paper can be found in Appendix 4

- Chapter 3: A Systematic Review of Huntington's Disease Like 2 Phenotype.

- Chapter 4: Absence of Acanthocytosis in Huntington's Disease Like 2: A Prospective Comparison with Huntington's Disease.

- Chapter 5: Emerging Differences Between Huntington's Disease Like 2 and Huntington's Disease: A Comparison Using MRI Brain Volumetry.

- Chapter 6: Comparison of Huntington's Disease Like 2 and Huntington's Disease Clinical Phenotypes.

CHAPTER 3

A Systematic Review of the Huntington's Disease Like 2 Phenotype

The first objective of this thesis was to review the literature and determine the existing description of the HDL2 phenotype. A systematic review of the literature was thus performed and is reported in this chapter. Initially, the study describes the molecular and historical background of HDL2 and notes the need for further clarity of the HDL2 phenotype. The methodology is then briefly described and illustrated. The results of the literature search are subsequently analysed, reported and, where appropriate, tabulated. The discussion of the findings is divided into demographic and genetic features, the HDL2 motor disorder, cognitive dysfunction, psychiatric disorder, the initial clinical signs, disease progression, acanthocytes, and finally, the neuroimaging and pathology findings. This systematic literature review further analysed the quality of the available HDL2 clinical data from each study and what tools were used to measure the clinical signs. The aim of this study was to assess the current view of the HDL2 phenotype and strongly support the need to examine the HDL2 phenotype systematically.

Anderson, D.G., Walker, R.H., Connor, M., Carr, J., Margolis, R.L. & Krause, A. (2017). A systematic review of the Huntington's disease like 2 phenotype. *Journal of Huntington's Disease*, 6(1), pp. 37-46.

3 A Systematic Review of Huntington's Disease Like 2 Phenotype

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3.1 Abstract

Background: Huntington's disease like 2 (HDL2) is a neurodegenerative disorder similar to Huntington's disease (HD) in its clinical phenotype, genetic characteristics, neuropathology and longitudinal progression. Proposed specific differences include an exclusive African ancestry, lack of eye movement abnormalities, increased parkinsonism and acanthocytes in HDL2.

Objective: The objective was to determine the similarities and differences between HD and HDL2 by establishing the clinical phenotype of HDL2 with the published cases.

Methods: A literature review of all clinically described cases of HDL2 until the end of 2016 was performed and a descriptive analysis was carried out.

Results: Sixty-nine new cases were described between 2001 and 2016. All cases had likely African ancestry, and most were found in South Africa and the USA. Many features were found to be similar to HD, including a strong negative correlation between repeat length and age of onset. Chorea was noted in 48/57 cases (84%). Dementia was reported in 74% patients and parkinsonism in 37%. Psychiatric features were reported in 44 of the 47 cases. Patients with chorea had lower expanded repeat lengths compared to patients without chorea. Eye movements were described in 19 cases, 8 were abnormal. Acanthocytes were detected in 4 of the 13 patients tested. There were 19 out of 20 MRIs which reported abnormal findings similar to HD.

Conclusion: This review clarifies some aspects of the HDL2 phenotype and highlights others which require further investigation. Features that are unique to HDL2 have been documented in a minority of subjects and require prospective validation.

3.2 Introduction

Huntington's disease like 2 (HDL2) is the Huntington's Disease (HD) genocopy* (see footnote below) that most strongly resembles HD clinically and radiologically (Margolis *et al.,* 2001; Wild *et al.,* 2008). Both are trinucleotide repeat disorders. HD is caused by a CAG repeat expansion occurring in exon 1 of the *huntingtin* gene on chromosome 4p16.3, while HDL2 is due to a CTG/CAG expansion mutation in exon 2A of the *junctophilin-3* (*JPH3*) gene on chromosome 16q24.3 (Holmes *et al.,* 2001). Since the discovery of the disease, there have been significant advances in the understanding of the genetics of HDL2, including data generated on the length of the repeat expansion and the haplotype associated with the mutation (Krause *et al.,* 2015). However, the clinical description so far is derived from reports of small case series, scattered families or isolated individuals. The phenotype has not been systematically or objectively reviewed.

* The terms 'genocopy' and 'phenocopy' have been used interchangeably to define diseases of similar phenotype with different genetic causes. Strictly speaking, this is the definition for the term 'genocopy', where 'phenocopy' refers to similar phenotypic traits caused by environmental factors. The term 'phenocopy' will be used in this instance because it is most commonly used in the literature and in most cases, assumed to mean diseases with similar phenotypes of different genetic origins.

3.3 Historical overview / background

The first eight cases of HDL2 were described in an African-American pedigree from the southeastern USA in 2001 (Margolis *et al.,* 2001). The initial cases were found to have symptoms starting in the fourth decade of life with a movement disorder, psychiatric symptoms, weight loss, dementia and a progressive and fatal course that was very similar to that of HD. The same authors identified the causative mutation in JPH3 (Holmes *et al.,* 2001). The gene encodes a junctional membrane protein (junctophilin-3) that binds the plasma membrane to endoplasmic reticulum in neurons. JPH3 is expressed predominantly in the brain and testes (Takeshima *et al.,* 2000).

In 2002, a family with three cases was published and described as “autosomal dominant chorea–acanthocytosis” (Walker *et al.,* 2002). These cases showed dementia, chorea and parkinsonism but lacked the orolingual dystonia and other typical features commonly seen in chorea-acanthocytosis. The first patient in this family was initially clinically diagnosed with HD. The clinical phenotype, autosomal dominant inheritance pattern and presence of acanthocytes in all three cases, suggested that the disorder was a novel variant of chorea-acanthocytosis. Subsequently, the affected individuals were found to have a mutation in the JPH3 gene (Walker, Rasmussen *et al.,* 2003).

In Johannesburg, in 2002, seven additional cases were identified from a laboratory-based cohort (Krause *et al.,* 2002). In black South African patients who tested positive for an HD-causing mutation, approximately 35% had a JPH3 mutation and 65% an HD mutation. This added further weight to the hypothesis that HDL2 is an African disease. Larger studies have confirmed the high frequency of JPH3 mutations in patients of African ancestry with a HD phenotype (Krause *et al.,* 2015).

The HDL2 phenotype was established as an autosomal dominant inherited disorder that manifests in the third or fourth decade, characterised by chorea, dystonia and/or parkinsonism, progressive weight loss and cognitive deficits. The disease progresses to death over the course of approximately 20 years (Walker, Jankovic *et al.,* 2003).

Knowledge of the HDL2 phenotype remains fragmentary and incomplete, with the likely consequence that HDL2 is underdiagnosed. Improved understanding of the phenotype is essential for the treatment and education of patients and their families. To achieve this, it is necessary to better describe the phenotype, including its presentation, course and complications.

We have systematically reviewed all the available published cases of HDL2 and analysed the available clinical data to provide a more detailed description of the phenotype. This review is designed to assist the clinician in recognising clinical features of HDL2, differentiating HDL2 from HD, and defining aspects of the HDL2 phenotype which are not yet clear and will therefore require future study.

3.4 Methodology

The details of the review process are summarised in Figure 3.1. We searched MEDLINE, Google Scholar, EMBASE and Scopus in December 2016. The terms ‘Huntington’s disease like 2’ or ‘HDL2’ or ‘junctophilin 3’ or ‘JPH3’ or ‘Huntington disease’ were always used in combination with the terms ‘phenotype’ or ‘case report’ or ‘case series’ or ‘clinical’ or ‘report’ or ‘acanthocytosis’ or ‘neuroacanthocytosis’ or ‘Africa’ or ‘African’ or ‘phenocopy’. Where possible, filters were set for studies pertaining to humans, and included articles written in all languages. No time limits were set to gather all articles published to the end of December 2016.

Articles were excluded if they did not have an association with HDL2, were purely laboratory-based, did not describe HDL2 clinically, relied on previously described cases or did not add to the clinical description of previously described cases. Available clinical data was extracted, and descriptive statistical analysis was performed.

Descriptive analysis of the data was carried out using SAS version 9.4 for Windows. We analysed repeat size, age of onset, duration of disease, presenting symptoms (cognitive, psychiatric or movement disorder) and the presence or absence of parkinsonism, bradykinesia, chorea, dystonia, dementia, psychiatric symptoms and acanthocytes. Age of onset was defined as the age of first onset of symptoms stated or implied in a publication.

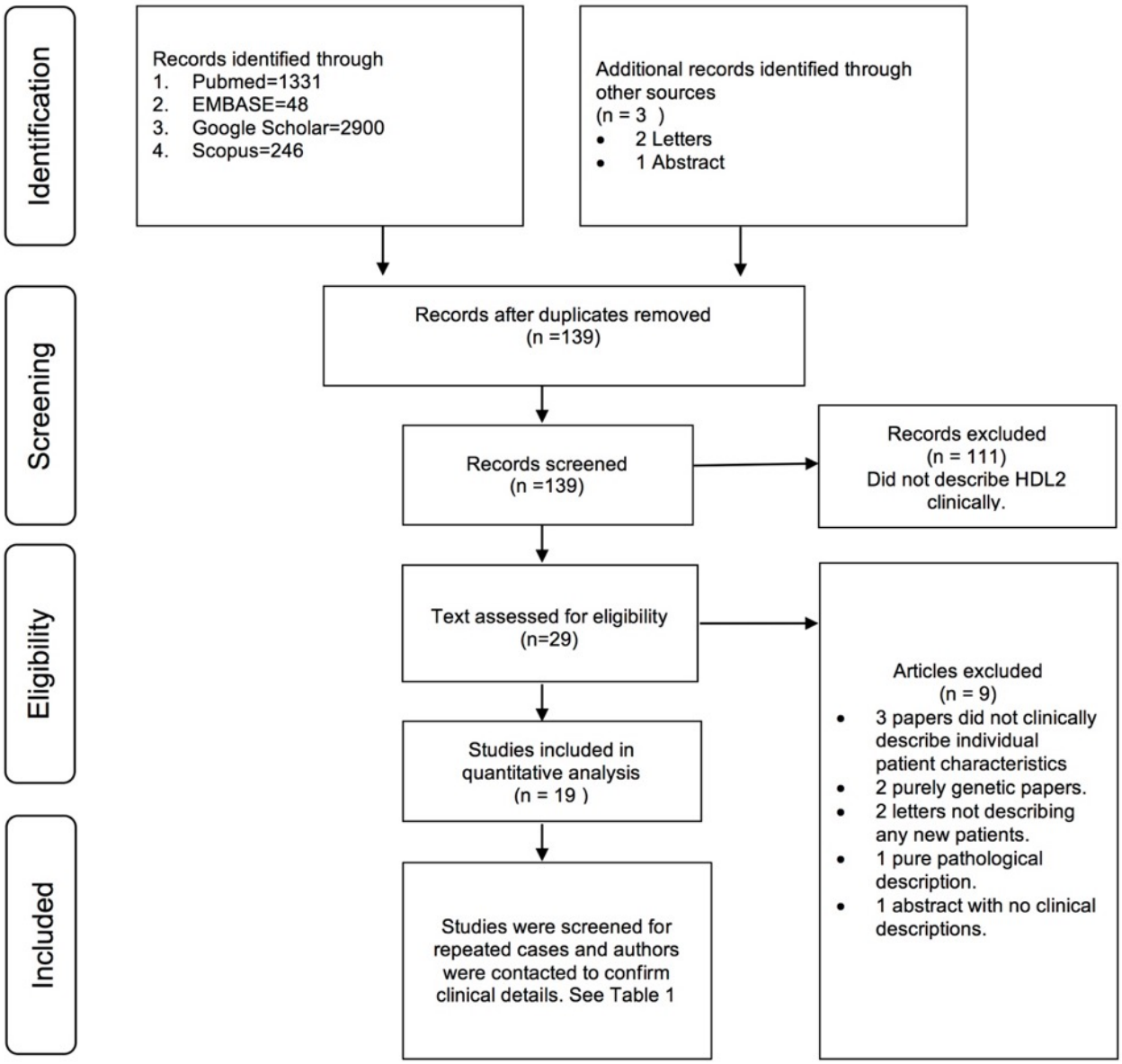

Figure 3.1 Huntington's disease like 2 systematic review record selection

Strategy used to systematically review the HDL2 literature.

3.5 Results

We initially identified 29 articles in which new clinical features or new cases of HDL2 were described. Once publications with repeat descriptions of the same patients were excluded, 19 articles remained. These articles provided descriptions of clinical features in 69 novel cases of HDL2. All of these cases were confirmed to have the causative genetic mutation, although the size of the abnormal repeats was not always reported. Table 3.1 shows a breakdown of the origins of the cases. Authors were contacted for confirmation where doubt remained about duplicated cases.

While clinical descriptions were available for the 69 cases, many of these were incomplete. Positive clinical features were noted, but there were few records of negative or absent features. The majority of publications were case reports with minimal longitudinal follow-up data available. Table 3.2 summarises the available clinical data on the 69 patients. Publications seldom provided family history or inheritance pattern information, and insufficient transmissions were documented to comment on anticipation in HDL2.

Table 3.1 Sources of HDL2 patients after the completed review process

Publications	Year	Number of patients	Number of new patients
(Margolis *et al.,* 2001)	2001	8	8
(Holmes *et al.,* 2001)	2001	6	4
(Stevanin *et al.,* 2002)	2002	1	0
(Walker *et al.,* 2002)	2002	3	3
(Stevanin *et al.,* 2003)	2003	2	1
(Walker, Rasmussen *et al.,* 2003)	2003	9	4
(Walker, Jankovic *et al.,* 2003)	2003	5	0
(Teive *et al.,* 2007)	2007	1	1
(Greenstein *et al.,* 2007)	2007	2	2
(Bardien *et al.,* 2007)	2007	3	3
(Wild *et al.,* 2008)	2008	1	1
(Santos *et al.,* 2008)	2008	1	1
(Rodrigues *et al.,* 2008)	2008	4	4
(Fischer and Licht, 2012)	2012	1	1
(Schneider *et al.,* 2012)	2012	6	6
(Paradisi *et al.,* 2012)	2012	4	4
(Krause *et al.,* 2015)	2015	22	22
(Mariani *et al.,* 2016)	2016	3	3
(Vasconcellos *et al.,* 2017)	2016	1	1
Total			69

Table 3.2 Clinical results

	Characteristic	n [1]			
A	Gender Ratio (M: F)	35	23:12		
			Range	**Mean**	**Median**
	Repeat size	65	39-59	48	47
	Age of onset (years)	54	12-66	41.0	35
	Duration of disease (years)	27	1-22	9.1	17
B	**First sign**	24	**Reported**		
	Cognitive dysfunction		13		
	Psychiatric symptoms		14		
	Movement disorder		11		
C	**Sign**		**Present**	**Absent**	
	Dementia	51	50	1	
	Psychiatric features	47	44	3	
	Chorea	57	48	9	
	Parkinsonism	26	26	-	
	Dystonia	16	16	-	
	Dysarthria	21	21	-	
	Dysphagia	4	4	-	
	Abnormal eye movements	19	8	11	
	Myoclonus	4	4	-	
	Primitive reflexes	16	10	6	
	Acanthocytes	13	4	9	

KEY

n [1]: Number of cases for which information was available out of possible 69 cases.

A: Demographic and genetic results

B: First sign findings

C: Clinical features

3.5.1 Demographic and genetic findings

The majority of the patients were identified in South Africa and the USA and were of African or African-American ancestry respectively (Table 3.3). The ancestry of patients with HDL2 is likely African in all cases, even if the ancestry is relatively distant. The shared common origin of these mutations is supported by studies that have shown a shared core haplotype in all patients tested, including those from South Africa, the USA, Mexico and the French West Indies (Krause *et al.,* 2015). Although haplotyping was not performed in the patients from Brazil and Venezuela, the historical links of these countries with Africa would suggest the origin of these patients is similar (Walker, Jankovic *et al.,* 2003; Paradisi *et al.,* 2012).

Table 3.3 Countries of origin of described HDL2 cases

Country	**Number of HDL2 reported cases**	**Percentage of total**
South Africa	25	36%
United States of America	24	35%
Brazil	7	10%
Venezuela	4	6%
Mexico	3	4%
West Indies	2	3%
Congo	1	1%
Haiti	1	1%
Morocco	1	1%
United Kingdom	1	1%

The repeat length distribution is shown in Figure 3.2. Although the median repeat expansion size (47 repeats) in HDL2 appears somewhat larger than in HD (43 repeats) (Langbehn *et al.,* 2009), the mean age of onset for HDL2 is 41 years, very similar to that of HD of 40 years (Ross and Tabrizi, 2011). In HD, the age of onset is defined as the age when movement abnormalities reportedly begin. In HDL2 publications, initial symptoms included psychiatric and cognitive features. The age of onset showed a normal distribution with a range of 12 to 66 years. Gender was reported in 35 cases (12 females and 23 males). The ratio of 1.9:1 was not significantly different from 1:1 as seen in HD (chi-square test; $p<0.10$).

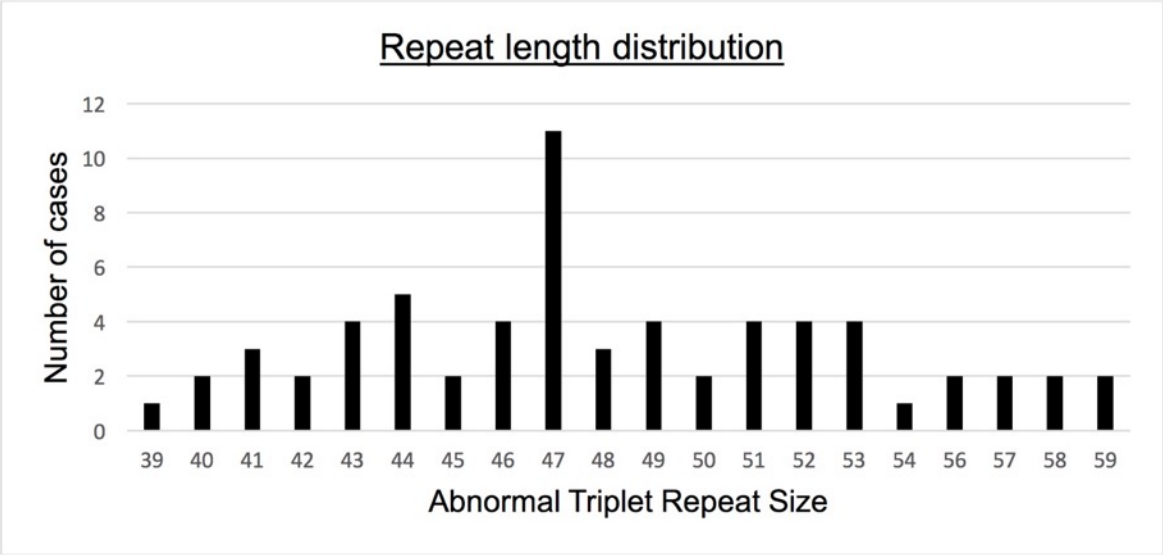

Figure 3.2 Repeat length distribution

Graph showing the frequency of abnormal triplet repeats in *JPH3* from the described cases in the literature.

There is a strong negative correlation between repeat length and age of onset in HDL2 (Pearson's r=-0.76; p<0.0001; large effect size) which is shown in Figure 3.3. This supports the findings of smaller HDL2 studies which showed similar correlations (Margolis *et al.,* 2001; Schneider *et al.,* 2012; Krause *et al.,* 2015). The co-efficient of determination (r^2) value in this HDL2 group is = 0,585 as seen in Figure 3.3This is quite similar to the r^2 value of 0.642 in HD in a North

American, European and Australian sample (the HD MAPS cohort) (Andresen *et al.,* 2006).

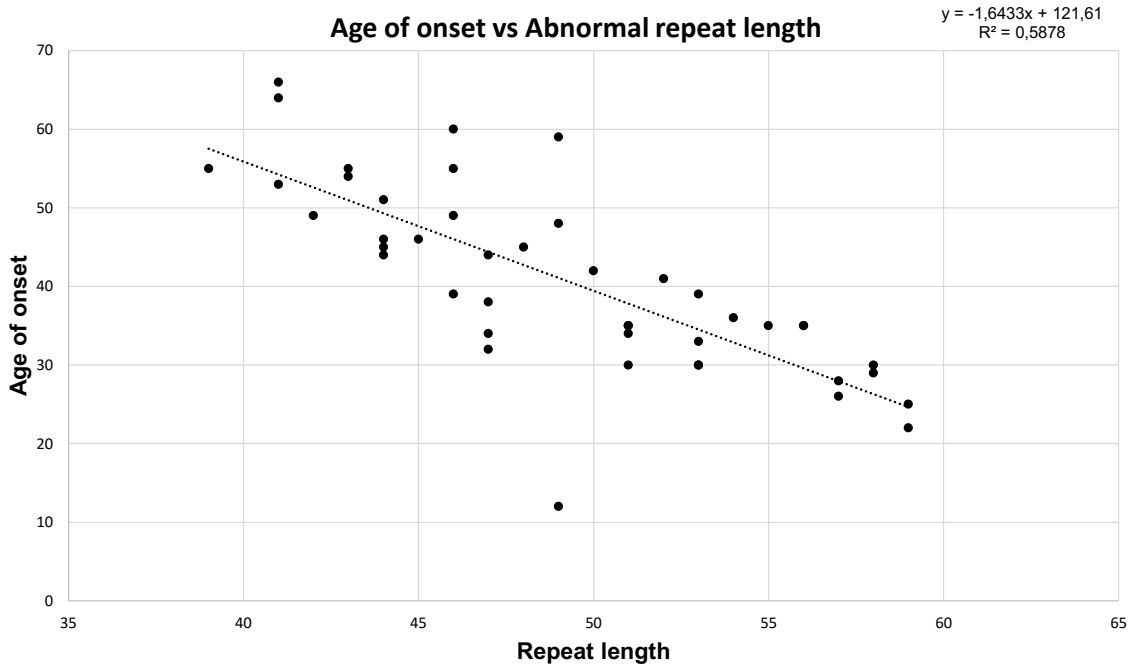

Figure 3.3 Age of onset vs repeat length

Graph showing an inverse correlation of age of onset and triplet repeat length in HDL2 from all described cases in the literature.

The youngest age of onset, 12 years, was reported in a patient from Mexico with a repeat length of 49 repeats and a presentation of depression, mild chorea and aggressiveness (Walker, Rasmussen *et al.,* 2003). The case is unusual for two reasons: the much younger than expected age at onset based on repeat length, and the absence of the parkinsonian symptoms typically found in juvenile onset HD (Walker, 2007). This case may demonstrate that patients with HDL2 may present at younger ages than would be predicted by their triplet repeat size and with unexpected clinical features. Alternatively, clinical signs unrelated to HDL2 may have led to the serendipitous finding of the HDL2 mutation.

3.5.2 Clinical features

The presence or absence of chorea was reported in 57 cases. Chorea was present in 48 cases (84%). Parkinsonism was reported in seven of the cases with chorea,

and dystonia was reported in six of these seven. The median expanded repeat length was significantly lower for those with chorea (47; IQR 44-51) compared to those without chorea (52.5; IQR 50-54) (n=51; Wilcoxon rank sum test; p=0.030). The average repeat size for the group with chorea was 48 and age of onset was 41 years of age. The average disease duration for this group was 6.5 years.

Parkinsonism was reported present in 26 (38%) cases. Of the 26, 23 were reported to have bradykinesia, 17 rigidity and 10 dystonia. The mean repeat length for this group was 51 triplets, age of onset 39 years, and disease duration 8.7 years.

Dysarthria was reported in 21 of the 69 cases and was noted as moderate or mild. Eye movements were reported to be abnormal in only 8/19 cases (Vasconcellos *et al.,* 2017; Margolis *et al.,* 2001; Walker, Rasmussen *et al.,* 2003; Schneider *et al.,* 2012). Generalised epilepsy was not reported, but four cases were reported to have myoclonus (Bardien *et al.,* 2007; Schneider *et al.,* 2012)

The presence of dementia was noted in 51 of the 65 cases, with only one case from a retrospective record review from South Africa specifically recording its absence (Krause *et al.,* 2015). Prominent dementia is described in most cases, and in the single report which explored this further, there were prominent frontal lobe features, working memory deficits and psychiatric features, similar to HD (Fischer and Licht, 2012). The presence of primitive reflexes was reported in 10/16 cases, all in patients with an associated dementia. The Unified Huntington Disease Rating Scale (UHDRS) has only been reported in three more recently published papers (Schneider *et al.,* 2012; Mariani *et al.,* 2016; Vasconcellos *et al.,* 2017). In one paper, the verbal fluency, Stroop and symbol digit modalities tests could not be completed due to mutism or severe dementia (Schneider *et al.,* 2012) .

There were 44 out of 47 cases reported to have psychiatric findings, and only three cases in which there were no psychiatric features present. These three were again from the South African retrospective record review (Krause *et al.,* 2015). The psychiatric findings included depression (seven reports), hallucinations (two reports) and aggression (four reports). It has been suggested that the heterogeneous psychiatric phenotype is similar to that found in HD (Fischer and Licht, 2012)

From the original family described, worsening of the chorea and an increase in bradykinesia was reported with disease progression (Margolis *et al.,* 2001). With longitudinal descriptions in patients, there have been reports of generalised spasticity, hyperactive deep tendon reflexes and extensor plantar responses. Clinical progression has been described in only five of the papers reviewed (Margolis *et al.,* 2001; Greenstein *et al.,* 2007; Santos *et al.,* 2008; Rodrigues *et al.,* 2008; Fischer and Licht, 2012).

3.5.3 Acanthocytes

Acanthocytes were detected in only four of the thirteen patients tested (Walker, Jankovic *et al.,* 2003; Walker *et al.,* 2002; Bardien *et al.,* 2007; Rodrigues *et al.,* 2008; Mariani *et al.,* 2016; Vasconcellos *et al.,* 2017). In those four patients, more than 30% of the erythrocyte population were acanthocytes, significantly greater than the normal population (less than 6.3% of erythrocytes are acanthocytes on a blood smear) but similar to the findings in chorea-acanthocytosis (typically higher than 28% (Storch *et al.,* 2005)). To the best of our knowledge, acanthocytosis has never been reported in HD.

3.5.4 Neuroimaging

There have been 20 reported MRIs in HDL2 cases; 19 were reported as abnormal. Generalised atrophy was described in all the cases, with more severe atrophy of the caudate nucleus and putamen (Margolis *et al.,* 2001; Stevanin *et al.,* 2002; Bardien *et al.,* 2007; Santos *et al.,* 2008; Rodrigues *et al.,* 2008; Fischer and Licht, 2012; Schneider *et al.,* 2012). The single normal MRI is unexpected as this patient had a CT scan of the brain three years later which showed diffuse atrophy (Walker *et al.,* 2002). This is remarkable as this patient was found with two triplet expansion diseases and was also diagnosed with Fragile X syndrome. White matter lesions and atrophy are MRI features in Fragile X syndrome as well as in Fragile X-Associated Tremor/Ataxia Syndrome (Rodriguez-Revenga *et al.,* 2012). In two advanced cases, atrophy was generalised but severely affecting the parietal-occipital regions

(Schneider *et al.,* 2012). The brainstems and cerebellar regions were comparatively well-preserved when described.

3.6 Discussion

Since HDL2 was first described in 2001, there have only been a small number of cases reported and the descriptions have often been incomplete. There are, however, a number of consistent observations that are useful.

3.6.1 Demographic and genetic findings

Mutations of *C9orf72*, spinocerebellar ataxia (SCA) 17 and HDL2 are variably reported as the most common HD phenocopies globally (Wild *et al.,* 2008; Moss *et al.,* 2014). In South Africa, HDL2 accounts for one third of patients of black and mixed ancestry with an HD phenotype and a confirmed diagnosis (Krause *et al.,* 2015). In a recent study of HD and HDL2 in South Africa, the estimated combined minimum disease frequency was found to be higher than previously thought. A figure of 0.25:100 000 in black and 2.10:100 000 in mixed ancestry populations was described as an underestimate due to the probable under-reporting of both diseases in an under-resourced country (Baine *et al.,* 2016). HDL2 is therefore an important consideration in the differential diagnosis of HD phenocopies.

HDL2 joins many of the other repeat expansion neurodegenerative diseases with an inverse correlation of repeat length and age of onset, including the SCAs, spinobulbar muscular atrophy (SMA), Dentatorubral-pallidoluysian atrophy (DRPLA) and HD (Stevanin *et al.,* 2000; Piccioni *et al.,* 2001; Walker, 2007). This has important implications in an attempt to understand the pathophysiology and potentially unravel the mechanism whereby these diseases have similar phenotypic features (Margolis and Ross, 2001).

Huntington's disease and HDL2 have very similar repeat ranges associated with age of onset. Other CAG-repeat disorders have closely related, but somewhat different, repeat ranges associated with age of onset. Examples are the HD phenocopies due

to expansions in SCA17 and DRPLA with ranges of 47–63 and 49–88 respectively (Stevanin and Brice, 2008; Hasegawa *et al.,* 2010).

There is a similar strong negative correlation between repeat length and age of onset in polyglutamine repeat diseases (Margolis and Ross, 2001). We have shown that the co-efficient of determination values in HDL2 and HD is similar when compared to SCA17 (r^2- 0.41) and DRPLA (r^2- 0.865) (Stevanin and Brice, 2008; Hasegawa *et al.,* 2010).

3.6.2 Movement disorder

Chorea was the most commonly reported movement abnormality in all published cases. Chorea was described as generalised or axial in most cases where the distribution was mentioned. Orofacial chorea was highlighted in two cases (Greenstein *et al.,* 2007; Fischer and Licht 2012). In the original pedigree, chorea and dystonia were present in all of the patients (Margolis *et al.,* 2001), although this does not hold true in all subsequent descriptions.

In the HD phenotype, chorea is not always present throughout the progression of the disease. In most cases it is a dominating feature. However, as the disease progresses, dystonia and rigidity become more prominent and chorea less noticeable (Walker, 2007). In early onset of the disease, with a high number of repeats, chorea may be absent. Parkinsonism has been reported to be more common in HDL2 than in HD (Margolis *et al.,* 2001; Schneider *et al.,* 2012). Bradykinesia has long been recognised as a feature of HD when chorea is present and is aggravated by antipsychotic medication (van Vugt *et al.,* 1996). A speculative picture emerges for HDL2 patients in which patients with shorter repeat lengths are more likely to have chorea, but the confounding factors of duration of disease, possibly coincident bradykinesia and the use of dopamine-blocking medications need to be taken into account.

Mild to moderate dystonia was reported in 14 cases, however, the presence or absence of dystonia was not mentioned in the majority of reports. In the few cases

with dystonia, it was usually noted in conjunction with other features of parkinsonism. Dystonia is reported in 95% of HD cases (Louis *et al.,* 1999). It may be that in the HDL2 cases, it was not noted due to the prominence of other movement abnormalities, specifically chorea. Alternatively, dystonia may not be as common as it is in HD.

Dysarthria was reported in less than a third of the HDL2 cases in the literature and was classified as moderate or mild. Dysarthria is common in HD, occurring in up to 89% of cases (Young *et al.,* 1986). Dysphagia was reported only in one study of six patients, where it was present in four cases, and absent in two (Schneider *et al.,* 2012). The frequency and characteristics of dysphagia in HD are poorly described (Heemskerk and Roos, 2010).

Preserved ocular motor function was suggested in the original family reported with HDL2 (Margolis *et al.,* 2001). Abnormal eye movements are a cardinal and early feature of HD (Lasker and Zee, 1997). A third of cases from the described patients with HDL2 in the literature had abnormal eye signs. Whether this is a potential distinguishing feature between the two conditions, or if the eye signs change with the progression of HDL2, warrants further observation. When described, the specific eye movement abnormalities were hypometric or jerky saccades and interrupted ocular pursuit (Schneider *et al.,* 2012; Vasconcellos *et al.,* 2017; Walker, Jankovic *et al.,* 2003). It has also been suggested that there may be two phenotypes of HDL2 – the first an early onset form which is more parkinsonian and lacks ocular motor involvement and the second, a late onset variant which is more typical of HD, with the typical jerky horizontal saccades (Margolis, 2012). Eye movements are not often reported in juvenile HD but have been described as abnormal in individual cases (Nance and Group, 1997). If eye movements are normal in HDL2, it would predict that the disease spares the brainstem and ocular motor control centres. This needs to be investigated on imaging and in neuropathological studies and dedicated systematic oculographic recordings.

Myoclonus has been reported in four cases. In one case, somatosensory evoked potentials (SEP) showed enlarged N20-P25 and P25-N33 components, indicating a

possible cortical origin (Bardien *et al.,* 2007). Epilepsy and myoclonus are unusual features in HD and usually occur in the juvenile variant of the disease. When myoclonus does present itself, large SEPs (Caviness and Kurth, 1997) are usually not found.

3.6.3 Cognition

Dementia is likely a universal feature in HDL2, but there are several unanswered questions. Is HDL2 dementia similar to the frontal and subcortical dementia seen in HD, as has previously been suggested? Is HDL2 dementia always a heralding sign of disease onset, and does the dementia progress more rapidly than in HD? A significant challenge, where cognitive testing was performed, was that the cases were reported late, when the cognitive impairment was already advanced. In order to describe the spectrum of cognitive dysfunction in HDL2, premanifest patients and early onset patients will need to be studied

3.6.4 Psychiatric features

The psychiatric features of HDL2 are poorly documented but have been suggested to be similar to HD. In HD, depression is 4-6 times more common than in the general population but there is still no evidence to adequately guide antidepressant use (Moulton *et al.,* 2014). Aggression is also a common feature, occurring in 66% of HD cases in some studies, with no evidence-based treatment currently available (Fisher *et al.,* 2014).

3.6.5 First sign/s of disease

For the 24 cases in which presenting signs and symptoms are described, similar frequencies of cognitive, psychiatric and movement features are documented. Some patients reported more than one presenting sign. It is important to define features of disease onset in HDL2. The TRACK-HD study defined clinical manifestations of HD

and biomarkers longitudinally and has allowed for defined endpoints for therapeutic trials as well as a better understanding of the disease (Shannon and Fraint, 2015).

3.6.6 Disease progression

The papers that have described HDL2 patients longitudinally indicate a clinical picture of increasing parkinsonism, corticospinal tract signs and worsening dementia, similar to that in HD (Margolis *et al.,* 2001; Greenstein *et al.,* 2007; Santos *et al.,* 2008; Rodrigues *et al.,* 2008; Fischer and Licht, 2012). This might be expected as *post mortem* findings have shown that there is atrophy and disease pathology in the basal ganglia and both cortical and subcortical motor structures (Rudnicki *et al.,* 2008). A similar progression has been noted in HD (Ross and Tabrizi, 2011).

3.6.7 Acanthocytes

Neuroacanthocytosis syndromes are syndromes where acanthocytes are a cardinal feature, in conjunction with neurological features. Mcleod Syndrome and Chorea Acanthocytosis are considered the 'core' neuroacanthocytosis syndromes. Acanthocytes are not specific to these conditions and have also been described in other conditions such as pantothenate kinase-associated neurodegeneration (Hayflick *et al.,* 2003).
HDL2 is currently classified as both an HD genocopy and a neuroacanthocytosis syndrome, and often features in reviews of both disorders (Danek and Walker, 2005; Wild *et al.,* 2008; Baeza, 2015). However, the association of HDL2 and acanthocytes remains unverified in the majority of patients (Walker, Jankovic *et al.,* 2003). No data exist on the relationship of acanthocytes to the trinucleotide repeat length or to duration of disease.

The association of HDL2 with acanthocytes may provide important clues about pathogenesis. Junctophilins are not thought to be present in red blood cell membranes (Nishi *et al.,* 2000). In HDL2 and the core neuroacanthocytosis syndromes, chorea-acanthocytosis and McLeod syndrome, abnormal phosphorylation of the proteins involved in connecting the membrane and

cytoskeleton in the erythrocytes may be a common link to the production of acanthocytes (Adjobo-Hermans *et al.,* 2015)

3.6.8 Neuroimaging and neuropathology

Radiologically and pathologically, HDL2 is broadly similar to HD in its involvement of the neostriatum and basal ganglia and general atrophy with progression of disease (Rudnicki *et al.,* 2008). However, HDL2 may differ, at least in some cases, in its degree of focal cortical involvement, notably of the occipital lobe. In one case with occipital lobe atrophy, visual failure was described. This patient had decreased visual acuity and was unable to follow visual commands (Greenstein *et al.,* 2007). HD was initially thought to preserve the nucleus accumbens but a strong correlation with poor functional and motor scores has now been found, with atrophy of the nucleus accumbens (van den Bogaard *et al.,* 2011). In the HDL2 cases that have been studied, this nucleus was at least partially or significantly involved (Greenstein *et al.,* 2007). These differences between HD and HDL2 have been noted pathologically but only focal occipital atrophy has been noted radiologically in the cases of HDL2 and not nucleus accumbens involvement (Schneider *et al.,* 2012).

Research using an HDL2 mouse model has found overlapping polyQ-mediated mechanisms of pathogenesis in HD and HDL2 (Wilburn *et al.,* 2011). A more detailed understanding of the HDL2 phenotype, an HD phenocopy caused by mutation in a gene with an entirely different function from huntingtin*,* may offer further evidence of a common pathogenesis and lead to the development of novel therapeutic approaches for both diseases.

3.7 Conclusion

The rarity of HDL2 makes describing the phenotype in a statistically meaningful way a challenge. Only 69 cases have been described over 15 years. This review has evaluated all published cases to the end of 2016 to consolidate the available data and to provide the current view and understanding of the clinical phenotype. The data is all retrospective, of varying quality, largely anecdotal and based on small

patient groups. It also tends to be biased by the reporting of positive features, with the failure to report the absence of features. There is clearly a need for a further systematic and focused prospective study of cases. To address this need, the South African Huntington's Disease Like 2 Study (SAHDLS), based in Johannesburg, where the HDL2 prevalence is the highest in the world, is currently collecting standardised clinical neuropsychological and MRI data and blood samples.

3.8 Acknowledgments

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CHAPTER 4

The Absence of Acanthocytosis in Huntington's Disease Like 2 : A Prospective Comparison with Huntington's Disease

An aim of this study was to determine the frequency of acanthocytes in HDL2. This chapter reports a blinded, case-controlled study, comparing the blood smears of 12 HDL2 patients with 13 HD cases. The cases with HDL2 and HD were compared against an unaffected control cases using standardised methods. No acanthocytes were found in the HDL2 subjects. The lack of acanthocytes in the HDL2 subjects from this research clarifies aspects of the HDL2 phenotype and suggests that HDL2 is not a neuroacanthocytosis syndrome.

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4 Absence of Acanthocytosis in Huntington's Disease Like 2: A Prospective Comparison with Huntington's Disease

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4.1 Abstract

Background: Huntington's disease like 2 (HDL2) is classified as a neuroacanthocytosis, however, this remains unverified. We aim to determine if acanthocytes are present in HDL2 and whether acanthocytes can differentiate HDL2 from Huntington's disease (HD).

Methods: We prospectively compared 13 HD and 12 HDL2 cases against 21 unaffected controls in Johannesburg. Blood smears were prepared using international standards and reviewed by at least two blinded reviewers. An acanthocytosis rate of greater than 1.2% in the dry smear or greater than 3.7% in the wet smear was designated *a priori* as the threshold for clinical significance based on previously established standards. Flow cytometry was performed on all but four of the cases. Red cell membrane protein analysis was performed on all participants.

Results: There were 12 HDL2, 13 HD and 21 controls enrolled. None of the HD or HDL2 participants had defined acanthocytosis or other morphological abnormalities. None of the HD or HDL2 cases had evidence of an abnormal band 3.

Discussion: Acanthocytosis was not identified in either HDL2 or HD in our patient population. Our results, based on the first prospective study of acanthocytes in HDL2 or HD, suggest that screening for acanthocytes will not help establish the diagnosis of HD or HDL2 nor differentiate between the two disorders. These results raise the question of whether HDL2 should be categorised as a neuroacanthocytosis syndrome.

4.2 Introduction

Huntington's disease like 2 (HDL2) is an autosomal dominant neurodegenerative disorder caused by a CTG/CAG expansion mutation in exon 2A of *junctophilin-3 (JPH3)* on chromosome 16q24.3, which occurs in patients of African ancestry (Holmes *et al.,* 2001). HDL2 is clinically and pathologically similar to Huntington's disease (HD), with progressive dementia and movement abnormalities characterising the predominant clinical phenotype (Margolis *et al.,* 2001). Anecdotal case reports suggested that, compared to HD, eye movement abnormalities are less common and parkinsonism more common in HDL2 (Anderson, Walker, *et al.,* 2017).

Acanthocytes were documented in some of the early reported cases of HDL2, resulting in diagnostic confusion with chorea-acanthocytosis (Walker *et al.,* 2002; Walker, Rasmussen *et al.,* 2003). Acanthocytes are erythrocytes with spiky deformations of their membranes, and when found in high numbers, are associated with many diseases. The source of acanthocytes in chorea-acanthocytosis, McLeod syndrome and pantothenate kinase-associated neurodegeneration (PKAN) (and possibly also aceruloplasminemia) appears to be complex. Abnormal phosphorylation of the proteins connecting the membrane and cytoskeleton of erythrocytes, erythrocyte metabolism, the involvement of lipids in underlying signalling pathways, changes in lipid composition and signal transduction have all been implicated (Adjobo-Hermans *et al.,* 2015). The relationships of these potential mechanisms to neurodegeneration are unclear (Siegl *et al.,* 2013).

A systematic review of all reported HDL2 patients showed acanthocytes were detected in 4 of 13 cases, however, the haematological findings in most HDL2 cases are unknown or unreported (Anderson, Walker *et al.,* 2017). In three of these four cases, the affected individuals were members of the same pedigree, and one individual had a Fragile X mutation (660 triplet repeats), while the two affected relatives had repeats in the Fragile X pre-mutation range (57 and 56 repeats). Peripheral smears and electron microscopy revealed that 30 to 35% of the red blood cells were acanthocytes and red blood cell membrane extract showed an abnormal breakdown of band 3 proteins (Walker *et al.,* 2002). The fourth case was from a

Mexican pedigree of likely African descent. Only one of the three family members with HDL2 had acanthocytes of 35% on peripheral smear, but cell membrane extracts did not show an abnormal band 3 protein (Walker, Rasmussen *et al.,* 2003). These findings have led to the suggestion that HDL2 be classified as one the neuroacanthocytosis syndromes, and that the presence of acanthocytes might provide important insight into HDL2 pathogenesis, with implications for understanding HD and the neuroacanthocytoses. To our knowledge there have been no systematic efforts to document acanthocytes in HD.

We therefore designed a prospective systematic investigation to study acanthocytosis in HD and HDL2 patients of the same ethnicity, taking advantage of our finding that the area around Johannesburg, South Africa, contains the world's highest known concentration of HDL2 patients (Krause *et al.,* 2015).

4.3 Methods

HD and HDL2 patients with African or mixed ancestry were identified over 30 months at the Charlotte Maxeke Johannesburg Academic Hospital Huntington Disease Clinic as part of a larger HDL2 phenotype study (Anderson, Walker *et al.,* 2017). Ethics clearance was obtained from the Human Research Ethics Committee of the University of the Witwatersrand (M140872) and informed consent was obtained from all study participants. Diagnoses were confirmed by mutation testing for the specific triplet repeat expansion in the genes causing HD and HDL2 at the Division of Human Genetics, National Health Laboratory Service (NHLS). Control cases, also of African or mixed ancestry, were genetically unrelated family members or friends of the affected patients.

Blood samples were collected from both the control and either HD or HDL2 subject at the same study visit. Two 5 ml blood samples were collected, one in EDTA and the other in ACD (BD Vacutainer Systems, UK). Each tube was labelled using a non-identifying study number and was processed within 20 minutes of the venepuncture. The laboratory was blinded to the diagnosis. Smears were prepared using the same techniques.

Methods for wet and fixed smears were performed according to previously established protocols (Storch *et al.,* 2005). In brief, an aliquot (~5µl) of whole blood, collected in EDTA tubes, was used to prepare a fixed smear on a glass slide and stained using the Rapidiff Stain (Diagnostic Media Products, South Africa). In parallel, unfixed wet smears were prepared within an hour of blood draw. A minimum of ten fields of each preparation was examined with a Zeiss Axiostar (Carl Zeiss Jena GmbH, Germany) microscope under oil immersion at 1,000X magnification. Blood was obtained from an unaffected control at the same time and smears were prepared using the same techniques. An acanthocytosis rate of greater than 1.2% in the dry smear or greater than 3.7% in the wet smear was designated a *priori* as the threshold for clinical significance based on previously established standards (Storch *et al.,* 2005).

As a third approach to detecting abnormal haematological features in HDL2, red cell membranes were prepared from blood samples drawn in ACD tubes, as described by Coetzer and Palek (1986). The protein concentration was determined using the Coomassie Plus™ Protein Assay Reagent (Pierce Biotechnology Incorporated, USA). Aliquots of solubilised erythrocyte membrane proteins were prepared by boiling for one minute in 10mM Tris-HCl pH 8, 1mM EDTA, 1% SDS, 5% sucrose, 2% β-mercaptoethanol, and 20-50µg of protein was resolved on a 12% Laemmli SDS-polyacrylamide gel and a 4-17% Fairbanks gradient gel. Gels were stained with 0.05% Coomassie Blue (BDH Laboratory Supplies, UK) in 25% isopropanol, 10% acetic acid) and the amount of band 3 was quantitated using a Hoefer GS300 transmittance/reflectance scanning densitometer and GS365W software (Hoefer Scientific Instruments, USA).

Fisher's exact test was used to assess the relationship between categorised acanthocyte screening test results and groups. Data analysis was carried out using SAS version 9.4 for Windows. The 5% significance level was used.

4.4 Results

All HDL2 or HD cases enrolled had African or mixed ancestry. Control cases were genetically unrelated family members or acquaintances with African or mixed ancestry of the affected patients. The HD group included 11 separate pedigrees; two pedigrees each contributed two individuals to the study. The HDL2 group consisted of 12 unrelated individuals. Two HDL2 patients were also HIV positive and receiving antiretroviral treatment. The results are presented in Table 4.1.

The acanthocyte counts remained below the defined normal thresholds in all of the HD, HDL2 and control cases in multiple biological replicates for both the wet and dry smears. There were no other abnormal erythrocyte features noted on blood smear. The rest of the assessed flow cytometry for each group can be seen in Table 4.1 an all were within normal ranges. The flow cytometry evaluation was not available in four cases where there was a blood smear available. These were in three controls and one HD case. The amount of band 3 in the red cell membranes of all the HD, HDL2 and control cases was normal, which correlates with the normal red cell morphology.

Table 4.1 Comparative results between HDL2, HD and control groups

	HDL2	HD	Control
Number of participants	12	13	21
Age (in years)			
Mean age (Std Dev)	49.2(±9.5)	46.8(±13.2)	49.5(±14.9)
Median age	49	47	52
Range	32-66	32-68	21-79
Disease Duration (in years)			
Mean duration of disease (Std Dev)	5.9(±4.3)	8.2(±4.7)	-
Median duration of disease	6	6	
Range	1-18	4-18	
Triplet Repeat length			
Mean abnormal repeat length (Std Dev)	46.3(±3.3)	44.3(±4.5)	-
Median abnormal repeat length	45	46	-
Range	43-53	40-49	
Haematology results			
Smear results			
Wet smear (defined as >3.7% erythrocytes being acanthocytes)			
Positive (number of patients)	0	0	0
Dry smear (defined as >1.2% erythrocytes being acanthocytes)			
Positive (number of patients)	0	0	0
Median % of acanthocytes	0.21	0.06	0.06
Interquartile range (IQR)	0.06-0.44	0.00-0.11	0.02-0.14
Erythrocyte analysis (Std Dev)			
Haemoglobin (g/dl)	14.62(±1.40)	14.28(±2.05)	15.56(±3.9)
Red cell count (10^{12}/l)	5.01(±0.63)	4.91(±0.47)	5.08(±0.56)
MCV (fl)	88.1(±4.2)	87.1(±5.6)	87.4(±4.9)
MCH (pg)	29.5(±2.0)	29.1(±2.6)	29.1(±2.0)
MCHC (g/dl)	33.3(±1.5)	33.3(±1.5)	33.3(±1.2)
RDW (%)	13.3(±0.9)	13.7(±1.4)	13.6(±0.8)

Abbreviations: IQR, Interquartile Range; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentrate; MCV, Mean Corpuscular Volume; RDW, Red Cell Distribution Width; SD, Standard Deviation.

4.5 Discussion

There was no evidence of acanthocytosis in any of the HLD2 or HD patients. Assessments were performed using three different measures and with a normal control group. We therefore conclude that acanthocytosis is not a significant component of HDL2 or HD in our patient population.

The presence of acanthocytosis in four previous cases of HDL2 remains intriguing. In the first three cases, the technique for obtaining the smear described not stressing the erythrocytes with saline or aging them. Up to 5% acanthocytes/echinocytes was accepted as being within the normal range (Walker, Rasmussen *et al.,* 2003). Echinocytes are difficult to differentiate from acanthocytes on light microscopy and are commonly caused from smear processing errors whereas acanthocytes are of pathological relevance. The method we employed for the detection of acanthocytes by Storch *et al.* (2005) was not published until 2005. This method is now considered to be the gold standard in testing for acanthocytes in neuroacanthocyte syndromes and reduces echinocytic stress (Storch *et al.,* 2005). The method used to detect acanthocytes in the original four cases where acanthocytes were seen was not described in sufficient detail to duplicate and therefore the Storch method was used by our group.

Acanthocytes may be an infrequent feature of HDL2 or may only develop in the presence of other genetic or environmental modifiers. Three of the four HDL2 cases with acanthocytes were related, and all carried either the Fragile X mutation or premutation. With hindsight, it may have been useful to have had a Fragile X control group, however, acanthocytosis has not been reported in either Fragile X or FXTAS patients. The fourth case did not have abnormal band 3 proteins and had HDL2-affected siblings without acanthocytes. All four cases have subsequently died and are therefore unfortunately not available for further study.

The pathogenesis of HDL2 appears to be complex, with potential contributions from a toxic gain-of-function of abnormal *JPH3* RNA (Rudnicki *et al.,* 2007), loss of *JPH3* expression (Seixas *et al.,* 2012) and potentially, expression of an antisense gene encoding expanded polyglutamine repeats (Wilburn *et al.,* 2011). The present study, although of a relatively small sample size, has nearly doubled the number of HDL2 patients tested for acanthocytes in the literature and for the first time examined acanthocytes in HD. Our results, based on the first prospective study of acanthocytes in HDL2 or HD, suggest that screening for acanthocytes will not help establish the diagnosis of HD or HDL2 nor differentiate between the two disorders. Further, the processes underlying acanthocyte formation are unlikely to substantially contribute to the pathogenesis of most cases of HDL2 and HD. The question is now raised as to whether HDL2 should be grouped with the neuroacanthocytosis syndromes. Finally, our results continue to demonstrate the remarkable phenotypic similarities between HD and HDL2.

4.6 Acknowledgements

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CHAPTER 5

Emerging Differences Between Huntington's Disease Like 2 and Huntington's Disease: A Comparison Using MRI Brain Volumetry

The MRI scans of HDL2 subjects have been described as having the same features as the MRI scans of HD subjects. However, these reports are rare, anecdotal and qualitative. Therefore, one of the objectives of this research was to quantitively and systematically compare the MRI scans of HDL2 and HD subjects. This chapter addresses this aim by comparing the MRI scans of HDL2 and HD subjects with unaffected control cases. In this paper, a blind case control design was used to acquire and analyse the images. The volumes of grey and white matter and subcortical structures were measured using Brain Research: Analysis of Images, Networks, and Systems (BRAINS). A description is then provided of the MRI sequence used in this study, how the images were analysed and the details of the statistical method that was applied. The results section initially compares the HDL2 brain volumes with the those of the unaffected control subjects. The HD brain volumes are then compared with the normal control group and finally, the two affected groups are compared. This study found that generally, the brain volumes of the two affected groups have similar volume loss compared to the normal control group. However, the HDL2 groups had significantly smaller thalamic volumes compared to those of the HD group. The novel finding of smaller thalamic volumes in the HDL2 subjects poses questions about the HDL2 pathogenesis and phenotype.

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5 Emerging Differences Between Huntington's Disease Like 2 and Huntington's Disease: A Comparison Using MRI Brain Volumetry

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5.1 Abstract

Huntington's Disease Like 2 (HDL2), caused by a CTG/CAG expansion in *JPH3* on chromosome 16q24, is the most common Huntington's Disease (HD) phenocopy in populations with African ancestry. Qualitatively, brain MRIs of HDL2 patients have been indistinguishable from those of HD patients. To determine brain regions most affected in HDL2, a cross-sectional study using MRI brain volumetry was undertaken to compare the brains of nine HDL2, eleven HD and nine age-matched control participants. Participants were sourced from the region in South Africa with the world's highest HDL2 incidence. The HDL2 and HD patient groups showed no significant differences with respect to mean age at MRI, disease duration, abnormal triplet repeat length or age at disease onset. Overall, intracerebral volumes were smaller in both affected groups compared to the control group. Comparing the HDL2 and HD groups across multiple covariates, cortical and subcortical volumes were similar with the exception that the HDL2 thalamic volumes were smaller. Consistent with other similarities between the two diseases, these results indicate a pattern of neurodegeneration in HDL2 that is remarkably similar to HD. However, smaller thalamic volumes in HDL2 raise intriguing questions into the pathogenesis of both disorders and how these volumetric differences relate to their respective phenotypes.

5.2 Introduction

Huntington's disease like 2 (HDL2) is the most common Huntington's disease (HD) phenocopy in populations with African ancestry. It is emerging as the most common HD phenocopy in South America, the USA and parts of Europe (Margolis *et al.,* 2004; Mariani *et al.,* 2016; Walker *et al.,* 2018). South Africa has the highest number of HDL2 cases described despite the under-ascertainment of patients due to lack of resources in rural communities (Anderson, Walker *et al.,* 2017; Baine *et al.,* 2016).

Huntington's Disease is caused by a CAG/CTG repeat expansion in exon 1 of the *huntingtin* gene on chromosome 4p16.3, while HDL2 is caused by a similarly sized CTG/CAG expansion in exon 2A of *junctophilin-3* (*JPH3*) on chromosome 16q24.3 (Holmes *et al.,* 2001). Both are autosomal dominant disorders characterised by

psychiatric disease, movement disorder and cognitive dysfunction which progress and inevitably result in death, approximately 15 to 20 years after clinical onset (Margolis *et al.,* 2001). Observations of the HDL2 phenotype are from retrospective and anecdotal cases and reviews (Anderson, Walker *et al.,* 2017). Notwithstanding these genetic differences and the need for systematic study of the HDL2 clinical phenotype, HDL2 is the disease that has been described as most resembling HD (Walker, Jankovic *et al.,* 2003).

Qualitative MRI studies have not been able to differentiate between HDL2 and HD. To date, these rare reports suggest similar patterns of atrophy between the two disorders (Anderson, Walker *et al.,* 2017). A systematic review of 69 published cases of HDL2 reported 20 MRI scans of HDL2 patients, described as indistinguishable from HD (Anderson, Walker *et al.,* 2017). An exception was a patient with HDL2 who presented with parkinsonism and myoclonus. The MRI of this case showed typical cerebral atrophy but relative sparing of the caudate nucleus (Bardien *et al.,* 2007). A report of a single case of HDL2 having two MRIs five years apart described progressive atrophy of the same areas as seen in HD (Rodrigues *et al.,* 2008). Hyperintense putaminal rims on T2 imaging have been described in three cases with HDL2 from the same family (Schneider *et al.,* 2012). The putaminal rim sign has also been detected in a number of other degenerative movement disorders including Multi-System Atrophy (MSA), Neuro-Acanthocytosis, Dentatorubral-Pallidoluysian Atrophy (DRPLA) and Spinocerebellar Ataxia Type 17 (SCA17).

Despite the fact that HD and HDL2 are caused by mutations in different genes which are linked to different functions within the cell, the majority of the qualitative MRI reports, neuropathological analysis and clinical phenotype, highlight the similarities between both disorders. We therefore hypothesised that quantitative MRI analysis of HDL2 patients would reveal similar findings to those detected in HD patients. To test this hypothesis, we performed a blinded systematic imaging study of matched HD and HDL2 patients with a normal control group, taking advantage of the relatively high number of HDL2 cases that have been reported in the region surrounding Johannesburg, South Africa (Krause *et al.,* 2015; Anderson, Walker *et al.,* 2017). Comparing the brain MRIs of these clinically similar diseases has the potential to guide further investigation into the differences and similarities between HD and

HDL2 and form part of an ongoing, systematic characterisation of the phenotype of HDL2.

5.3 Methods

Patients with HDL2 and HD were identified and diagnosed through the Division of Human Genetics, National Health Laboratory Service and the School of Pathology at the University of the Witwatersrand in Johannesburg, South Africa. Controls were unrelated, healthy acquaintances matched for gender, age and race. Participants were enrolled between January 2014 and June 2017 and all subjects had African or mixed ancestry, including a significant African component. The Human Research Ethics Committee of the University of the Witwatersrand approved this cross-sectional study (M140872).

MRIs of subjects were performed at the University of the Witwatersrand Donald Gordon Medical Centre Radiology Department on a 1.5T Philips Intera MR scanner (Philips Medical Systems, Eindhoven). There were no relevant scanner upgrades during the study. The MRI sequences used in this study are available in the supplement.

We used the Brain Research: Analysis of Images, Networks and Systems (BRAINS) protocol, which has been validated and extensively used to measure intracerebral volumes for HD in the large PREDICT-HD study (Paulsen *et al.,* 2008). Scans were processed by Brain Image Analysis, LLC using the cross-sectional analysis stream of NeuroAnalytica version 1.3.09, a commercial image analysis platform based on AutoWorkup (Pierson *et al.,* 2011). A discriminant tissue classification was then performed (Harris *et al.,* 1999). Artificial neural networks for each subcortical structure were applied (Powell *et al.,* 2008). Additional information about this technique is available in the supplement.

Comparison of age at MRI, disease duration, abnormal repeat length and age at onset among the groups was performed using a one-way ANOVA. Comparison of variables among the three groups, controlling for age at MRI and separately, for

disease duration, expanded repeat length, and age of disease onset was performed using a General Linear Model. Post-hoc comparisons were performed using the Tukey-Kramer adjustment. The false discovery rate was controlled by the Benjamini-Hochberg procedure and significance was calculated at $p<0.0084$. Data analysis was carried out using SAS version 9.4 for Windows.

5.4 Results

Images of nine HDL2, eleven HD and nine normal control participants were included in the study. None of the HDL2 participants were related. Three of the HD pedigrees each contributed two participants to the study. There were no significant differences among the groups with respect to mean age at MRI ($F [2,26]=0.54$; $p=0.59$), disease duration ($F [1,18] =0.57$; $p=0.57$), abnormal repeat length ($F [1,18] =0.18$; $p=0.18$) or age at onset ($F [1,18] =0.63$; $p=0.63$) (Table 5.1.a).

The main aim of the study was to contrast HD and HDL2 MRIs. Initially, a qualitative comparison of MRIs could not distinguish between both pathologies (Figure 5.1.b). The quantitative comparison was conducted using BRAINS. We conducted an initial comparison between HD and healthy controls in order to ensure comparability with previous studies and validation of the present methodology. Subsequently, the HDL2 group was compared to controls with the objective of providing the first MRI brain volumetry characterisation of HDL2. Lastly, HD and HDL2 MRIs were compared.

Table 5.1 Table 5.1a and 5.1b showing subject demographic and genetic data and MRI volumes.

5.1.a Demographics and Genetics	HDL2	HD	Controls
Number of participants	9	11	9
Male: Female	5:4	6:5	5:4
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
Age at MRI (years)	47.6 (9.1)	46.0 (12.4)	42.6 (9.1)
Abnormal repeat length (n)	47.0 (3.5)	44.9 (3.2)	-
Duration of disease (years)	5.6 (3.0)	6.6 (4.8)	-
Age at onset(years)	42.0 (10.2)	39.4 (13.1)	-
5.1.b Region Volume (cm^3)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Left thalamus	5.156 (0.671)	6.453 (0.868)	6.628 (0.698)
Right thalamus	4.885 (0.805)	6.232 (0.745)	6.269 (0.643)
Left putamen	2.147 (0.452)	2.615 (0.233)	4.771 (0.637)
Right putamen	2.249 (0.389)	2.698 (0.263)	4.714 (0.502)
Left caudate	1.010 (0.455)	1.528 (0.485)	3.359 (0.548)
Right caudate	1.080 (0.475)	1.667 (0.488)	3.392 (0.629)
Left globus pallidus	0.812 (0.190)	0.749 (0.103)	1.297 (0.311)
Right globus pallidus	0.772 (0.150)	0.766 (0.101)	1.371 (0.323)
Left amygdala	0.747 (0.230)	0.743 (0.143)	0.841 (0.171)
Right amygdala	0.665 (0.170)	0.755 (0.141)	0.834 (0.266)
Left nucleus accumbens	0.159 (0.050)	0.174 (0.043)	0.266 (0.075)
Right nucleus accumbens	0.179 (0.067)	0.232 (0.068)	0.289 (0.059)
Intracranial volume	1 437.9 (112.7)	1 424.1 (118.5)	1 424.6 (156.5)

Table 5.1.a: Subject ages, age of disease onset, duration of disease and triplet repeat length. Table 5.1.b: Unadjusted volumetric analysis of selected subcortical structures, hippocampi and total intracranial volumes in cm^3. HD = Huntington's Disease, HDL2 = Huntington's Disease Like 2, n = number, SD = Standard Deviation.

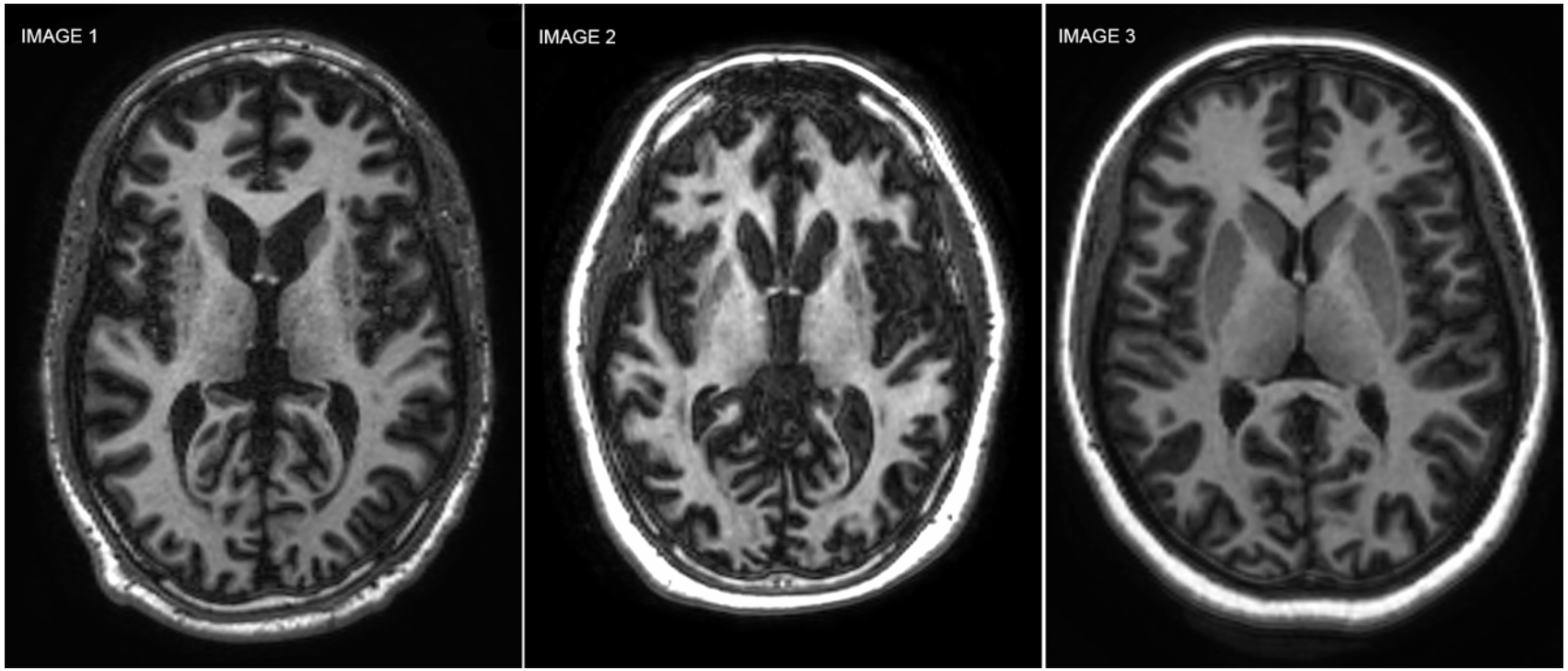

Figure 5.1 Qualitative MRI Comparison

Three T1 axial 1.5T MRIs comparing HDL2, HD and a normal control case. Image 1: 35-year-old HD subject with abnormal *HTT* allele = 49 triplet repeats. Image 2: 32-year-old HDL2 subject with abnormal *JPH3* = 53 triplet repeats. Image 3: 32-year-old normal control subject. The HDL2 and HD subjects show qualitatively similar grey and white matter atrophy with caudate nuclei and putaminal volume loss compared to the normal control.

5.4.1 Comparison between patients with HD and normal controls

When compared to the normal control group and controlling for the age at the time of the MRI, the HD group showed significantly less grey matter volume in both of the parietal and left temporal lobes, with significantly smaller caudate nuclei, putamen and globus pallidum bilaterally (Figures 5.2, 5.3 and Table 5.1.b). These smaller grey matter volumes were reflected in corresponding increases in CSF volumes (Figure 5.4). Notably, the mean thalamic volumes were not statistically different between the HD group and the normal control group ($p < 0.01$) (Table 5.2).

5.4.2 Comparison between patients with HDL2 and normal controls

Grey matter volume tended to be less in the HDL2 group compared to control groups when controlling for age at the time of the MRI (Figure 5.2). The HDL2 group showed significantly smaller grey matter volumes for both the parietal and temporal lobes and the left occipital lobe and significantly smaller white matter volumes for both the frontal and occipital lobes and the left parietal lobe compared to the normal control participants ($p<0.008$). Almost all of the CSF spaces were significantly larger in the HDL2 group compared to the control group (Figure 5.4). Most of the basal ganglia, including the thalamus, were significantly smaller in the HDL2 group compared to the control group ($p<0.008$) (Table 5.1.b and Figure 5.3).

5.4.3 Comparison between patients with HDL2 and HD

When comparing the HDL2 and HD groups, a similar pattern of grey and white matter volume was noted (Figure 5.2). The mean volumes of the caudate nucleus, globus pallidum, amygdala and the nucleus accumbens tended to be smaller in the HDL2 group compared to the HD group but this did not reach statistical significance when compared across all covariates (Table 5.2 and Figure 5.3). When controlling for age at the time of the MRI, duration of disease, abnormal repeat length and age of onset, the HDL2 groups mean thalamic volumes were significantly smaller compared to the HD participants (Mean [STD] left and right thalamic volumes; HDL2

= left 5.156 cm^3 [0.671], right = 4.885 cm^3 [0.805], HD left = 6.453 cm^3 [0.86], right = 6.232 cm^3 [745]).

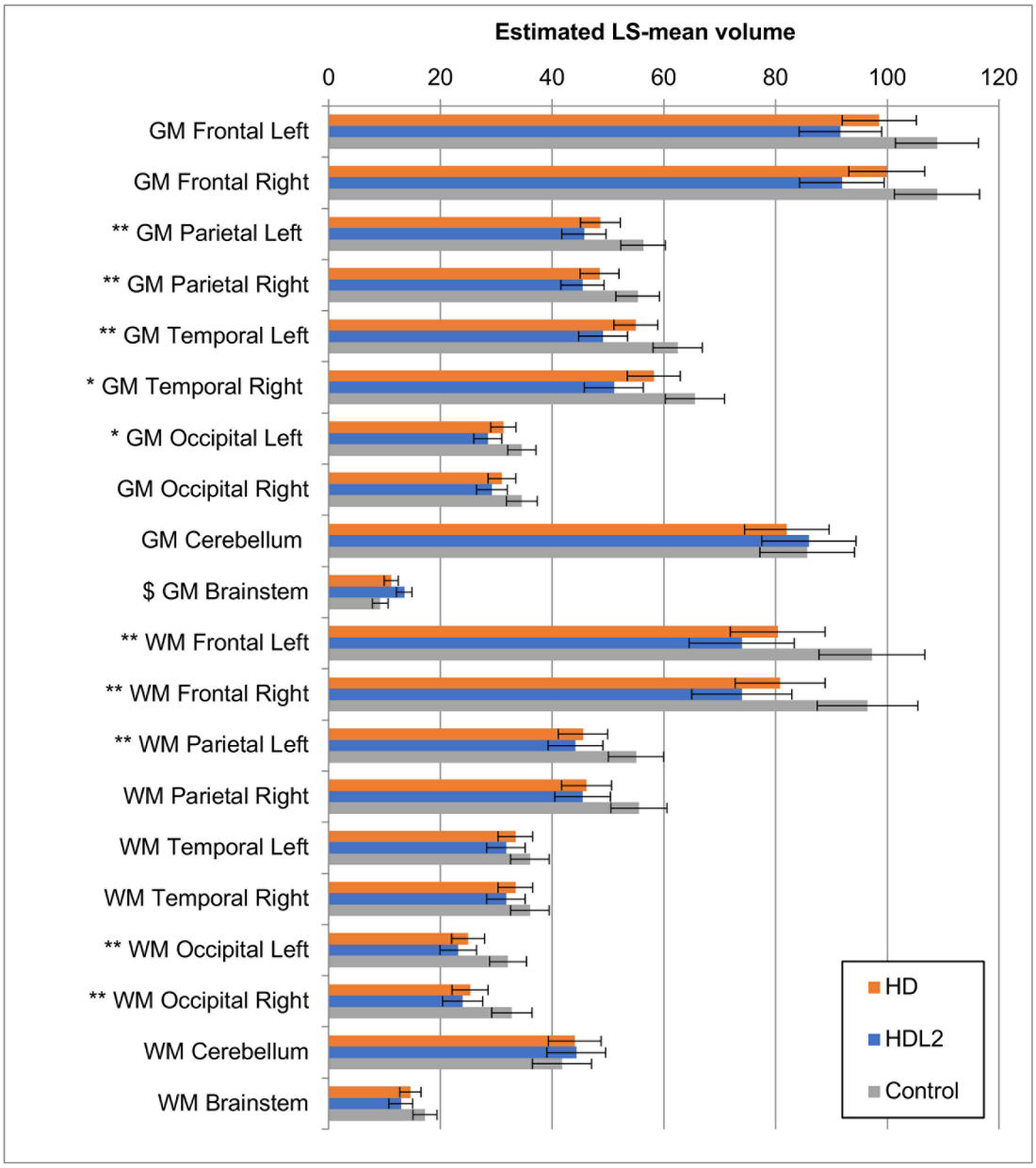

Figure 5.2 HD, HDL2 and control groups' grey matter and white matter volumes

The estimated least-squares (LS) means from the regression are depicted in a three-way comparison of grey and white matter volume. Error bars denote the 95% confidence intervals for the mean. Significance was set at $p < 0.008$. Symbols denote significant differences: **: HD, HDL2 < Control; *: HDL2 < Control; $: HD, Control< HDL2. GM = grey matter, WM = white matter.

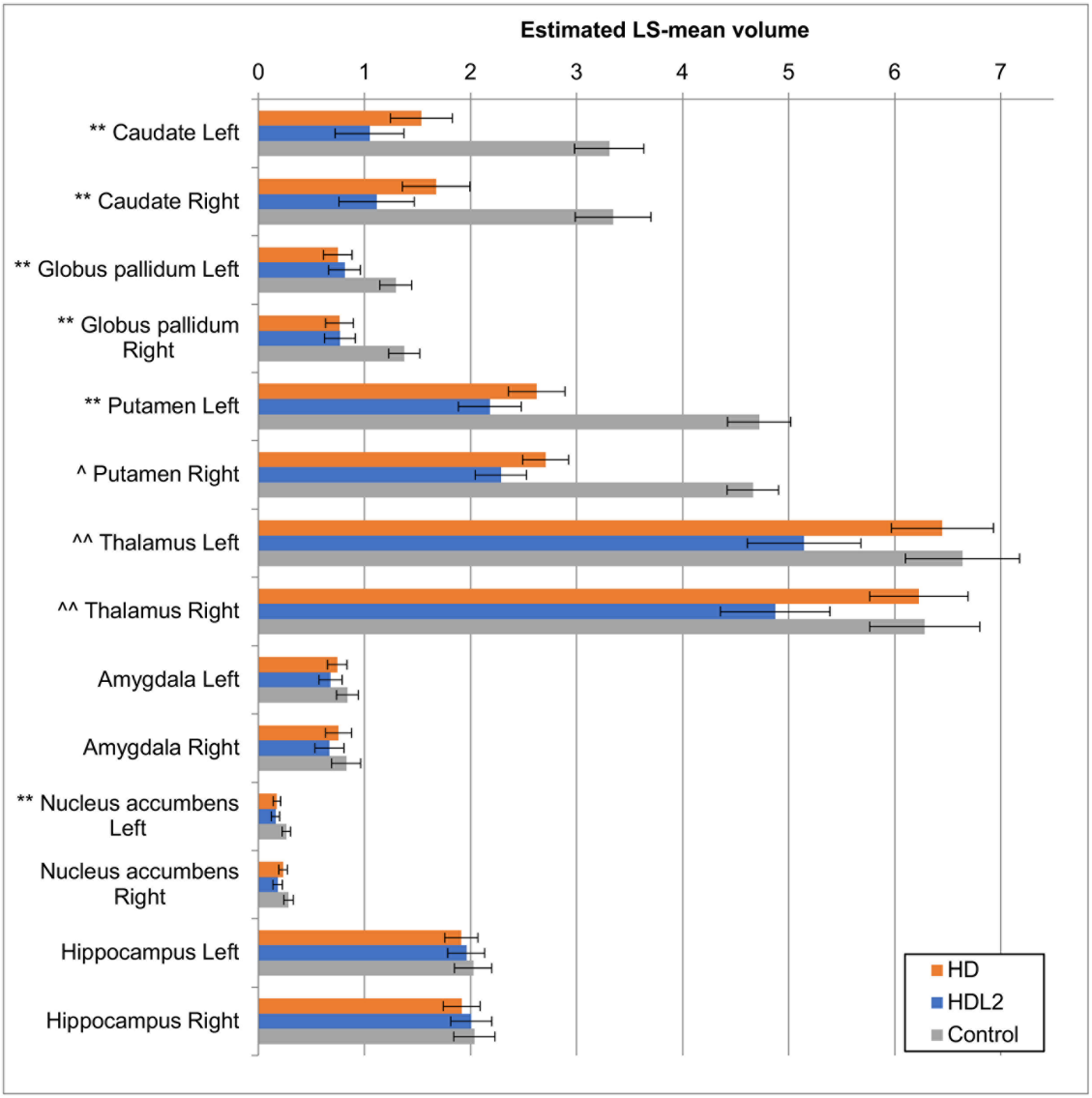

Figure 5.3 HD, HDL2 and control groups' basal ganglia, thalamic and hippocampal volumes

The estimated least-squares (LS) means from the regression are depicted in the figure in a three-way comparison among HD, HDL2 and control groups. Statistical significance is denoted by a symbol. Error bars denote the 95% confidence interval for the mean. Significance was set at $p < 0.008$. Symbols: **: HD, HDL2< Control, ^: HDL2 < HD <Control, ^^: HDL2<Control, HD where significance was reached. LS = Least-squares means, Nucleus acc = Nucleus accumbens.

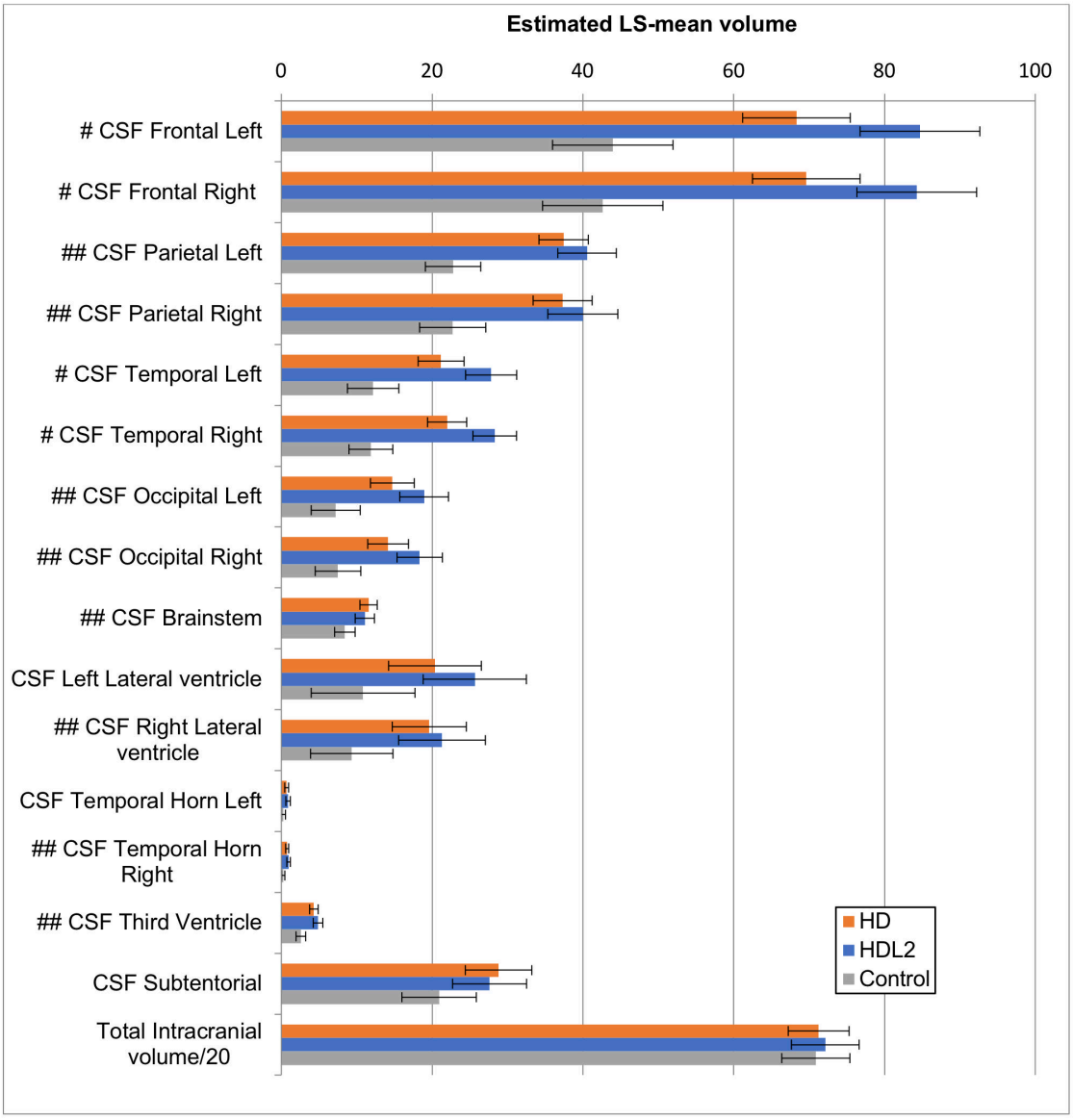

Figure 5.4 HD, HDL2 and control cerebrospinal fluid and total intracranial volumes

The estimated least-squares (LS) means from the regression are depicted in the graph in a three-way comparison among the HD, HDL2 and control groups showing cerebrospinal fluid volumes and total intracranial volumes. Note that total intracranial volume was divided by 20 to fit to the graph scale. Statistical significance is shown with a symbol. Error bars denote the 95% confidence interval for the mean and significance was set at $p < 0.008$. Symbols denote #: Control < HD < HDL2. ##: HD, HDL2 < Control where significance was reached. LS = least squares mean, CSF=Cerebrospinal fluid.

Table 5.2 Table comparing the Huntington's disease like 2, Huntington's disease and the normal control groups' basal ganglia mean volume significant differences across multiple covariates

Region	Comparison between HDL2, HD and control groups controlling for age at MRI		Comparison between HDL2 and HD groups controlling for duration of disease		Comparison between HDL2 and HD groups controlling for abnormal repeat length		Comparison between HDL2 and HD groups controlling for age at onset	
	Significant differences	P value	Significant differences	P value	Significant differences	P value	Significant differences	P value
Left Thalamus	HDL2 < HD, Control	0,0007	HDL2 < HD	**0,0015**	HDL2 < HD	**0,0049**	HDL2 < HD	**0,0012**
Right Thalamus	HDL2 < HD, Control	0,0005	HDL2 < HD	**0,0008**	HDL2 < HD	**0,0032**	HDL2 < HD	**0,0006**
Left Putamen	HDL2, HD < Control	<0.0001	HDL2 < HD	**0,0083**	HDL2 < HD	**0,0063**	Not Significant	0,011
Right Putamen	HDL2 < HD < Control	<0.0001	HDL2 < HD	**0,0064**	HDL2 < HD	**0,0014**	HDL2 < HD	**0,0081**
Left Caudate	HDL2, HD < Control	<0.0001	Not Significant	0,023	Not Significant	0,027	Not Significant	0,034
Right Caudate	HDL2, HD < Control	<0.0001	Not Significant	0,010	Not Significant	0,018	Not Significant	0,019
Left G. Pallidus	HDL2, HD < Control	<0.0001	Not Significant	0,45	Not Significant	0,41	Not Significant	0,42
Right G. Pallidus	HDL2, HD < Control	<0.0001	Not Significant	0,81	Not Significant	0,80	Not Significant	0,97
Left Amygdala	Not Significant	0,11	Not Significant	0,35	Not Significant	0,27	Not Significant	0,35
Right Amygdala	Not Significant	0,27	Not Significant	0,22	Not Significant	0,16	Not Significant	0,25
Left N. Accumbens	HDL2, HD < Control	0,0016	Not Significant	0,38	Not Significant	0,16	Not Significant	0,54
Right N. Accumbens	Not Significant	0,011	Not Significant	0,018	Not Significant	0,10	Not Significant	0,10

Table comparing the Huntington's disease like 2, Huntington's disease and the normal control groups' basal ganglia mean volume significant differences across multiple covariates (controlling for age at MRI, controlling for duration of disease, controlling for abnormal repeat length and controlling for age at onset). Two-tailed significance: P<0.008. G. Pallidus = Globus Pallidus, HDL2 = Huntington's Disease Like 2, HD = Huntington's Disease, N. Accumbens = Nucleus accumbens.

The HDL2 volumes were smaller when evaluating differences in the unadjusted putamen volumes in HDL2 and HD cases (Mean [STD] left and right putamen volumes; HDL2 = left 2.147 cm^3 [0.452], right = 2.249 cm^3 [0.389], HD left = 2.615 cm^3 [0.233], right = 2.698 cm^3 [0.263]). However, when applying the Benjamini-Hochberg procedure and applying a $p<0.0084$, significance was not seen bilaterally for the putamen as was with the thalamus when controlling for age of onset. Furthermore, the thalamus (Left: $p<0.0012$, Right: $p<0.0006$) in comparison to the putamen (Left: $p<0.011$, Right: $p<0.0081$) had greater significant volume difference between the two diseases when controlling for age of disease onset (Table 5.2).

The unadjusted left and right caudate volumes suggest similar trends as seen in the thalamus and putamen when the unadjusted data is observed, with the HDL2 volumes being smaller compared to the HD group caudate volumes (Mean [STD] left and right putamen volumes; HDL2 = left 1.010 cm^3 [0.455], right = 1.080 cm^3 [0.475], HD left = 1.528 cm^3 [0.485], right = 1.667 cm^3 [0.488]) (Table 5.1.b). However, the LS mean caudate volumes are small: 3.2 for HD and 2.2 for the HDL2 groups and when applying the same procedure, the difference between the HDL2 and HD caudate volumes did not reach significance when controlling for any of the covariates as noted in Table 5.2.

The relationship between duration of disease, abnormal repeat length and age at onset was separately determined for each volume measurement in both the HDL2 and HD groups. The trend was an inverse correlation for most measurements, as expected, but significance was not reached. In both diseases, smaller volumes of the grey matter structures were seen, with increasing duration of disease and larger repeat lengths.

Lastly, on blinded clinical review, T2 images demonstrated bilateral putaminal rim hyperintensities in HD and HDL2 patients, but not in the control group.

5.5 Discussion

This is the first quantitative volumetric MRI study in patients with HDL2 and the first comparison to an HD group and an unaffected control group, done blind to the patient's diagnosis. Consistent with the previously reported qualitative review of individual cases (Margolis *et al.,* 2001; Anderson, Walker *et al.,* 2017), the analysis indicated similar atrophy of the grey and white matter structures, including the basal ganglia and enlarged CSF spaces, in the two disease groups compared to the normal control group. However, smaller thalamic volumes were noted in the HDL2 group compared to the HD group. There was no evidence of relative caudate sparing, as observed in one previous HDL2 case (Bardien *et al.,* 2007).

The HD group showed white and grey matter loss, enlarged CSF spaces and basal ganglia changes that replicated the findings of previous studies using similar analytic methods but much larger sample sizes. These previous studies provided support for the methods and patient population used in this study (Paulsen *et al.,* 2008; Tabrizi *et al.,* 2009; van den Bogaard *et al.,* 2011). The key finding of this analysis is the similarity between the MRIs of HD and HDL2 patients, with the notable exception that the average thalamic volume was 21% smaller in the HDL2 group compared to the HD group. In contrast to this difference, the mean thalamic volume in the HD group was similar to that recorded in the normal control participants.

Initial neuroimaging studies found no difference in thalamic volumes between HD patients and controls (Rosas *et al.,* 2003). Later studies have demonstrated thalamic volume loss in HD but have suggested that this reflects disease progression. Studies comparing MRI brain volumes in HD with normal controls often used different volumetric methods and showed HD thalamic volumes being between 2-8% less than the unaffected control groups (Kassubek *et al.,* 2004; Coppen *et al.,* 2018). Thalamic atrophy in HD appears to reflect whole brain atrophy and is proportional to overall degeneration (van den Bogaard *et al.,* 2011). Therefore, in keeping with previous reports of HD thalamic volumes, the mean thalamic volumes of the HD group in this study were 2% less than the control group. However, in the HD and HDL2 groups, regions like the putamen and caudate showed typically smaller

volumes compared to the normal control group, as has been described previously in HD (Tabrizi *et al.,* 2009; van den Bogaard *et al.,* 2011). The striking finding from this study was that HDL2 thalamic volume was significantly smaller than in the HD group when controlling for age at the time of the MRI, duration of disease, abnormal repeat length and age of onset of disease.

Attempts to find features differentiating HDL2 from HD are available, but findings have been anecdotal, inconsistent or disproved. For example, the hypothesis that HDL2 is a neuroacanthocytosis syndrome was recently refuted by a blinded controlled study (Anderson, Carmona *et al.,* 2017). Descriptions of the HDL2 phenotype have introduced the possibility that HDL2 may disproportionately present with bradykinetic/parkinsonian features which likely reflect the unique phenotype and relatively long expanded repeat length. However, this remains unverified (Margolis *et al.,* 2001; Schneider *et al.,* 2012). The characterisation of the clinical phenotype of HDL2 will remain incomplete while based only on retrospective case series (Anderson, Walker *et al.,* 2017). Thus, future research is needed. Our findings regarding the differences in thalamic volumes between HDL2 and HD may provide a new and exciting guide to differentiating between these two mutations.

The possible clinical implications and pathogenic mechanisms causing greater thalamic atrophy in HDL2 would need to be accounted for. The pathogenesis of HDL2 is complex, with likely contributions from the toxic properties of *JPH3* RNA with the repeat expansion, loss of expression of *JPH3* and perhaps expanded tracts of polyglutamine, expressed from a cryptic gene on the antisense strand at the HDL2 locus (Rudnicki *et al.,* 2007; Wilburn *et al.,* 2011; Seixas *et al.,* 2012). The finding of smaller putamen and thalamic volumes in HDL2 compared to HD suggests differential neuronal and/or glial vulnerability in these areas to the HDL2 mutation compared to the HD mutation. This may have implications, firstly, in terms of phenotype. It has been suggested that there are clinical differences such as more myoclonus and parkinsonism although this needs further validation (Margolis *et al.,* 2001; Anderson, Walker *et al.,* 2017). Secondly, although little difference has been reported in the gross or histopathological findings between five patients with HDL2 compared to matched cases with HD (Rudnicki *et al.,* 2008), it is possible that the thalamic differences may be the consequence of histopathological discrepancies

between these mutations which require larger samples of HDL2 specimens (Rudnicki *et al.,* 2008).

This study is based on the uniquely high prevalence of HDL2 in South Africa and would be difficult to accomplish in any other location. Even in this region, however, HDL2 is a rare disorder and therefore the sample size is small. Nonetheless, the results do establish the general neuroradiological similarity of HD and HDL2 and are consistent with their reportedly analogous clinical phenotypes. The selectively smaller thalamic volumes in HDL2 are an interesting finding, but the sample size imposes limits on data interpretation. In both HD and HDL2, the relationship between intracerebral volumes and duration of disease and abnormal repeat length does not reach significance, though most measurements tend to show smaller volumes with increasing duration of disease and larger abnormal repeat lengths. These are well established negative correlations for HD demonstrated in larger studies (Tabrizi *et al.,* 2009). We would predict that in HDL2, like HD, these trends would be confirmed with larger cross-sectional cohorts of patients or with longitudinal analyses of smaller cohorts. In support of this, sequential MRIs five years apart have been reported for a single HDL2 case which qualitatively demonstrated progressive brain atrophy in a similar pattern to HD (Rodrigues *et al.,* 2008).

Lastly, we found putaminal rim hyperintensities in all of the HD and HDL2 cases. This sign has been described before in HDL2 (Schneider *et al.,* 2012). Other neurodegenerative disorders and normal controls have been shown to have putaminal rim hyperintensities and this is therefore unlikely to help distinguish HDL2 from other conditions (Tha *et al.,* 2012; Lee *et al.,* 2005). To our knowledge, the putaminal rim sign has not been commented on in HD previously.

5.6 Conclusion

HDL2 and HD are remarkably similar clinically and radiologically. When HDL2 is objectively compared to an HD group that is matched for age, abnormal repeat length and duration of disease, a similar pattern emerges of smaller volumes in the grey and white matter as well as in the basal ganglia. The results presented here,

now demonstrate the similarities between HDL2 and HD extend to spatial patterns of neurodegeneration, with the interesting exception of the thalamus, where selective thalamic volume loss tends to be greater in HDL2.

These findings raise intriguing possibilities about both the convergent and the divergent pathogenic pathways of the two diseases and open future paths of research into how this relates to their phenotypes.

5.7 Acknowledgements

We wish to thank the Department of Radiology at the University of the Witwatersrand Donald Gordon Medical Centre, Dr TJ Nel and Partners for generously donating all the MRI scans for this study and all the staff at the Department for their helpfulness and participation. We would also like to acknowledge and thank Marianne Gomes for co-ordinating and counselling the patients and families in this study and Dr Petra Gaylard for her assistance with the statistical analysis of the data. Most importantly, we would like to thank the patients and their families for their time and kind participation in this study.

5.8 Disclosures and conflict of interest

Ronald Pierson is a shareholder in NeuroAnalytica and received fees from the University of the Witwatersrand for analysis of the MRIs.

5.9 Supplementary information

This section describes the MRI sequences and Brain Research: Analysis of Images, Networks and Systems (BRAINS) protocol used to analyse the images in this study.

5.9.1 Methods

5.9.1.1 Sampling and recruitment

Patients with HDL2 and HD were identified and diagnosed through the Division of Human Genetics, National Health Laboratory Service and the School of Pathology at the University of the Witwatersrand in Johannesburg, South Africa. Controls were unrelated, healthy acquaintances matched for gender, age and race. Participants were enrolled between January 2014 and June 2017 and all subjects had African or mixed ancestry. The Human Research Ethics Committee of the University of the Witwatersrand approved this cross-sectional study (M140872).

5.9.1.2 Magnetic Resonance Imaging (MRI)

MRIs of subjects were performed at the University of the Witwatersrand Donald Gordon Medical Centre Radiology Department on a 1.5T Philips Intera MR scanner (Philips Medical Systems, Eindhoven). There were no relevant scanner upgrades during the study. The following sequences were included in all cases: T1 Sagittal (TE 4.6 msec, TR 25 msec, FA 30°, slice thickness 1mm), FLAIR Sagittal (TE 350 msec, TR 4800 msec, TI 1660 msec, slice thickness 1.2mm), T2 Sagittal (TE 277 msec, TR 2250 msec, slice thickness 2mm), T1 Sagittal (TE 3.4 msec, TR 7.3 msec, Inversion prepulse delay 627ms, FA 8°, slice thickness 1.3mm), T2 FFE axial (TE 18 msec, TR 593 msec, FA 18°, slice thickness 5mm).

Variable IR sequences were also obtained as a backup for basal ganglia detail, depending on the subject's ability to keep still: 3D IR coronal, TE 350 msec, TR 4800 msec, TI 1660 msec, slice thickness 1.2mm, NSA 2., 3D IR axial through basal ganglia, TE 15 msec, TR 2000 msec, TI 400 msec, slice thickness 1.8mm, NSA 1.

We used the Brain Research: Analysis of Images, Networks and Systems (BRAINS) protocol, which has been validated and extensively used to measure intracerebral volumes for HD in the large PREDICT-HD study (Paulsen *et al.,* 2008). Scans were processed by Brain Image Analysis, LLC using the cross-sectional analysis stream of NeuroAnalytica version 1.3.09, a commercial image analysis platform based on AutoWorkup (Pierson *et al.,* 2011). In processing with NeuroAnalytica, scans were reoriented into a standard space centred on the anterior commissure and resampled to 1 mm resolution. The T1 images were then intensity-normalised and inhomogeneity-corrected. A discriminant tissue classification was then performed (Harris *et al.,* 1999). Artificial neural networks for each subcortical structure were applied to segment thalamus, caudate nucleus, putamen, globus pallidus, nucleus accumbens, hippocampus and amygdala (Powell *et al.,* 2008). Measures of grey matter, white matter and regional CSF volumes were then completed using an atlas-based cortical lobe segmentation. Processing, quality review and analysis were completed, blinded to the diagnosis of the participant.

5.9.2 Data analyses

Comparison of age at MRI, disease duration, abnormal repeat length and age at onset among the groups was performed using a one-way ANOVA. Comparison of variables among the three groups, controlling for age at MRI and separately, for disease duration, expanded repeat length and age of disease onset was performed using a General Linear Model. *Post hoc* comparisons were performed using the Tukey-Kramer adjustment. The false discovery rate was controlled by the Benjamini-Hochberg procedure and significance was calculated at $p<0.0084$ (Benjamini and Hochberg, 1995). Data analysis was carried out using SAS version 9.4 for Windows.

CHAPTER 6

Comparison of the Huntington's Disease Like 2 and Huntington's Disease Clinical Phenotypes

The main objective of this study was to examine the clinical phenotype of HDL2 and systematically compare it to that of HD. Particular attention was paid to the movement disorder of HDL2. To achieve this aim, a blinded case-control design was applied. This paper initially presents the historical background and genetic features of HDL2. The need for this study is then explained in light of the current and unsubstantiated description of the HDL2 phenotype. The study design, data ascertainment and analysis are subsequently described. A detailed explanation of the method and outcome for the interrater reliability analysis is referred to and can be found in the supplement of this chapter. The results of this study are then reported. Subject demographic data are illustrated and tabulated with the genetic findings. Results for the Unified Huntington's Disease Rating Scale (UHDRS) are then tabulated and illustrated. The discussion of this chapter consolidates the findings form this research and notes that HDL2 and HD are virtually indistinguishable when examining an individual subject in a clinical setting. However, there may be early evidence of HDL2 having a greater degree of severity when comparing the two patient groups. This chapter concludes by suggesting the UHDRS may a useful instrument to monitor HDL2 and that this study supports common pathogenic pathways between HDL2 and HD.

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6 Comparison of the Huntington's Disease Like 2 and Huntington's Disease Clinical Phenotypes

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6.1 Abstract

Background: Huntington's disease like 2 (HDL2) is the most common Huntington's disease (HD) phenocopy in many countries and is described as the phenocopy with the greatest resemblance to HD. The current clinical description of HDL2 is based on retrospective data. It is unknown whether HDL2 has clinical features which distinguish it from HD.

Objective: To describe the HDL2 phenotype and to systematically compare it to HD.

Methods: A blinded, cross-sectional design was used to compare the HDL2 (n=15) and HD (n=13) phenotypes. African ancestry participants underwent assessments including the Unified Huntington's Disease Rating Scale (UHDRS). The UHDRS motor component was video-recorded and evaluated by blinded experts and the interrater reliability calculated.

Results: Both groups were homogeneous in terms of demographics and disease characteristics. However, HDL2 subjects presented with more prominent dysarthria and dystonia three years earlier. Raters could not distinguish between the two diseases with a high level of agreement. No significant differences in the TMS between HDL2 and HD were found. In both disorders, disease duration correlated with motor scores except for chorea. Psychiatric and cognitive scores were not significantly different between groups.

Conclusions: The HDL2 phenotype is similar to HD insofar as it is initially characterised by dementia, chorea and oculomotor abnormalities, progressing to a rigid and bradykinetic state. This suggests that the UHDRS is useful in monitoring disease progression in HDL2. Although HDL2 patients scored higher on some UHDRS domains, this did not differentiate between the two diseases. It may, however, be emerging evidence of HDL2 having a more severe clinical phenotype.

6.2 Introduction

Since its discovery (Margolis *et al.,* 2001), Huntington's disease like 2 (HDL2) has emerged as the most common Huntington's Disease (HD) phenocopy in subjects with African ancestry (Krause *et al.,* 2015). In the South African black population, for every two cases diagnosed with HD there is one diagnosed with HDL2 (Baine *et al.,* 2016). Importantly, HDL2 is the most frequent HD phenocopy in South America, the USA and parts of Europe (Margolis *et al.,* 2004; Mariani *et al.,* 2016; Walker *et al.,* 2018).

The index HDL2 pedigree was followed in the Baltimore Huntington's Disease clinic for many years as a family believed to have HD. The HD mutation caused by a CAG repeat expansion occurring in exon 1 of the *huntingtin (HTT)* gene on chromosome 4p16.3 was reported in 1993 (MacDonald, 1993). When genetic testing became available, this African American family was tested and was found not to have the HD mutation (Margolis *et al.,* 2001). Subsequent study led to the discovery that the disease in this family was caused by a CTG/CAG expansion mutation in exon 2A of *junctophilin-3* (*JPH3*) on chromosome 16q24.3. This resulted in the designation of the disease as HDL2 (Holmes *et al.,* 2001).

Both diseases present with movement abnormalities, progressive subcortical dementia and psychiatric symptoms, ending in death 15 to 20 years after onset (Margolis *et al.,* 2001; Walker, Jankovic *et al.,* 2003). However, previous reports suggested that HDL2 presents with two different phenotypes (Margolis, 2012) – a parkinsonian, earlier-onset form without oculomotor involvement, or a later-onset variant, akin to the classical HD phenotype with typical oculomotor involvement. Overall, the HDL2 phenotype has been considered to have greater parkinsonism, less dysarthria and relatively preserved oculomotor function compared to HD (Walker, Jankovic *et al.,* 2003; Schneider *et al.,* 2012). A systematic review of 69 cases of HDL2 corroborated some of these findings (Anderson, Walker *et al.,* 2017). Parkinsonism was reported in 37% of HDL2 cases. However, this was without the specific parkinsonian features of rigidity and bradykinesia being described. Previous

definitions of parkinsonism have implied the presence of tremor, with other classic hypokinetic features (Jankovic, 2008). Therefore, defining specific features of hypokinesia would allow for a more accurate description of the HDL2 phenotype. Dystonia was described in conjunction with parkinsonism and/or chorea in only 16 cases of HDL2. Dysarthria was identified in one third of the HDL2 cases and was reported as mild to moderate. Only 8 of the 19 cases in which an oculomotor exam was recorded had abnormal oculomotor function, potentially differentiating HDL2 from HD, as early loss of oculomotor function is considered a cardinal sign of HD (Lasker and Zee, 1997; Anderson, Walker *et al.,* 2017). Myoclonus has been reported in four cases of HDL2 (Bardien *et al.,* 2007; Schneider *et al.,* 2012). Like HD, dementia was seen in almost all cases, chorea was the most common motor finding and a strong negative correlation was observed between repeat length and age of onset of the disease (Anderson, Walker *et al.,* 2017).

Descriptions of the HDL2 phenotype have consisted of small case series with limited detailed documentation of particular clinical signs being either present or absent. To date, there have been only three reports where the Unified Huntington's Disease Rating Scale (UHDRS) was used to assess HDL2 subjects (Schneider *et al.,* 2012; Vasconcellos *et al.,* 2017; Mariani *et al.,* 2016). Therefore, the current understanding of the HDL2 phenotype remains incomplete (Anderson, Walker *et al.,* 2017). Systematic examination of specific phenotypic features has yielded findings which were not consistent with initial reports. For instance, reports of acanthocytosis in HDL2 were not confirmed in a blinded, controlled study (Anderson, Carmona *et al.,* 2017). Furthermore, volumetric comparisons between matched HD and HDL2 subjects' MRIs have demonstrated remarkable similarities, as expected from previous case series of HDL2 neuroimages. However, an exception of greater thalamic atrophy in HDL2 was previously reported in a blinded study for the first time, and could potentially distinguish HDL2 from HD when using quantitative MRI image analysis (Anderson, 2018).

To compare the HDL2 and HD clinical phenotype systematically, we took advantage of the relatively high prevalence of HDL2 in South Africa (Krause *et al.,* 2015). We developed a protocol for rating clinical phenotypes, using standardised rating scales

and video examinations by experienced clinicians who were blinded to the subjects' genetic diagnosis. Our results demonstrate remarkable similarities between the two disorders and go some way to clarifying the HDL2 phenotype.

6.3 Materials and methods

6.3.1 Study design

A cross-sectional design with blinded assessment was used to compare the phenotypes of subjects with HDL2 (n=15) and HD (n=13). Subjects with a genetically confirmed diagnosis of HDL2 or HD, tested at the the Division of Human Genetics, National Health Laboratory Service (NHLS) in Johannesburg, South Africa, were invited to enroll into an ongoing prospective study of the HDL2 phenotype, as previously described (Anderson, Walker *et al.,* 2017). To date, HDL2 has been exclusively identified in subjects with African and mixed ancestry (Krause *et al.,* 2015). Mixed ancestry individuals, in South Africa, are those whose gene pool is derived from one or more of the indigenous African populations (San, Khoikhoi or Bantu-speaking), European immigrants from western Europe and/or slaves and indentured labourers from Madagascar, the Malaysian archipelago or India (Krause *et al.,* 2015). Therefore, both cohorts were restricted to these ethnic groups to avoid potential unblinding. The Human Research Ethics Committee of the University of the Witwatersrand approved this study (M140872).

6.3.2 Data ascertainment

A movement disorder neurologist (DGA) interviewed all the participants as well as a friend/family member. The age of disease onset was defined as the age at which the subject initially experienced abnormal movements. This data was obtained from interviewing the subjects and their accompanying friends/family members. They were then asked whether the initial symptom of the disease was cognitive, psychiatric or a movement abnormality. Participants were then examined using the UHDRS Total Motor Score (TMS), with the examination recorded on video. Three raters (AA, DGA, FBR), experienced in the clinical examination of HD, independently

viewed the videos and scored the TMS for each subject. Raters were blinded to the genetic diagnosis of the subjects. All TMS raters are Enroll-HD TMS certified and have at least three years of experience using the scale. After reviewing the videos, evaluators also assigned each participant with a disease diagnosis of HDL2 or HD. The TMS scores were averaged among the three examiners. The subjects underwent a cognitive assessment using the cognitive components of the UHDRS by an experienced neuropsychologist (AFC). A subgroup of subjects could not complete cognitive components of the UHDRS due to the presence of important confounding variables on neuropsychological performance or inability to take the cognitive tests. Specifically, eleven cases were excluded; four of them presented with advanced dementia, three were Human Immunodeficiency Virus positive (HIV+), three were not proficient in English and one had motor aphasia.

6.3.3 Data analysis

A comparison of abnormal repeat length, age at onset, age at diagnosis and disease duration between HD and HDL2 was performed using the Wilcoxon rank sum test. Comparison of the difference between age at onset (defined as the age at which the subject initially experienced abnormal movements) and age at diagnosis between phenotypes was done using the independent samples t-test. The relationship between age at onset and abnormal repeat length, overall and by group, was determined by a General Linear Model (GLM), with age at onset as the dependent variable and abnormal repeat length and disease group, and their interaction, as the independent variables. Comparison of manifesting symptom, ethnicity and gender between phenotypes was performed using Fisher's exact test. Interrater reliability (IR) analysis is discussed in section 6.8, Supplementary data.

Composite motor assessment scores for each dimension were calculated by averaging the scores of the three raters. Comparison of these motor assessment dimensions and total scores as well as the UHDRS functional assessment scores between phenotypes was done with a GLM, with the score as the dependent variable and phenotype and disease duration as the independent variables. The phenotype-disease duration interaction was non-significant in all cases and was

removed from the model. We controlled for disease duration (rather than age of onset or repeat length) since this had the largest difference between the two phenotypes. The z-test for proportions was used to determine the accuracy of the clinician assigning the subject diagnosis as either HDL2 or HD. A 5% significance level was used.

6.4 Results

6.4.1 Demographic and genetic data

Thirteen subjects with HD and 15 with HDL2 were studied from predominantly the north of South Africa (Figure 6.1). In the HD group, three of the ten pedigrees each contributed two participants to the data. The HDL2 group consisted of 15 unrelated individuals. There was no significant difference between the abnormal triplet repeat length, age of disease onset, age at diagnosis, gender, ethnicity or initial manifesting symptom between the two groups (Table 6.1). The median duration of disease was longer in the HD group (7y; IQR 6-8) compared to the HDL2 group (4y; IQR 3-7y) (p=0.029).

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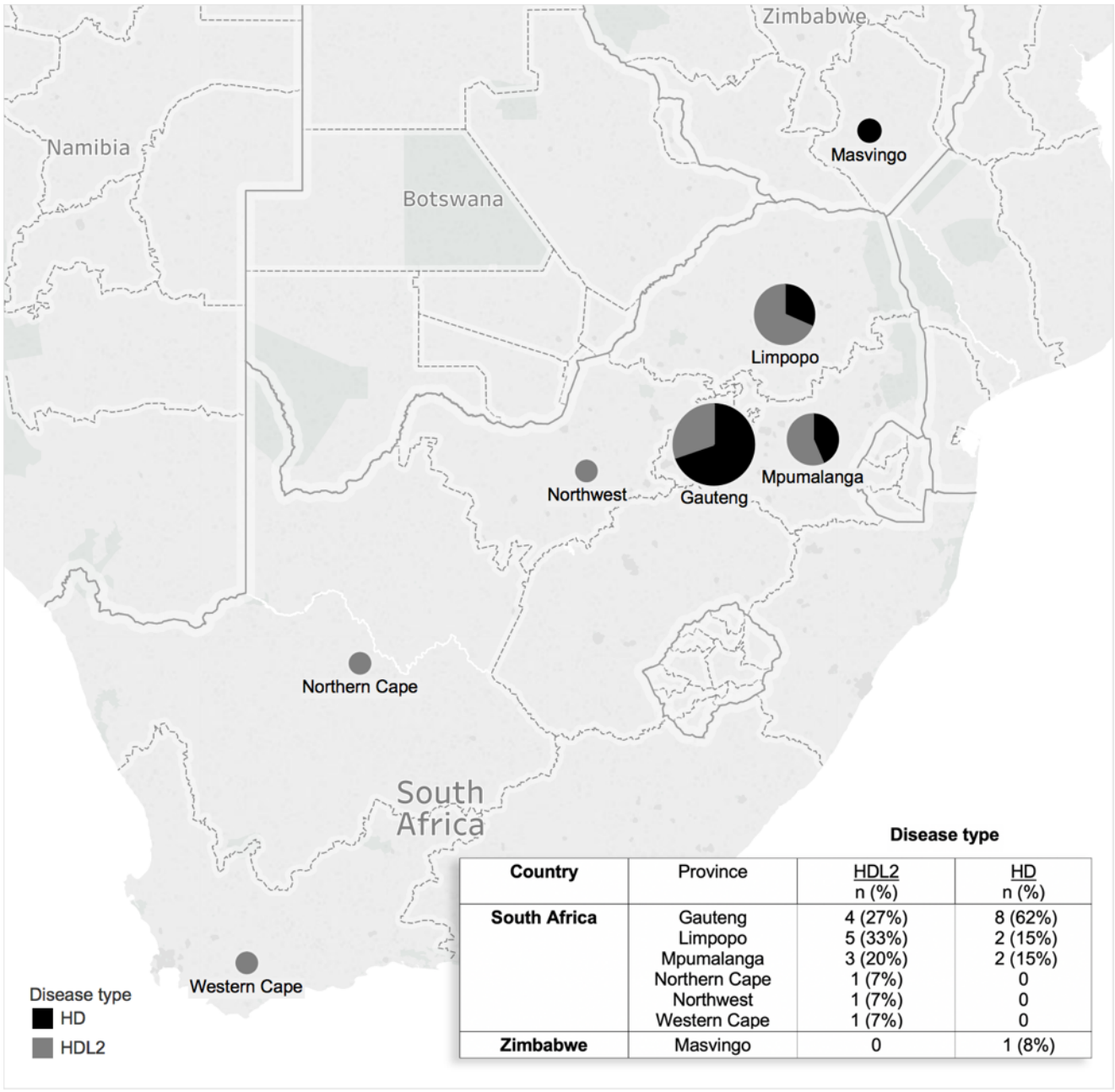

Country	Province	HDL2 n (%)	HD n (%)
South Africa	Gauteng	4 (27%)	8 (62%)
	Limpopo	5 (33%)	2 (15%)
	Mpumalanga	3 (20%)	2 (15%)
	Northern Cape	1 (7%)	0
	Northwest	1 (7%)	0
	Western Cape	1 (7%)	0
Zimbabwe	Masvingo	0	1 (8%)

Figure 6.1 Distribution of Huntington's disease like 2 and Huntington's disease subjects

Map of Southern Africa showing the distribution of Huntington's disease like 2 and Huntington's disease subjects. Huntington's disease like 2 = HDL2, Huntington's disease = HD, n= number of participants.

Table 6.1 Comparison of demographic, genetic and manifesting symptom data between Huntington's disease like 2 and Huntington's Disease groups

Demographic / Genetic		HDL2 (n=15)		HD (n=13)		P value between groups
		Median	**IQR**	**Median**	**IQR**	
Abnormal triplet repeat length		46	44-50	46	42-47	0.19
Disease duration (y)		4	3-7	7	6-8	**0.029**
Age at onset (y)		41	35-50	40	28-43	0.41
Age at diagnosis (y)		47	38-54	45	34-50	0.68
Years of education		12	9-15	12	12-14	0.80
		n (%)		**n (%)**		
Gender	Female	7 (47%)		7 (54%)		0.71
	Male	8 (53%)		6 (46%)		
Race	Black	12 (80%)		6 (46%)		0.061
	Mixed	3 (20%)		7 (54%)		
Manifesting Symptom	Cognitive	3 (20%)		2 (15%)		0.57
	Movement	10 (67%)		7 (54%)		
	Psychiatric	2 (13%)		4 (31%)		

Table comparing the demographic, genetic and manifesting symptom data between HDL2 and HD groups. Note: Two-tailed significance at $p<0.05$ for p-value between groups with statistically significant findings noted in bold font. HDL2 = Huntington's disease like 2, HD = Huntington's disease, IQR = interquartile range, y = years.

Age at onset (defined as the age at which the subject initially experienced abnormal movements) was strongly associated with the abnormal repeat length in both diseases ($p<0.0001$). The slope of the relationship varied with the disease group ($p=0.022$). For every triplet increase in abnormal repeat length, the age at onset decreased by 3.2 years for the HD group, but only by 1.8 years for the HDL2 group (Figure 6.2). The estimated delay between onset and molecular diagnosis was 6.0 ± 1.6y (95% CI) for the HD group, compared to 3.3 ± 1.5y for the HDL2 group ($p=0.021$). This suggests that the time to present for medical assistance and diagnosis from the time of disease onset in the HDL2 subjects was approximately three years earlier compared to HD subjects. However, the age of disease onset is

later for a given abnormal triplet repeat length in HDL2 compared to HD subjects. The CAG repeat size on the normal allele has been shown to influence the age of disease onset in HD (Djoussé *et al.,* 2003). However, the age at onset was not significantly related to the normal triplet repeat length when controlling for either disease.

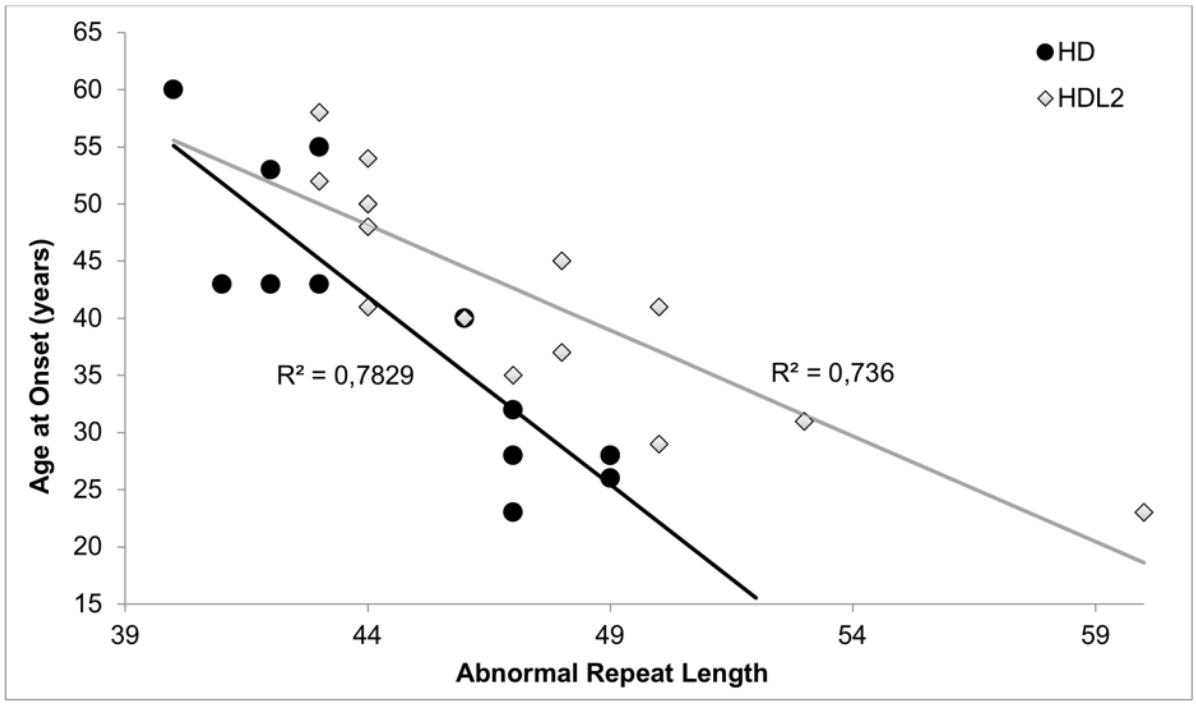

Figure 6.2 Age of disease onset vs. abnormal repeat length

Scatterplot showing inverse relationship between age of onset and abnormal triplet repeat length for both HDL2 and HD. HDL2 = Huntington's disease like 2, HD = Huntington's disease.

6.4.2 Comorbidities and concomitant medications

Two of the HDL2 and one of the HD subjects were HIV+; none of them had other infections. The HIV+ cases had MRI scans at the time of the study showing atrophic changes, in keeping with HD. The MRIs did not show any focal lesions or HIV-associated infections. One of the HDL2 HIV+ cases had a CD4 cell count below 200 cells/μL. This subject was awaiting HIV treatment. The other two HIV+ cases had CD4 cell counts of over 500 cells/μL and were receiving antiretroviral treatment. Eight HDL2 and eight HD subjects were taking dopamine antagonists. Three HD subjects and none of the HDL2 group had myoclonus. Two were brothers who also

had generalised seizures. Both brothers had an abnormal expanded HD allele with a relatively long 47 triplet repeats. The disease onset was also relatively young at 23 and 28 years of age for each subject with chorea as the manifesting symptom. However, apart from the myoclonus and seizures, they did not differ significantly from the rest of the HD cases. EEG reports for both brothers were reported as generalised spike and wave discharges, and both were treated with sodium valproate. Their MRIs at the time of the study showed typical HD changes with striatal atrophy without focal brain lesions or other radiological findings that could account for the seizures. All three reported on history that the myoclonus and seizures began after the onset of HD motor symptoms.

6.4.3 Motor phenotype

Across all subjects, the Brennan-Prediger coefficient IR for the TMS was 0.80 (95% CI = 0.70 - 0.90), in the 'substantial-to-almost-perfect' range, with an intraclass correlation (ICC) = 0.95, nearly identical to the standard set in the original development of the UHDRS (Huntington Study Group, 1996). IR ranged from moderate to near-perfect for other sub-scores, similar to previously established standards (Winder, Roos *et al.,* 2018) (Section 6.8 Supplementary data and Table 6.3).

When controlling for duration of disease, there was a significant increase in motor scores with increased disease duration in both disease groups. The rate of increase was similar between diseases (Figure 6.3). The total motor score increased by 4.4 ± 1.3 points. This did not differ significantly between disease groups ($p=0.37$). However, for a given disease duration, the HDL2 subjects had TMS scores that were higher on average although this difference did not reach statistical significance ($p=0.06$). In both disease groups, most individual component scores increased by an estimated 0.2 ± 0.1 units for every year of disease ($p<0.0001$). The chorea scores were the exception to this trend and were not higher in individuals with longer disease duration in either disease. This finding is consistent with previous reports in HD where the severity of chorea tends to plateau from the early to moderate stages of the disease (Rosenblatt *et al.,* 2011).

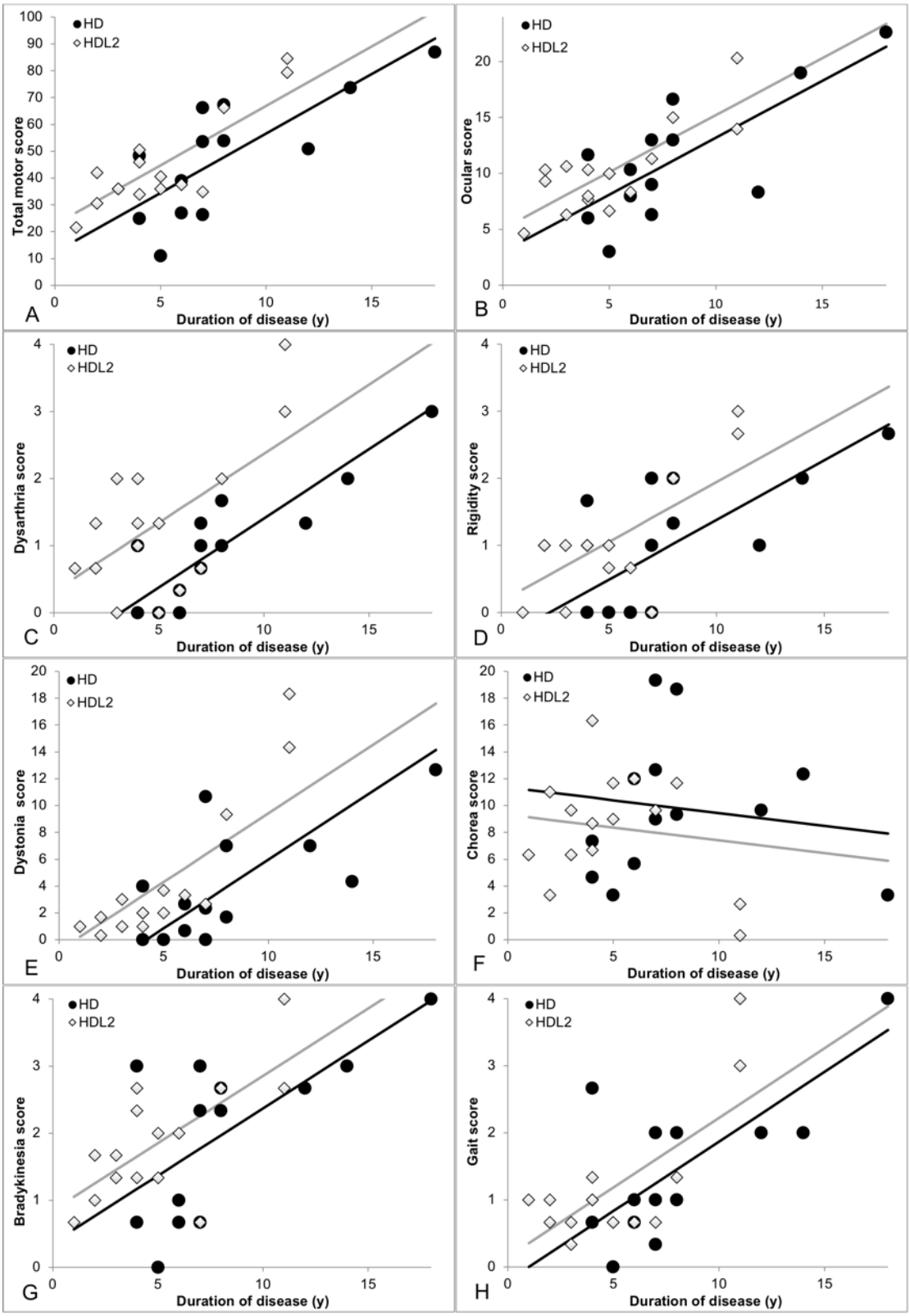

Figure 6.3 Scatterplots for Unified Huntington's Disease Rating Scale Total Motor Score and sub-scores vs. Duration of disease

Scatterplots demonstrating the TMS and some sub-scores from the UHDRS. A. Total Motor Score, B. Ocular Score, C. Dysarthria Score, D. Rigidity Score, E. Dystonia, F. Chorea, G. Bradykinesia, H.

Gait. HD = Huntington's disease, HDL2 = Huntington's disease like 2, UHDRS TMS = Unified Huntington's Disease Rating Scale Total Motor Score, y = year.

The HDL2 group on average had worse results for the combined dystonia score (t=-2.6; p=0.015), dysarthria score (t=-3.33; p=0.003) and the Luria test scores (t=-3.38; p=0.002) when compared to the HD group and controlling for duration of disease (Table 6.2.A). No other significant differences in the TMS, either overall or on any sub-score, between the two groups were found.

Blinded raters' success at assigning a diagnosis of HD or HDL2 to each case was no better than chance (p=0.28).

6.4.4 Behaviour and functional components

All three UHDRS functional assessments demonstrated increased impairment in each disease with longer disease duration, but no difference between the two diseases controlling for duration of disease (Table 6.2.B).

The behavioural component score of the UHDRS, designed to measure psychiatric symptoms in HD, did not significantly differ between the two groups and did not change with disease duration (Table 6.2.C).

6.4.5 Cognition

Cognitive scores were not significantly different between the two groups except for a higher score on the Stroop Colour-Word test for the HD subjects when controlling for covariates as described (p=0.04) (Table 6.2.D).

Table 6.2 Comparisons of the Unified Huntington's Disease Rating Scale between Huntington's disease like 2 and Huntington's disease groups

UHDRS	HDL2			HD			P value between groups
A. UHDRS-TMS	**N**	**Mean**	**STD**	**N**	**Mean**	**STD**	
Total Motor Score	15	45.1	18.0	13	48.4	22.1	0.06
	N	**Median**	**IQR**	**N**	**Median**	**IQR**	
Combined Ocular Score	15	10.0	7.7-11.3	13	10.3	8.0-13.0	0.12
Dysarthria	15	1.3	0.7-2.0	13	1.0	0.3-1.3	**0.003**
Tongue protrusion	15	1.0	0.0-2.7	13	1.3	1.0 -2.0	0.13
Combined Rigidity	15	1.0	0.7-1.0	13	1.0	0.0-2.0	0.06
Luria	15	4.0	2.3-4.0	13	2.3	1.0-3.7	**0.002**
Combined Dystonia	15	2.0	1.0-3.7	13	2.7	0.7-7.0	**0.015**
Combined Chorea	15	9.0	6.3-11.7	13	9.3	5.7-12.3	0.31
Bradykinesia	15	2.3	0.7-3.0	13	2.3	0.7-0.3	0.16
Retropulsion	15	1.7	1.0-2.0	13	2.0	2.0-2.0	0.71
Gait	15	1.0	0.7-1.3	13	1.0	0.7-2.0	0.26
Tandem gait	15	1.0	0.3-3.3	13	2.3	1.0-3.0	0.70
B. Functional Scores	**N**	**Median**	**IQR**	**N**	**Median**	**IQR**	
Functional Capacity Assessment	15	5.0	3.0-9.0	13	5.0	2.0-7.0	0.23
Functional Checklist	15	15.0	7.0-21.0	13	14.0	9.0-18.0	0.11
Independence Score	15	70	60- 90	13	70	60-90	0.05
C. Behavioural Assessment	**N**	**Median**	**IQR**	**N**	**Median**	**IQR**	
Behaviour Score	15	20.0	9.0-23.0	13	19.0	13.0-33.0	0.34
D. Cognitive Assessment	**N**	**Median**	**IQR**	**N**	**Median**	**IQR**	
MoCA	7	13	11-16	10	16.5	13-20	0.15
SDMT Written	5	14	10-20	10	15	11-21	0.82
SDMT Oral	5	20	11-24	10	17.5	10-21	0.57
% Speed/Accuracy	4	88.5	80.5-89.5	10	87.5	76-92	0.68
Stroop Word	7	44	39-81	10	52	30-61	0.60
Stroop Colour	7	19	16-50	10	26	23-35	0.60
Stroop Interference	7	7	2-12	10	18.5	15-21	**0.04**

Table comparing the UHDRS findings of the HDL2 and HD subjects. A. UHDRS Total Motor Scores - Noting some of the scores are combined, B. Functional Scores, C. Total Behavioural Score, D. Cognitive Assessments. Note: Two-tailed significance at $p<0.05$ for p-value between groups with

statistically significant findings noted in bold font. HDL2 = Huntington's disease like 2, HD = Huntington's disease, IQR = interquartile range, N = number, MoCA = Montréal Cognitive Assessment, SDMT = Symbol Digit Modality Test, STD = Standard deviation, UHDRS TMS = Unified Huntington's Disease Rating Scale Total Motor Score.

6.5 Discussion

This is the first comparison of the HDL2 and HD clinical phenotypes by blinded raters using the UHDRS. In summary, the demographic features of HD and HDL2 subjects were comparable. The results support the previously described phenotypic similarity between HDL2 and HD despite their genetic differences (Margolis *et al.,* 2001; Krause *et al.,* 2015; Anderson, Walker *et al.,* 2017) and that the diseases remain clinically indistinguishable at various points of disease duration.

When comparing the HDL2 and HD subjects using the UHDRS, all four components of the scale showed similar scores. After scoring the UHDRS TMS, blinded specialists were unable to differentiate HDL2 from HD. This has important implications for clinical practice. Clinicians should consider testing for the *JPH3* mutation in patients with African ancestry with a HD phenotype but no *HTT* expansion mutation.

Dementia is a prominent feature in HDL2. Nevertheless, identifying a more refined neurocognitive profile in HDL2 has been challenging because many subjects presenting with advanced disease are unable to complete comprehensive testing (Fischer and Licht, 2012). Although this remains to be further investigated, the present study detected no quantitative difference between HDL2 and HD except that the Stroop Colour-Word test median score for the HD subjects was significantly higher than that of the HDL2 subjects. In addition, HDL2 subjects performed worse than HD subjects on the Luria test, although this test has not been useful in differentiating different dementias (Weiner *et al.,* 2011). This suggests that the cognitive dysfunction in HDL2 may be similar to that of HD, although it introduces the possibility that HDL2 dementia may be different from HD in terms of severity and progression. Dedicated research to the neuropsychology of HDL2 is needed, as the

present study did not control for relevant neuropsychological cofounders (Watts and Shuttleworth-Edwards, 2016).

The behaviour and independence components of the UHDRS did not differentiate HDL2 from HD. The independence results worsened for both diseases with disease duration, as seen in the UHDRS cognitive and motor scores. The psychiatric measures showed no correlation with duration for either disease, a finding which has previously been described in HD (Huntington Study Group, 1996). This likely reflects the unpredictable and non-linear nature of the psychiatric syndromes in these disorders.

The motor phenotype was of particular interest to the authors given the lack of systematic descriptions from the literature and reports of motor features that have been described to differentiate HDL2 from HD. Eye movements, initially reported as relatively preserved in HDL2, were abnormal in all HDL2 cases. Abnormal eye movements were detected in subjects in the early stages of HDL2, and appeared worse with longer disease duration, mirroring the findings in HD.

Chorea was not significantly different between the HDL2 and HD groups and was present in all but one HDL2 case. In both diseases, the chorea score was the only clinical sign that did not increase with disease duration. This has been described previously in HD (Rosenblatt *et al.,* 2011).

Previous accounts of HDL2 have highlighted a higher rate of parkinsonism (Anderson, Walker *et al.,* 2017). This was not shown in this study, as bradykinesia and rigidity were similar in the two diseases. Previous descriptions of HDL2 subjects with a more parkinsonian phenotype may be attributable to evaluations performed later in the course of disease when parkinsonism is more prominent (Margolis *et al.,* 2001; Schneider *et al.,* 2012).

Myoclonus has been described in HDL2 previously (Anderson, Walker *et al.,* 2017). All cases of myoclonus from this current study were in the HD group. The distribution of subjects with myoclonus only in the HD group and none in the HDL2 group is possibly due to the small sample size. This may result in a larger margin of error and

therefore this finding may not be a true reflection of the myoclonus in HD and HDL2. The existence of generalised epilepsy in two of our adult-onset subjects is of interest as epilepsy associated with HD has only been described in the juvenile onset form of the disease (Cloud *et al.,* 2012). Both subjects with epilepsy from this study were brothers and both had 47 CAG repeat expansions in the HD locus. The disease manifested relatively in their twenties in both cases. However, phenotypically, the HD did not differ from the other HD study subject with predominant chorea and their MRI scans showed typical HD changes with no focal cause for seizures.

While most movement abnormalities were similar in the HD and HDL2 groups, the dysarthria and dystonia scores were worse in the HDL2 subjects. Dystonia is recognised as the TMS item with the poorest interrater reliability due to the subjectivity and difficultly with interpretation (Winder, Roos *et al.,* 2018). Our interrater agreement for dystonia showed substantial agreement (0.67), which is much higher than recently reported (0.34) (Winder, Roos *et al.,* 2018).

The age of disease onset in the HDL2 group was later for a given abnormal triplet repeat length compared to HD subjects. This was previously reported in a retrospective analysis of 41 HDL2 cases (Krause *et al.,* 2015). However, HDL2 subjects in this study presented for diagnosis on average three years sooner once the disease had manifested when compared to the HD subjects. This finding suggests that once the disease manifests in HDL2, there may be more prominent features resulting in these subjects seeking medical care sooner after onset. The Luria test, dysarthria and dystonia are more severe in HDL2 compared to HD as the UHDRS findings have shown in this study which may account for the HDL2 cases seeking aid earlier.

Given the similarity of HD and HDL2 phenotypically, identifying other features that HDL2 and HD share may help elucidate pathogenic pathways implicated in both diseases. Common features of triplet repeat disorders include a strong negative correlation between age of onset and the number of triplet repeats. The two groups had very similar inverse correlations (HDL2 [$R^2 = 0.74$], HD [$R^2 = 0.78$]), which has been previously noted in a systematic review of HDL2 cases (Anderson, Walker, *et al.,* 2017). Another feature that many CAG repeat disorders share is that they

manifest clinically at a threshold of 35-40 triplet repeats (Paulson, 2018). This has been shown to be the threshold that polyglutamine proteins begin to aggregate in *in vitro* studies (Brignull *et al.,* 2006). Although there is some debate as to the degree that polyglutamine proteins play in the pathogenesis of HDL2 (Seixas *et al.,* 2012), the identical number of 40 triplet repeats shared between HDL2 and HD at which the diseases manifest (Holmes *et al.,* 2001) may suggest some molecular overlap.

Current evidence suggests that the HD expansion mutation results in disease, primarily through the expression of a protein with an excessively long stretch of polyglutamine residues, with contributions from toxic properties of the mutant RNA and loss of normal protein function (Romo *et al.,* 2018). In HDL2, in which pathogenic pathways are less established, evidence suggests that loss of *JPH3* expression and toxic properties of the mutant *JPH3* RNA contribute to neurodegeneration, with a role for cryptic antisense expression of a transcript encoding a protein with an expanded stretch of polyglutamine residues (Rudnicki *et al.,* 2007; Wilburn *et al.,* 2011; Seixas *et al.,* 2012). The similarities between the two diseases genetically, clinically, pathologically and radiologically are striking and suggest common mechanisms of pathogenesis. However, it is likely that divergent aspects of their pathogenesis results in subtle phenotypic differences like dysarthria and dystonia, or more striking symptoms at onset, leading to an earlier diagnosis. We have shown in this study that differences between HDL2 and HD cannot be differentiated by examining individual subjects.

The key limitation of this study is the small sample size, reflecting the low prevalence of HDL2 even in a population in which the disease is more common, as well as the difficulty in ascertaining cases in areas with poor healthcare services (Baine *et al.,* 2016). Nonetheless, our analysis is the first systematic comparison of HD and HDL2. While subtle differences between the diseases cannot be excluded, our results strongly support that the clinical phenotype of the two diseases is indistinguishable in the clinical setting.

A possible limitation could be the inclusion of mixed ancestry subjects into the analysis as a way of introducing other genetic modifying variables into the study. However, HDL2 has been exclusively reported in black and mixed ancestry subjects

around the world (Krause *et al.,* 2015; Anderson, Walker *et al.,* 2017; Walker *et al.,* 2018). In Johannesburg, a significant proportion of the cases with HDL2 have mixed ancestry (Krause *et al.,* 2015). Therefore, the mixed ancestry group represents an important subsection of the HDL2 phenotype. Excluding this group would not be a true reflection of the HDL2 phenotype and furthermore, this would have reduced the number of cases available to study.

Another potential limitation of the study was the inclusion of HIV+ participants, however, there were important reasons for their inclusion. Firstly, the HIV+ cases are representative of this population as South Africa has the highest prevalence of HIV in the world, with seven million people infected (Statistics SA, 2017). Secondly, the HD phenotype has not been described as different in HIV from previous studies, although HD may manifest earlier in the presence of HIV (Schultz *et al.,* 2018). HIV-associated neurocognitive disorders are common in HIV. Therefore, the three HIV+ cases from this study were excluded from the cognitive analysis (Sacktor *et al.,* 2016). Comparatively, HIV-associated movement disorders are rare, affecting 2-3% of cases. Chorea and parkinsonism related to HIV often have secondary causes like toxoplasmosis or cerebral infarcts (Tse *et al.,* 2004), which were not present in these cases as evidenced by the MRI findings of the HIV+ cases.

6.6 Conclusion

Our results indicate that, like HD, HDL2 is heterogenous in its clinical phenotype and is characterised by dementia, chorea and oculomotor abnormalities as the manifesting symptoms, with progression to a rigid and bradykinetic state with worsening dystonia. However, compared to HD, HDL2 subjects presented three years earlier for diagnosis from the time of the disease manifesting, had poorer scores on dysarthria and dystonia testing and had worse results on the Luria and Stroop interference tests. These differences may suggest a more severe course in HDL2 compared to HD. However, this did not help in distinguishing the two diseases when subjects were assessed by blinded examiners. We therefore suggest testing for HDL2 if the HD mutation is negative in a subject with African ancestry.

Previous studies have shown the UHDRS to be a sensitive tool to measure motor changes in HD. We have now demonstrated that HDL2 and HD share the same clinical features and that their motor phenotypes are indistinguishable. Thus, with the current evidence, developing a separate rating scale for HDL2 appears unnecessary. Therefore, we suggest that the UHDRS, once further validated, may be a useful method of monitoring HDL2 longitudinally and for determining treatment outcomes, as it has been used for HD.

Finally, the findings of this study support common pathogenic pathways between HD and HDL2 which remain unknown. Detecting these pathogenic mechanisms could assist in identifying therapeutic targets for both diseases.

6.7 Acknowledgements

First and foremost, we would like to thank the patients and their families for their participation in this study. We would also like to acknowledge and thank Dr Petra Gaylard (DMSA) for the statistical analysis of the data and Marianne Gomes for co-ordinating and counselling the subjects and their families.

6.8 Supplement: Interrater reliability for the Unified Huntington's Disease Rating Scale comparing the motor scores of the Huntington's disease like 2 and Huntington's disease groups

Interrater agreement for individual dimensions of the TMS for all three raters, and each pair of raters, was estimated using the Brennan-Prediger coefficient of agreement for ordinal data, with ordinal weights. Interrater bias was assessed by the Stuart-Maxwell test. Interrater agreement for the total chorea, dystonia, ocular and overall scores for all three raters, as well as pairs of raters, was determined using the Brennan-Prediger coefficient of agreement for interval data, with linear weights. For the latter group of scores, the intraclass correlation coefficient (ICC) was also

calculated for comparison to the literature (Huntington Study Group, 1996). The magnitude of the IR statistics was interpreted according to the classification by Landis and Koch (1977).

The Brennan-Prediger coefficient of agreement was chosen to test the interrater reliability of the Total Motor Score (TMS) component of the Unified Huntington's Disease Rating Scale (UHDRS) amongst raters because it can be used for ordinal as well as continuous data whereas the intraclass correlation coefficient (ICC) can only be used for continuous data (Gwet, 2014).

The findings were compared with the original UHDRS reliability results that are available from the Huntington Study Group and some of the results from an interrater reliability study of the UHDRS TMS certification data (Supplementary Table) (Huntington Study Group, 1996; Winder, Roos *et al.,* 2018). The ICCs are higher than the Brennan-Prediger coefficients but follow the same trend. The overall ICC scores in our study were slightly higher than those from the Huntington Study Group and Winder, Roos *et al.* (2018).

The Brennan-Prediger coefficient of interrater reliability for the UHDRS TMS showed substantial agreement overall. Bradykinesia and the combined chorea scores were the only two that had the lowest agreement amongst raters. There was no systematic evidence of any pair of raters obtaining higher interrater reliability than any other pair of raters, nor was there any systematic evidence of interrater bias.

In a recent study investigating the interrater reliability of the UHDRS TMS from the online certification programme for Enroll-HD, dystonia and chorea were found to have lower agreement scores (Winder, Roos *et al.,* 2018). Bradykinesia has had the lowest reliability among all the items of the Unified Parkinson's Disease Rating Scale (UPDRS), with agreement scores of less than 0.5. This resulted in a modified bradykinesia rating scale, separating out its components and therefore reducing rater errors (Heldman *et al.,* 2011). Revaluation of the aforementioned components of the UHDRS TMS has been suggested (Winder, Roos *et al.,* 2018). However, we have demonstrated high levels of agreement with our interrater reliability coefficients and patterns that match both the Huntington Study Group (1996) and Winder, Roos *et al*.

(2018). This is reassuring and validates our results and the HD group as an appropriate control.

Table 6.3 Supplementary table comparing interrater reliability scores for the UHDRS TMS across different studies

	Current study		(Huntington Study Group, 1996)	(Winder, Roos *et al.,* 2018)
	BP (95% CI)	**ICC**	**ICC**	**ICC (95% CI)**
Total score	0.80 (0.70-0.90)	0.95	0.94	0.85 (0.77-0.93)
Combined dystonia score	0.67 (0.45-0.89)	0.77	0.62	0.37 (0.18-0.56)
Combined chorea score	0.54 (0.11-0.97)	0.67	0.62	0.67 (0.52-0.83)
Combined ocular score	0.68 (0.48-0.88)	-	Not published	Not published
Dysarthria	0.90 (0.84-0.96)	-	Not published	0.45 (0.21-0.70)
Tongue protrusion	0.91 (0.84-0.96)	-	Not published	0.27 (-0.03-0.56)
Rigidity right arm	0.87 (0.74-1.00)	-	Not published	0.60 (0.51-0.68)
Rigidity left arm	0.90 (0.74-1.00)	-	Not published	0.39 (0.17-0.61)
Finger tap right	0.85 (0.73-0.97)	-	Not published	0.41 (0.11-0.71)
Finger tap left	0.80 (0.53-1.00)	-	Not published	0.57 (0.31-0.82)
Pronate/supinate right	0.88 (0.77-0.97)	-	Not published	0.60 (0.37-0.82)
Pronate/supinate left	0.83 (0.67-0.99)	-	Not published	0.71 (0.55-0.88)
Luria test	0.82 (0.58-1.00)	-	Not published	0.48 (0.19-0.77)
Bradykinesia	0.56 (0.03-1.00)	-	Not published	0.59 (0.45-0.72)
Retropulsion pull test	0.95 (0.91-1.00)	-	Not published	0.71 (0.52-0.88)
Gait	0.89 (0.77-1.00)	-	Not published	0.64 (0.42-0.86)
Tandem walking	0.79 (0.54-1.00)	-	Not published	0.82 (0.69-0.96)

Table comparing the interrater reliability for the UHDRS TMS scores using the Brennan-Prediger coefficient and Intraclass Correlation Coefficients from the current study with the Huntington Study Group (1996) and Winder, Roos *et al.* (2018). Interpretation: Slight agreement = 0.00–0.20, Fair agreement = 0.21– 0.40, Moderate agreement = 0.41–0.60, Substantial agreement = 0.61–0.80, Almost perfect agreement = 0.81–0.99 (Gwet, 2014). BP = Brennan-Prediger coefficient, CI = Confidence interval, ICC = Intraclass correlation coefficient, UHDRS TMS, Unified Huntington's Disease Rating Scale Total Motor Score.

SECTION III

Section III consists of Chapter 7.

- Chapter 7 Discussion and Conclusion

CHAPTER 7

Discussion and Conclusion

This chapter summarises the important findings from Section II and discusses how this research has contributed to the understanding HDL2 clinical phenotype. Particular attention has been paid to the movement disorder of HDL2, together with the cognitive, haematological and radiological features. The demographic aspects of HDL2 are described with reference to the disease phenotype. The emergent themes from this research are then deliberated, including how the findings may relate to the pathogenic pathways of HDL2. The strengths and limitations of the study are then presented. This chapter concludes by describing avenues of potential future research which may ultimately lead to the treatment of people living with HDL2.

7 Discussion and Conclusion

7.1 Summary and consolidation of the study findings

The intention of this study was to clarify the clinical features of HDL2 and substantiate the reported resemblance of the HDL2 phenotype to that of HD. In order to achieve this, specific research objectives were defined, and the methodology described in Chapter 2 was implemented. The results from this study are presented in each of the published papers in Section II, Chapters 3 to 6. Each objective is presented below and the degree to which these were achieved is subsequently discussed.

7.1.1 Objective 1

The objective, to review the literature and determine the current description of the Huntington disease like 2 phenotype, was addressed in the published paper in Chapter 3 – A Systematic Review of the Huntington disease Like 2 Phenotype.

7.1.1.1 Key findings from the systematic literature review of Huntington disease like 2 (Chapter 3)

- The clinical descriptions of 69 unique HDL2 subjects were reported between 2001 and 2016.
- All subjects had African ancestry.
- Clinical signs of the subjects were inconsistently and incompletely reported.
- Many of the clinical features of HDL2 subjects were found to be similar to those of HD subjects, specifically:
    - Chorea - 48 out of 57 subjects
    - Dementia - 50 out of 51 subjects
    - Parkinsonism - 26 out of 26 subjects
    - Psychiatric features - 44 out of 47 subjects
    - A strong negative correlation between repeat length and age of onset.

- HDL2 patients were reported to have less oculomotor dysfunction (8 out of 19 subjects) and less dystonia and dysarthria than HD patients.
- Acanthocytes were detected in 4 of the 13 subjects tested.
- Nineteen out of 20 MRIs of HDL2 patients reported findings similar to HD.

7.1.1.2 Objective 1: Outcome

The systematic review consolidated all published cases of HDL2 and found clinical descriptions of HDL2 were retrospective, from single cases or case series. The. This review found that the HDL2 phenotype has not been systematically reported and the absence of clinical signs in HDL2 cases was generally not noted. Therefore, the findings from this review supported the need for a thorough evaluation to clarify the clinical, radiological and haematological features of the HDL2.

7.1.2 Objective 2

The objective to determine the frequency of acanthocytes in Huntington disease like 2 subjects was addressed in the published paper in Chapter 4 – Absence of Acanthocytosis in Huntington's Disease Like 2: A Prospective Comparison with Huntington's Disease.

7.1.2.1 Key findings from the haematological study of Huntington disease like 2 (Chapter 4)

- None of the HD or HDL2 participants had acanthocytosis when compared to normal controls in a blinded study.
- None of the HD or HDL2 subjects had evidence of an abnormal band 3 on red blood cell membrane analysis. Abnormal band 3 proteins are an associated feature of neuroacanthocytosis (Kay, 2004).

7.1.2.2 Objective 2: Outcome

Neither acanthocytes nor band 3 abnormalities were found in HDL2 subjects, contrary to previous reports in the initial HDL2 literature (Walker, Rasmussen *et al.,* 2003). This suggests that screening for acanthocytes will not help differentiate between HDL2 and HD subjects, nor will it assist in the diagnosis of HDL2. Furthermore, these findings suggest that HDL2 should not be classified with the neuroacanthocytosis syndromes without additional evidence, and that analysis of red blood cell membranes is unlikely to provide insight into the disease pathogenesis.

7.1.3 Objective 3

The objective of this study was to use structural MRI to quantitatively compare regional brain volumes of Huntington disease like 2 and Huntington disease subjects and was the focus of the published paper in Chapter 5 – Emerging differences between Huntington's Disease Like 2 and Huntington's Disease: A comparison Using MRI Brain Volumetry.

7.1.3.1 Key findings from the radiological study of Huntington disease like 2 (Chapter 5)

- Overall, volume loss was greater in subjects with HDL2 and HD compared to the control group.
- The distribution of volume loss was generally similar in the HDL2 and HD groups.
- Volume loss of the thalamus was greater in HDL2 compared to HD subjects.

7.1.3.2 Objective 3: Outcome

The MRI volumes of most structures of HDL2 and HD brains were similar when quantitatively compared, therefore supporting MRI findings from previous qualitative and anecdotal reports. However, the thalamic volumes of the HDL2 groups were significantly smaller than the HD groups. The smaller thalamic volumes in HDL2

could potentially differentiate HDL2 from HD and may have phenotypic and pathogenic significance.

7.1.4 Objective 4

The objective of this study was to define systematically the clinical phenotype of HDL2 and compare it to that of HD, with particular attention paid to movement disorder. This objective was addressed in the published paper in Chapter 6 – Comparison of the Huntington's Disease Like 2 and Huntington's Disease Clinical Phenotypes.

7.1.4.1 Key findings from the clinical comparison of the HDL2 and HD phenotype study (Chapter 6)

- The groups were similar in terms of age, gender and overall disease characteristics.
- The HDL2 group presented for diagnosis three years earlier than the HD group when controlling for age of onset of disease.
- Examiners, blinded to the subjects' diagnosis, could not distinguish between the two disease after reviewing the motor features of individual participants.
- No significant differences in the UHDRS Total Motor Score (TMS) were found between subjects with HDL2 and HD.
- Notable findings in the UHDRS motor sub-scores:
    - Dysarthria, dystonia and the Luria test were more severe in HDL2.
    - Oculomotor dysfunction was indistinguishable between subjects with HDL2 and HD.
- In both disorders, disease duration correlated with motor scores except the chorea score.
- The motor phenotypes of HDL2 and HD were clinically indistinguishable on the UHDRS when administered by blinded raters. Therefore, since all four components of the UHDRS were able to detect and measure the clinical features of HDL2, developing a separate rating scale for HDL2 may not be necessary.

- Psychiatric and cognitive scores were not significantly different between the two groups except the HDL2 group had lower Stroop Colour-Word test scores.
- UHDRS functional, independence and behavioural scores were similar when compared between the two diseases.

7.1.4.2 Objective 4: Outcome

Aspects of the HDL2 phenotype were clarified by this research and demonstrated that a clinician is unlikely to be able to differentiate between individual patients with HDL2 or HD on examination. This is because firstly, experienced raters blinded to the subject's diagnosis could not differentiate between an individual subject with HDL2 or HD after observing the participant and then rating the UHDRS. Secondly, when the UHDRS was applied, the HDL2 and HD groups were generally found to have similar features across all four components of the scale. Some differences did emerge when comparing the UHDRS sub-scores between the two disease groups. The Luria test, dystonia and dysarthria scores of the UHDRS motor component were worse in the HDL2 group, as were the Stroop Colour-Word test scores in the cognitive component. Furthermore, HDL2 subjects presented three years earlier for diagnosis than the HD group. These findings would suggest greater severity in the HDL2 group. However, these clinical features did not help to differentiate between the two diseases in the individual subject because they are shared between HDL2 and HD and the degree of severity cannot be discerned when examining an individual patient. These findings may have relevance in future when the pathogenesis of both diseases is studied further. This research supports the use of the UHDRS as an instrument to measure and assess HDL2 clinically, similarly to the way that it has been applied in HD.

7.2 The evolving understanding of the Huntington's disease like 2 phenotype

This section will give an overview of the study findings and how they relate to the HDL2 literature. The analysis of this study has been primarily concerned with the clinical features of the disease. Therefore, this discussion will not be a comprehensive description of the demographic and epidemiological characteristics of HDL2 although it will highlight these aspects when they are relevant or contribute to the phenotype of HDL2. The discussion regarding the cognitive aspects of HDL2 is limited to the findings in the UHDRS as a more extensive examination of the HDL2 cognitive profile forms the basis of another PhD research project performed in parallel to this study.

One of the study goals was to explore systematically whether HDL2 could be clinically differentiated from HD. The findings presented here strongly suggest that an individual patient with HDL2 cannot be distinguished from one with HD in a clinical setting. These findings are compatible with previous reports (Margolis *et al.,* 2001) where the two disorders could not be distinguished based upon clinical features alone. It was only when genetic testing was available that these phenotypically similar diseases were found to have different genotypes (Holmes *et al.,* 2001). Although many of the findings from this study are broadly in keeping with the current view that HDL2 is the disease with the greatest clinical resemblance to HD, there are several areas in which the two diseases differ. Table 7.1 summarises the relevant and specific phenotypic signs and features, highlighted in the systematic literature review in Chapter 3, that have been assigned to the HDL2 phenotype as 'characteristic' and that have now been objectively examined in this study.
These are discussed in detail in the following section.

Table 7.1 Review of the HDL2 phenotype: Relevant haematological, radiological and clinical signs reviewed in this study

Relevant sign/feature	Chapter 3: Literature review - Description of clinical sign or feature from the literature	Chapter in which the relevant sign or feature is addressed	Finding from this study
	Mean (SD, range)		Mean (SD, range)
Repeat size	48 (SD=5.1, Range=39-59)	Chapter 6	47 (SD=4.6, Range=43-60)
Age of disease onset (y)	41 (SD=11.2, Range=12-66)	Chapter 6	42 (SD=10, Range=23-58)
Duration of disease (y)	9 (SD=5.1, Range=1-22)	Chapter 6	5 (SD=3.1, Range=1-11)
Haematology			
Acanthocytes	• 4 of 13 subjects had acanthocytes Classified as neuroacanthocytosis syndrome in some reports and reviews.	Chapter 4	None of the 12 HDL2 subjects had acanthocytes in a blinded controlled study
Radiology			
MRI	• 19 of 20 HDL2 MRIs reported as qualitatively indistinguishable from HD.	Chapter 5	• Volumetric image analysis of 9 HDL2 and 11 HD MRIs showed similar volume loss. • The HDL2 thalamic volumes were significantly smaller compared to those in HD subjects.

Relevant sign/feature	Chapter 3: Literature review - Description of clinical sign or feature from the literature	Chapter in which the relevant sign or feature is addressed	Finding from this study
Clinical Signs			
Phenotype	• Two HDL2 phenotypes variants: 1. Early onset form which is more parkinsonian and lacks ocular motor involvement 2. Late onset variant which is more typical of HD with the typical jerky horizontal saccades.	Chapter 6	• Heterogenous clinical phenotype characterised by dementia, chorea and eye movement abnormalities as the manifesting motor symptoms, with progression to a rigid and bradykinetic state with worsening dystonia.
Motor Phenotype			
Oculomotor function	• Abnormal in 8 out of 19 subjects. • Potentially differentiates HDL2 from HD.	Chapter 6	• Abnormal eye movements were detected in subjects in the early stages of HDL2, and appeared more severe with longer disease duration, mirroring the findings in HD.
Dysarthria	• Dysarthria was reported in less than a third of subjects.	Chapter 6	• Dysarthria worse in HDL2 than in HD
Parkinsonism	• Parkinsonism reported in 26 subjects. However, rigidity and bradykinesia not evaluated in most subjects.	Chapter 6	• Rigidity and bradykinesia similar in HD and HDL2
Dystonia	• Mild to moderate dystonia was reported in 14 subjects.	Chapter 6	• Dystonia worse in HDL2 than in HD
Myoclonus	• Reported in 4 subjects.	Chapter 6	• No myoclonus in HDL2
Luria test	• Not reported	Chapter 6	• Luria test performance worse in HDL2 than in HD
Cognitive dysfunction	• Present in 50 of 51 reported subjects • Likely universal feature	Chapter 6	• Cognitive dysfunction is similar in HDL2 and HD
Psychiatric features	• Present in 44 of 47 subjects • Poorly described	Chapter 6	• UHDRS measures of psychiatric symptoms in HDL2 and HD are similar

7.2.1 Huntington's disease like 2: Demographics and epidemiology

7.2.1.1 Demographics

The HDL2, HD and control groups were not significantly different with regards to race, gender or age at the time of ascertainment. The HD subjects were primarily from Gauteng, an urban and wealthier province, than many others in South Africa. One subject with HD was originally from Zimbabwe and was living in Johannesburg at the time of the study. The HDL2 subjects originated from Gauteng, Limpopo and Mpumalanga provinces; the latter two are predominantly rural (Statistics SA, 2017). This study did not specifically document the income of each patient. However, all affected subjects were receiving a disability grant from the South African government except for one of the HDL2 subjects who was from Gauteng and an HD subject who was from Zimbabwe. The reason for the disproportionally higher number of HDL2 subjects from rural areas may be due to the small sample size and selection error, however, a founder effect strongest in the Limpopo and Mpumalanga regions for HDL2 could be considered.

South African rural provinces have poor health care services and therefore the HDL2 subjects likely had less access to specialist health care (Benatar, 2013; Baine *et al.,* 2016). Despite these challenges, HDL2 subjects presented three years earlier for diagnosis than the HD subjects ($p=0.021$). This finding could reflect that HDL2 is a more severe disorder and therefore it comes to attention earlier once the disease has manifested, forcing patients to seek care earlier. Evidence to support HDL2 have having some features that are more severe than HD will be discussed below in section 7.3.

The male to female gender ratio for HD is 1:1 (Bates et al., 2015). When using a Chi squared test and a 1:1 ratio the HDL2 groups gender ratio from the systematic review was similar to HD ($p<0.1$). Furthermore, HD group was not significantly different from the HDL2 groups gender ratio ($p=0.71$) in the comparative group study.

7.2.1.2 Age of disease onset

The age of disease onset in HDL2 subjects that is reported in the literature ranged from 12-66 years (mean = 41, SD = 11.2 years). In the current study, the age of disease onset in the HDL2 group ranged from 23-58 years (mean = 42, SD = 10 years) and was not significantly different from the HD group (p = 0.41). This was further confirmed when the co-efficient of determination of the HDL2 and HD cases were compared. The co-efficient of determination is the proportion of the variance in the dependant variable (the age of onset of disease) that can be predicted from the independent variable (abnormal triplet repeat length). The finding of HDL2 and HD having similar co-efficient of determinations (Anderson, Walker et al., 2017) suggests both diseases have similar ages of onset for a given abnormal triplet repeat length. Therefore, as the age of disease onset is not different between HDL2 and HD subjects, the age of the subject presenting with African ancestry and an HD clinical phenotype is not of diagnostic value.

In HD, the age of disease onset has clinical value when counseling and treating patients (Ross and Tabrizi, 2011). Evidence from the presented research and other studies support that the age of disease onset in HDL2 is similar to HD and therefore may have similar implications. Firstly, the age at onset was strongly associated with abnormal repeat length in both diseases ($p<0.0001$) as shown in Chapter 6. This association has been demonstrated before in HDL2 (Margolis *et al.,* 2001; Krause *et al.,* 2015). Secondly, the findings from this study show that there is increasing disability with disease duration in HDL2 that is not significantly different from HD ($p<0.0001$). This predictable progression from the onset of disease has been described in HD (Rosenblatt *et al.,* 2011). Therefore, counselling and planning for the care of patients with HDL2 should be similar to that in HD, which supports recommendations from Krause and Greenberg (2008).

7.2.2 Huntington's disease like 2: Motor phenotype

The motor findings from this study are broadly in keeping with the initial descriptions of the movement disorder of HDL2 (Margolis *et al.,* 2001, Walker, Jankovic *et al.,*

2003). The results presented here build on the idea that HDL2 is indistinguishable from HD in the clinical setting by demonstrating that, after observing the subjects or their video-recordings and then scoring the UHDRS motor component, experts could not differentiate between the two diseases. However, when analysing the group results for the UHDRS motor scores and comparing the two disease groups, there are important exceptions that run counter to the previously held views of the HDL2 phenotype. These are summarised in table 7.1 and discussed below.

Oculomotor function was previously reported as relatively preserved in HDL2. Therefore, this sign was proposed as one that could potentially differentiated HDL2 from HD (Margolis *et al.,* 2001; Walker, Jankovic *et al.,* 2003). In HD, oculomotor dysfunction is one of the first and most prominent features of the disease (Lasker and Zee, 1997). When directly comparing HDL2 and HD subjects in this study, abnormal oculomotor function was detected in both groups at all stages of the disease. Furthermore, the HDL2 group UHDRS eye movement scores increased with longer disease duration, indicating worsening oculomotor function with disease progression. Oculomotor function was not significantly different between the two groups. Abnormal oculomotor function is therefore unlikely to differentiate between the two diseases in a clinical setting.

Dysarthria and dystonia were previously underreported or reported as mild in HDL2 subjects, as demonstrated in the literature review. An exception to this was in the index family where these clinical features were reported as prominent (Margolis *et al.,* 2001). In this study, both dysarthria and dystonia were found to be more severe in the HDL2 subjects compared to the HD group. Although these signs were worse in HDL2, when compared between the two groups by experts blinded to the diagnosis, this degree of severity did not help to differentiate the two diseases in individuals.

HDL2 subjects also performed worse than HD subjects on the Luria test. This test is performed as part of the UHDRS motor component as it is a motor task requiring the subject to observe a sequence of three hand movements and then repeat them four times.(Huntington Study Group, 1996). However, the Luria test it is indicative of executive and frontal lobe function and is useful in measuring cognitive decline

(Weiner *et al.,* 2011) and is therefore explored further in the HDL2 cognitive section 7.2.3 below.

The hypokinetic features of HDL2 reported from this study provide important insights into the nuances of the pathophysiology and phenotype of HDL2. Parkinsonism was reported in 38% of HDL2 subjects from the literature, and it has been suggested as a dominant feature of the disease (Margolis, 2012). However, the current findings show that bradykinesia and rigidity are similar when comparing the HDL2 and HD group. These contrasting results from the HDL2 literature and this study may be reconciled to some extent when reviewing the current study findings with those from the literature especially studies that described the natural history of HD and will be discussed below.

It is important to note that hypokinesia is a prominent feature of HD even when chorea is present (van Vugt *et al.,* 1996) and it is aggravated with the use of medications that block dopamine receptors (Frank, 2013). These receptor antagonists were used in both disease groups equally and therefore were unlikely to have biased the hypokinetic features of either group.

The HD literature shows that the most important factors that affect the hypokinetic features of HD are the size of the triplet repeat length and the duration of the disease. Evidence for the repeat length and disease duration influencing HD hypokinetic features includes the longer repeat length which is associated with the juvenile parkinsonian variant of HD (van Dijk, 1985). Furthermore, there is a linear progression of hypokinetic features with a longer disease duration that corresponds with greater disability in HD (Rosenblatt *et al.,* 2011).

The findings of the current study show that the HDL2 hypokinetic features progress as chorea diminishes with increasing disease duration, similarly to HD. This was demonstrated by a significant increase in most of the UHDRS motor scores as the disease duration increased ($p<0.0001$). Specifically, bradykinesia and rigidity both increased by 0.2 per year (±0.1, CI 95%). There was no difference between the two disease groups with respect to bradykinesia ($p=0.16$) or rigidity ($p=0.06$). The notable exception to the motor scores was the chorea scores, which did not increase

with disease duration. The findings in the HDL2 group of progressive hypokinetic features and less chorea with increasing disease duration, are similar to the well-established features of the HD phenotype (Rosenblatt *et al.,* 2011).

The other factor affecting the hypokinetic features of HD, namely, effect of the triplet repeat length, was established from studies that describe the natural history of HD (Rosenblatt *et al.,* 2006, Dorsey, 2013, Tabrizi *et al.,* 2013). These studies reported that longer CAG expansions are associated with earlier disease onset, more rapid progression and shorter life expectancy. However, all of these studies had a longitudinal design and required a relatively large number of participants before the effect of the length of the abnormal triplet expansion could be demonstrated. The current study did not show a relationship between the abnormal triplet repeat expansion and UHDRS score in either the HDL2 or HD groups. Specifically, this study did not demonstrate an effect from the abnormal triplet repeat length on the motor scores (UHDRS TMS [p=0.70], rigidity [p=0.83] nor bradykinesia [p=0.70]) in either the HDL2 or HD groups. The sample size of the current study may have been too small to show the association between the HDL2 hypokinetic features and the CTG/CAG expansion size. This would therefore warrant a study with more participants to address this question.

There are two studies reporting greater parkinsonism in HDL2. In the original description of the index pedigree of HDL2, Margolis *et al.* (2001) suggested that the HDL2 phenotype had more prominent bradykinesia and rigidity compared to that of HD. This family had abnormal CTG/CAG triplet repeat expansions of greater than 50 repeats, which is higher than the mean from the current study (47 triplet repeats, SD=4.6, range 43-60) or from the HDL2 literature (48 triplet repeats, SD=5.1, range 39-59). The second study reporting greater parkinsonism in HDL2 described five subjects with parkinsonism, four of whom had a disease duration of ten years or more. All of these subjects had reported CTG/CAG expansions of 51 triplets or greater (Schneider *et al.,* 2012). These findings from the literature therefore are not inconsistent with the findings from this study and can now be viewed as a point in the spectrum of the HDL2 phenotype where bradykinesia and rigidity might be expected to become more severe as the disease progresses. This is because these subjects were generally reported after a longer disease duration.

The previously described view of HDL2 having two motor phenotypes is not supported by the findings of the current study. It was initially suggested that subjects with HDL2 either have an early onset more hypokinetic form without oculomotor involvement or a variant that that has a later onset and is more typical of HD (Margolis *et al.,* 2001). However, the current study found a strong correlation between the HDL2 motor findings and the duration of the disease suggesting that the HDL2 phenotype falls within a spectrum in a similar way to HD. Reports of the natural history of HD (Rosenblatt *et al.,* 2006; Dorsey, 2013) describe the HD phenotype as a spectrum which is influenced predominantly by the length of the triplet repeat expansion and disease duration. The findings from this study suggest that HDL2 has a similar clinical phenotype which is characterised by dementia, with chorea and oculomotor abnormalities as the initial motor symptoms. With longer disease duration there is progression to a rigid and bradykinetic state with worsening dystonia. Therefore, HDL2 is indistinguishable from HD in a clinical setting (Ross *et al.,* 2014).

The initial description of HDL2 further supports it having a phenotype which falls within a spectrum that is similar to that of HD (Margolis *et al.,* 2001). The proband from the index pedigree had a triplet expansion of 50 repeats and was described as an African American woman who initially had involuntary movements from the age of 26 years. These movements progressed to more prominent chorea by the age of 32 years and by the age of 37 she had predominant rigidity with little chorea.

The presented study has therefore provided some clarity of the overall HDL2 motor phenotype and specific HDL2 features. These motor features are the oculomotor dysfunction, dysarthria, dystonia and the hypokinetic features. Furthermore, using the HDL2 hypokinetic features as an important example, this study suggests that the HDL2 phenotype falls within a range which is strongly influenced by disease duration and abnormal triplet repeat length. Most of the HDL2 motor features are similar to HD with some differences. The HDL2 group had significantly worse results for the three sub-scores of dysarthria, dystonia and the Luria test. Interpreting this finding is limited due to the small sample. However, in conjunction with other results from this research that will be discussed in section 7.3.1 of this chapter, these motor findings

may be the initial clinical evidence for different pathogenic mechanisms between HDL2 and HD.

7.2.3 Huntington's disease like 2: Cognition

The findings from this study and from the literature support dementia as a cardinal feature of HDL2. The dementia of most subjects with HDL2 has been reported as similar to HD (Fischer and Licht, 2012). It should be noted, however, that published reports often omit cognitive assessments or the description of the dysfunction is limited because subjects presented late, with advanced disease and were unable to be formally evaluated. When the HDL2 and HD subjects from this study were compared using the UHDRS, their cognitive profiles were very similar. The UHDRS cognitive component is designed to measure progression of the dementia of HD and here showed no quantitative difference between HDL2 and HD except that the Stroop Colour-Word test median score for the HD patients was significantly higher than that of the HDL2 patients. The Stroop Colour-Word test is an extensively used instrument that indirectly measures executive function (Scarpina and Tagini, 2017). In addition, HDL2 subjects performed worse at the Luria test than the HD subjects. The Luria test is a measure of praxis and frontal lobe function that has not been useful in differentiating between different forms of dementia, but is sensitive to dementia progression (Weiner *et al.,* 2011). Therefore, this finding of worse Luria test scores and Stroop Colour-Word results in the HDL2 subjects compared to the HD subjects may reflect pathogenic differences.

This is an interesting result especially given the similar demographic profiles between the groups. However It is important to note that due to the diversity among the participants in terms of age, quality of education and English proficiency, which are significant covariates in cognitive performance, these results should be taken cautiously until our results from the single-case comparisons to a matched control group are available (Watts and Shuttleworth-Edwards, 2016). The preliminary finding from this study suggests HDL2 has a heterogeneous impact on cognitive function with preferential damage in the subcortical and frontal regions (Ferreira-Correia, 2019).

7.2.4 Huntington's disease like 2: Haematology

HDL2 was designated as one of the neuroacanthocytosis syndromes based on four subjects that were described shortly after the disease was recognised (Walker *et al.,* 2002; Walker, Rasmussen *et al.,* 2003; Walker, Jankovic *et al.,* 2003). Neuroacanthocytosis syndromes are a group of neurodegenerative genetic disorders characterised by atrophy of the basal ganglia and a movement disorder with acanthocytes on blood smears (Danek and Walker, 2005). Since the four subjects with acanthocytes were described, HDL2 has consistently appeared in haematology text books and reviews of neuroacanthocytosis syndromes (Jung *et al.,* 2011; Hoffman *et al.,* 2013; Adjobo-Hermans *et al.,* 2015).

Three of the HDL2 cases reported with acanthocytes were related, one of whom had Fragile X syndrome and the other two affected members of this family had triplet repeat numbers in the premutation range at the Fragile X locus (Walker *et al.,* 2002). Acanthocytes have not been reported in Fragile X syndrome. The relevance of Fragile X syndrome in these three related HDL2 subjects is unknown however the presence of two triplet repeat disorders in one family is noteworthy.

The finding of acanthocytes on blood smear has a high false positive rate due to artifacts produced in preparing the slides (Storch *et al.,* 2005). The current study was designed to address these technical errors and when comparing the number of acanthocytes in the blood smear samples between the groups there was no significance difference ($p=0.29$). Although the current cohort was small, other anecdotal reports from studies without controls have not found acanthocytes in HDL2, thus supporting the findings in Chapter 4 (Bardien *et al.,* 2007; Rodrigues *et al.,* 2008; Mariani *et al.,* 2016 Vasconcellos *et al.,* 2017).

The absence of acanthocytes in HDL2 subjects from this research should be seen as relevant for several reasons. Firstly, until there is further evidence, HDL2 should no longer be designated as one of the neuroacanthocytosis syndromes. Secondly, this highlights the importance of verifying phenotypic features of diseases even if a condition is rare. Thirdly, the analysis of red blood cell membranes is unlikely to be a

fruitful approach in understanding the HDL2 pathogenesis, especially given that *JPH3* expression is largely limited to the brain and testis in humans (Nishi *et al.,* 2000). Lastly, these findings do not support using screening for acanthocytes in the diagnosis of HDL2 or in differentiating HDL2 from HD.

7.2.5 Huntington's disease like 2: Radiology

The qualitative radiological findings from this study were generally compatible with previous reports of MRI scans from HDL2 subjects which found striatal volume loss similar to the changes seen in HD subjects. The blinded qualitative analysis of the MRIs, by a radiologist, reported smaller cerebral and striatal MRI volumes in the HDL2 and HD groups compared to the normal controls. Quantitative volumetric image analysis of the radiology component of this study further confirmed the similar striatal and cerebral volumes loss in the two affected groups, however, significantly smaller thalamic volumes were found in the HDL2 group compared to the HD group, with a similar trend in the putamen and caudate nuclei. The radiological findings from the current study show that smaller thalamic volumes may indicate greater atrophy in this region as volume loss on MRI has corresponded with increasing disease severity, worsening disability and greater atrophy in HD (Paulsen *et al.,* 2014). Therefore, the radiological findings from this study may similarly suggest HDL2 has greater atrophy of the thalamus although this has not been confirmed in HDL2 histopathological studies that are available (Rudnicki *et al.,* 2008).

Thalamic degeneration is now a well-established, if not prominent, feature of HD and is considered to be in keeping with the generalised atrophy of the brain (van den Bogaard *et al.,* 2011). In the TRACK HD study, the rate of thalamic atrophy followed the rate of the atrophy of the entire brain in HD (van den Bogaard *et al.,* 2011). Rüb *et al.* (2015) recently demonstrated that in HD, the thalamus is affected in specific nuclei that are associated with areas in the brain with a predilection for HD pathology, namely, the prefrontal cortex and striatum. The intralaminar centromedian-parafascicular complex of the thalamus is anatomically associated with the striatum while the mediodorsal nucleus of the thalamus is linked with the prefrontal cortex and integrated into the associative, limbic and oculomotor circuits.

These are thought to be related to cognitive, emotional and ocular motor features in HD (Rüb *et al.,* 2015). This may have clinical significance as thalamic atrophy has been shown to contribute to early executive decline in HD (Kassubek *et al.,* 2004). These reports demonstrate the growing interest in thalamic function in HD and that some clinical symptoms of HD may be influenced by thalamic dysfunction of which cognitive dysfunction may be of particular importance.

A possible limitation to this study could have been the inclusion of the HIV positive patients in the final MRI analysis as HIV has been associated the cerebral atrophy (Tesic et al., 2018). However, these were not included in the final MRI analysis as they were of insufficient quality or patients were unable to complete all the required MRI sequences.

This study did not demonstrate phenotypic features that could differentiate between individuals with HDL2 and HD. However, when combining and comparing the average between the two groups, some differences have emerged in the motor and cognitive scores, with the HDL2 group performing worse than the HD group. These poorer UHDRS results, which like the radiological findings of smaller thalamic volumes in the HDL2, may support differing pathogenic mechanisms compared to HD (Seixas *et al.,* 2012). The relationship between these findings and whether the thalamic volume may influence cognition in HDL2, as it does in HD, will require further comparative study and further radiological analysis of HDL2.

7.3 Themes emerging from this study

7.3.1 Differentiating clinically between Huntington's disease like 2 and Huntington's disease in individuals

Based on the evidence presented from this study, if an individual patient presents with African ancestry and an HD phenotype, a clinician would not be able determine whether that patient has HDL2 or HD by examination alone. This has clinical relevance and supports testing simultaneously for both diseases when faced with a subject with an HD phenotype in a population where there is a high probability of African ancestry (Krause and Greenberg, 2008).

7.3.2 Emerging differences when comparing between disease groups

The results from this study suggest that clinical features alone cannot differentiate between HDL2 and HD in an individual. However, comparing the averages between the two groups, some radiological and clinical differences have emerged. Specifically, the UHDRS scores were generally worse in the HDL2 group and the radiological finding of smaller thalamic volumes in the HDL2 subjects may suggest a greater degree of severity in HDL2. It has been suggested that HDL2 and HD likely share many pathogenic mechanisms as they are clinically very similar (Rudnicki *et al.,* 2008). However, the findings of subtle phenotypic differences when comparing the two disease groups suggests possible differences between HDL2 and HD pathogenic processes. These unique pathogenic mechanisms, such as the loss of *JPH3* function, have already been described in Chapter 1 (Seixas *et al.,* 2012).

On closer examination of the findings from Chapter 5, the MRI volumes in the HDL2 group tended to be smaller in all of the grey, white and subcortical regions when compared to HD subjects, although only the thalamus and, to some degree, the putamen volume differences reached statistical significance (Figure 5.2, Chapter 5). Significantly smaller thalamic volumes in the HDL2 group were found when

controlling for disease duration, abnormal triplet expansion length and age at disease onset. The HDL2 putamen volumes were significantly smaller than the HD groups for all covariates except when controlling for age of disease onset. The radiological similarities between HDL2 and HD are consistent with other evidence of convergent disease mechanisms, however, the novel findings from this study of smaller thalamic volumes in the HDL2 group and the emerging trend of smaller volumes in the HDL2 group overall may point to some disease mechanisms that are distinct to HDL2.

When comparing the HDL2 and HD groups using the UHDRS, further supportive evidence emerges of greater severity in the HDL2 subjects. The HDL2 and HD groups were closely matched with respect to age at diagnosis, abnormal triplet repeat length, gender and age of disease onset. The UHDRS did not assist in differentiating the two diseases in individual patients. However, when comparing the UHDRS groups scores, the HDL2 subjects generally had worse motor, functional, independence and cognitive results although statistical significance was reached for only three of the motor component tests (dystonia, dysarthria and Luria scores) and one of the cognitive components (Stroop Colour-Word test).

When controlling for the age of disease onset, HDL2 subjects presented three years sooner than in HD subjects, suggesting that the need to seek care may have been greater. Interestingly, HDL2 cases had a later disease onset than HD. This was reported in this study and has been previously described by Krause *et al.* (2015) where HDL2 subjects had a disease onset five years later than the HD group. Whether this later age of onset is due to different genetic factors, environmental influence, compensatory mechanisms or sampling error is currently speculative. However, the findings from the current study would suggests that even if HDL2 onset is at a later age, individuals with HDL2 may nonetheless seek medical attention more rapidly once the disease has manifested than patients with HD.

A shorter disease duration in HDL2 subjects in comparison to HD subjects has been previously reported by Rudnicki *et al.* (2008). This histopathology study compared a small cohort of five HDL2 cases to five HD cases. This report suggested that HDL2 may progress more rapidly than HD since there was a shorter duration of disease.

Therefore, there are now two reports of HDL2 having a shorter duration of illness, which is further supportive evidence of differences noted in the UHDRS and radiological components of this study being clinically relevant.

This study shows that HDL2 is phenotypically similar to HD supporting possible confluences of pathogenic pathways. However, some of the radiological and clinical results, together with the finding of a shorter disease duration before presenting for diagnosis, suggest that HDL2 has some unique pathogenic mechanisms and vulnerabilities compared to HD. This will be discussed in the following section (Seixas *et al.,* 2012).

7.3.3 Overlap and divergence of the Huntington's disease like 2 and Huntington's disease pathogenesis

The evidence presented in this study shows that although there are now emerging phenotypic differences between HDL2 and HD, they generally share many clinical features. Common pathogenic pathways resulting in HDL2 and HD having similar clinical features may support the findings from the current research. Some evidence of this overlap can be found in histological and animal model studies in the HDL2 literature (Margolis *et al.,* 2005; Rudnicki *et al.,* 2008; Wilburn *et al.,* 2011).

Rudnicki *et al.* (2008) reported that HDL2 is the disease with the greatest resemblance histopathologically to HD, which may suggest pathogenetic overlap between the two conditions. However, the HDL2 cohort from this histopathological study had a shorter disease duration when compared to the HD group, which suggests that the HDL2 had a more rapid progression. These findings have some resemblance to the clinical and radiological findings from the current study which suggest that there are overlapping pathogenic processes between HDL2 and HD but there may be further evidence of pathogenic mechanisms that are distinct to HDL2.

An important and consistent point of overlap in all histopathological studies of HD and HDL2 is selective cortical and striatal atrophy (Greenstein *et al.,* 2007; Rudnicki *et al.,* 2008; Seixas *et al.,* 2012). In both diseases, the greatest degeneration is

reported in the striatum. However, there are relatively few intracellular aggregates seen in this region in either disease (Rudnicki *et al.,* 2008). The mechanisms that underlie the predilection for striatal pathology that specifically target medium spiny neurons in HD are complex and are classified into two broad groups. Firstly, there are the cell-autonomous mechanisms which are the unique intrinsic susceptibilities of the medium spiny neurons. The second group consists of the non-cell-autonomous mechanisms which require communication from cell to cell and from region to region (Ehrlich, 2012). Non-cell-autonomous mechanisms involve cellular transport and molecular mechanisms from mutant HTT (mHTT), polyQ and BDNF and vulnerability of the striatum from associated cortical regions with HD pathology (Ehrlich, 2012). These latter mechanisms have prion-like qualities that have been implicated in the spread of mHTT and HD pathogenesis.

Medium spiny neurons are targeted initially in the indirect pathway, leading to a hyperkinetic movement disorder. Later in the disease, the medium spiny neurons in the direct pathway are targeted, resulting in more parkinsonism (Plotkin and Surmeier, 2015). Striatal atrophy has been noted as evidence of pathogenic overlap between HDL2 and HD by Seixas *et al*. (2012). Furthermore, previous reports have found the striatum and medium spiny neurons to be similarly affected in both HDL2 and HD (Rudnicki *et al.,* 2008). Therefore, as Seixas *et al.* (2012) have suggested, this is an important potential point of pathogenic overlap between HDL2 and HD. The pathology of the medium spiny neurons can explain many of the clinical and radiological findings from this study. Furthermore, comparisons between HDL2 and HD may reveal a better understanding of the striatal medium spiny neuron and cell-autonomous and non-cell-autonomous mechanisms of disease.

As noted, the histopathological findings of HDL and HD are very similar. However, there are some differences reported between their neuronal aggregates (Greenstein *et al.,* 2007; Rudnicki *et al.,* 2008). The aggregates in HDL2 are more frequent in the cortical layers II and III whereas in HD they are found in the deeper cortical layers V and VI (Greenstein *et al.,* 2007). Furthermore, HDL2 differs from HD in that the aggregates are absent in the pons and medulla, HDL2 aggregates form as nuclear inclusions and not as neuropil inclusions (Greenstein *et al.,* 2007; Rudnicki *et al.,* 2008), and lastly, the HDL2 aggregates do not contain HTT. Therefore, comparing

the constituents and formation of these aggregates may be key to revealing the processes unique to each disease (Seixas *et al.,* 2012).

Research has begun to address the possible pathogenic mechanisms of HDL2. These studies have been discussed in Chapter 1 and indicate some differences in the HDL2 pathogenesis compared to that of HD. Specifically, there is loss of JPH3 function in HDL2 (Seixas *et al.,* 2012) and the trinucleotide repeat expansion of *JPH3* may result in toxic RNA foci (Rudnicki *et al.,* 2008). This is a mechanism that has been found in HD. Furthermore, the polyQ strands that have been associated with HD pathology are not found in HDL2 brain samples (Seixas *et al.,* 2012) although toxic polyQ stands have been demonstrated in HDL2 animal models (Wilburn *et al.,* 2011).

Discovering the molecular mechanisms shared by both diseases is central to understanding the pathogenesis of each of these disorders. In light of the novel findings from this study, comparing genotype-phenotype correlations between HDL2 and HD may provide insight into their overlapping and divergent pathogenic mechanisms. This would need to include new *post mortem* studies because HDL2 histology reports are rare (Margolis *et al.*, 2001; Greenstein *et al.*, 2007; Rudnicki *et al.,* 2008). With the MRI findings now noted from this study, further insight into the pathogenesis of HDL2 may be found with focused examination of the thalamus in new HDL2 histopathological research. This study provides some clarity on the phenotypic similarities between HDL2 and HD and gives context and direction to future research of pathogenesis for both diseases. Figure 7.1 illustrates how this study has clarified some aspects of the HDL2 phenotype in terms of correlating genotype to phenotype.

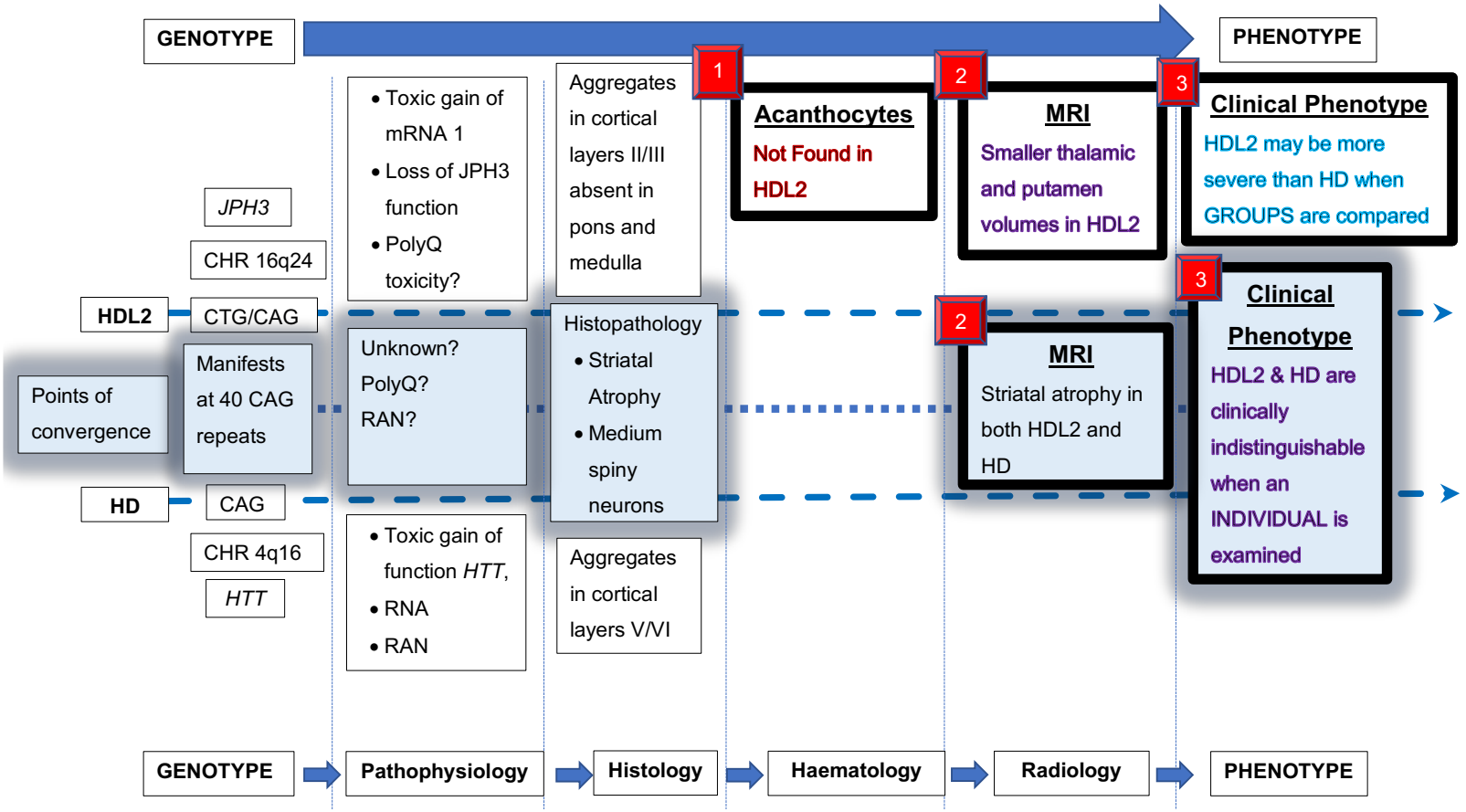

Figure 7.1 HDL2 and HD genotype-phenotype correlation in the context of the study findings

Possible points of convergence between diseases are shadowed. Components and the findings of the study are labelled: 1 - Haematology, 2 - Radiology, 3 - Clinical Phenotype. Purple script highlights novel findings from this study that contribute to the phenotype of HDL2. Scarlet script highlights findings from this study that may change previously unsubstantiated HDL2 features. Blue script highlights emerging findings that are supported by this research and warrant further study.

7.4 Strengths and limitations of the study

7.4.1 Sample population and size

HDL2 cases from the literature have either been ascertained by screening large cohorts sent for HD genetic testing or from subjects with an HD phenotype. The participants in the current research were identified from a similar genetic data base of samples sent for HD or HDL2 genetic testing and therefore this study may have a similar bias to previous HDL2 reports. Reports of subsequent generations of patients with HDL2 have described phenotypes with similar clinical features to those of HD (Margolis *et al.,* 2001; Greenstein *et al.,* 2007). The findings of the current study would suggest that the HDL2 clinical profile is generally similar to that of HD; an extended HDL2 phenotype would likely only be found by researching preclinical subjects ascertained in a prospective study.

HDL2 is a rare disorder and the prevalence of the disease is unknown. The highest number of subjects reported in the world is from the area surrounding Johannesburg. Every attempt was made to contact all the subjects recorded on the NHLS (WITS) database, however, the sample size remained small with 15 cases of HDL2 and 13 cases of HD enrolled. The limitation of the small sample size was therefore unavoidable. Interpretation of the results presented here would need to take the sample size into account (Strasak *et al.,* 2007). Nevertheless, these cases constitute the largest cohort of HDL2 subjects to have been systematically studied and collecting a sample of this size would have not been possible in another setting. The findings from this study assist in benchmarking features of the HDL2 phenotype.

7.4.2 Unified Huntington's Disease Rating Scale

The study design resulted in data being available from multiple domains, as described in Chapter 2. Where possible quantitative assessments were performed using relevant scales. Clinical rating scales have the advantage that they can be used in most locations and are inexpensive (Klempír *et al.,* 2006). In HD, the UHDRS is the most widely used rating scale and is a primary outcome measure for

therapeutic interventions in the disease (Ross and Tabrizi, 2011). This is because the UHDRS has been shown to be sensitive to motor and cognitive changes as the disease progresses (Klempír *et al.,* 2006).

The literature review found that most reported cases of HDL2 were phenotypically similar to HD and therefore the UHDRS was an appealing rating scale to use in this study. However, using the UHDRS as the single tool to compare the clinical HDL2 and HD phenotypes could potentially limit the observations in this study insofar as only HD-like motor symptoms would be identified and assessed. Consequently, a wide range of rating scales was used from the beginning of the study. These scales, which were video-recorded, included the Scale for Assessment and Rating of Ataxia (SARA) (Weyer *et al.,* 2007), the Unified Myoclonus Rating Scale (UMRS) (Frucht *et al.,* 2002) and the 'timed get up and go' test (Podsiadlo and Richardson, 2015). As previously noted, the Unified Parkinson's Disease Rating Scale (UPDRS) (Goetz *et al.,* 2008) was considered but excluded due to the considerable overlap with the UHDRS. Early in the study, it became clear that trying to assess ataxia and myoclonus using these rating scales in subjects with significant chorea and dystonia produced false positive results. These scales were performed in all cases but not included in the final analysis. For these reasons, the UHDRS was designated as the primary study instrument.

Interrater variability limits the usefulness of rating scales and was another potential concern for using the UHDRS (Mestre *et al.,* 2018). Computerised motor measuring techniques that quantitatively measure components of the UHDRS can be used to avoid rater subjectivity (Reilmann *et al.,* 2013). However, these quantitative motor assessment tools are not available in South Africa and are expensive. For these reasons, they were not used in the current study. To ensure that the interrater variability of the UHDRS motor component was within an acceptable range for our study, the interrater reliability was extensively assessed. The interrater reliability was high for the motor component of the UHDRS and considerably better than previously reported (Winder, *et al.,* 2018). The interrater reliability from this study is reported in the supplement to Chapter 6.

7.4.2.1 The UHDRS as a tool to differentiate between individuals with Huntington's disease like 2 and Huntington's disease

After observing the affected subjects and scoring the UHDRS, experienced UHDRS raters who were blinded to the participants diagnosis, could not differentiate between individuals with HDL2 or HD. The UHDRS therefore has limited diagnostic value in a clinical setting to differentiate between the two diseases. However, this aspect of the study highlights the phenotypic similarity between the two diseases.

7.4.2.2 The UHDRS as a tool to differentiate between groups with Huntington's disease like 2 and Huntington's disease

Although the UHDRS did not differentiate between individuals with HDL2 or HD, when the UHDRS scores for the groups were analysed and compared, important clinical features of the HDL2 phenotype were clarified. Oculomotor function is such an example. Furthermore, some clinical features that are shared with HD, such as dystonia, were found to be worse in HDL2. This was only seen when comparing the UHDRS scores between the two groups. The finding in the HDL2 group of worse UHDRS scores correlated with a shorter disease duration and radiological findings from this study. This study has therefore shown some evidence that the UHDRS, when used to compare the two disease groups, may be a useful instrument to differentiate between groups of patients with HDL2 and HD. A prospective study with larger cohorts would be required to substantiate these findings.

7.4.2.3 The UHDRS as a tool to assess and monitor Huntington's disease like 2

This study has demonstrated that HDL2 and HD share similar clinical features and that their motor phenotypes are indistinguishable in an individual patient. Thus, with the current evidence, developing a separate rating scale for HDL2 appears to be unnecessary. The UHDRS may be a useful method for monitoring HDL2 longitudinally and for determining treatment outcomes, as it has been for HD (Klempír *et al.,* 2006). However, this would require validation in a longitudinal study to determine if this scale is sensitive to clinical changes as the disease progresses.

7.4.3 Huntington's disease like 2 and HIV

A possible limitation was the inclusion of three HIV positive cases in the motor assessment of this study. South Africa has the highest prevalence of HIV in the world, with seven million people affected in a total population of 57 million (Statistics SA, 2017). Therefore, these three cases are representative of the population that would be seen in this setting. The HIV positive cases in this study were referred for genetic testing because they had a typical HD phenotype and history. Furthermore, subjects with HIV were present in both the HDL2 (n=2) and HD (n=1) groups. In HIV-infected individuals with HD, the clinical HD phenotype has not been reported as different when compared to those not infected with HIV, although HIV positive subjects with HD may have an earlier age of onset (Schultz *et al.,* 2018). There was no difference between the HDL2 HIV positive subjects ages of onset compared to the mean age onset of the HDL2 cases of 42.3 (SD=10 years) (p=0.97). The single HD HIV positive case had an age of onset of 43 years compared to the mean HD ages of onset of 38.6 (SD=12.2) years from this study. Furthermore, when using a one-way ANOVA there were no significant differences between the HIV positive and negative groups combined (p=0.17) or when the TMS scores of the HIV positive and negative participants where separately compared in the HDL2 (p=0.98) or HD (p>0.99) groups. These numbers are small and therefore a conclusion cannot be drawn about the age of onset from this group within this study.

The HIV positive subjects were excluded from the UHDRS cognitive analysis because neurocognitive dysfunction is found in one third of HIV positive subjects (Sacktor *et al.,* 2016). HIV-associated movement disorders are comparatively rare, affecting less than 3% of cases. Furthermore, chorea and parkinsonism that are related to HIV are usually associated with secondary focal causes such as infections or cerebral infarcts (Tse *et al.,* 2004). The MRI findings from our study found no focal lesions in the HIV positive subjects. The inclusion of these three cases therefore contributes to understanding the HDL2 and HD phenotype and did not show motor differences due to the presence of HIV.

7.5 Relevance and context

This study demonstrates that HDL2 has unique characteristics among diseases with an HD-like phenotype.

Firstly, the setting of this study provided a relatively large number of unrelated subjects in one area with this rare disorder. Most of the other HD phenocopies are not concentrated in one area or are restricted to single pedigrees (Wild *et al.,* 2008).

Secondly, this research has shown that the HDL2 phenotype is very similar to that of HD. Studying diseases with similar phenotypes could lend insight into shared pathogenic mechanisms and potential therapeutic targets (Margolis *et al.,* 2005).

7.6 Future research

Potential areas for future research in this field have emerged from this study.

7.6.1 Longitudinal comparison of Huntington's disease like 2 and Huntington's disease

The current study has established a research framework and database to monitor HDL2 and HD subjects longitudinally and address outstanding and new questions that have arisen from this research. A longitudinal examination of the HDL2 phenotype would be further enriched by including more objective measurement tools such as quantitative motor measuring techniques from the Q battery used in TRACK HD (Reilmann *et al.,* 2013; Tabrizi *et al.,* 2013) .

Validation of the UHDRS for the use in HDL2 as an outcome measure for therapeutic interventions would be an important objective in a longitudinal study of the HDL2 phenotype. If found to be sensitive to the changes of disease progression over time, the UHDRS could be established as a clinical assessment tool for HDL2. A

longitudinal study could aim to establish a radiological biomarker for HDL2 and confirm the smaller thalamic volume in HDL2 compared to HD.

A prospective longitudinal study of premanifest HD and HDL2 subjects would provide valuable information in a number of clinical and anatomical domains. Prospective evaluation of HDL2 patients may verify a more rapid presentation in HDL2 after disease onset.

Anticipation has been established in many triplet repeat disorders (Telenius *et al.,* 1994). Anticipation was reported in one HDL2 case through the paternal line (Greenstein *et al.,* 2007). In most of the HDL2 cases from this study, there was no reported preceding family history of HDL2. Therefore, the age of onset and abnormal repeat length was not available from the preceding generations to determine the existence of anticipation. A prospective study, with the use of the data from this study, could establish the behavior of the triplet repeat and if there is anticipation in HDL2.

7.6.2 The Huntington's disease phenotype in subjects with African ancestry

The prevalence and phenotype of HD in Africa is not well established, with much of the available data obtained before the availability of genetic testing (Lekoubou *et al.,* 2014; Rawlins *et al.,* 2016). Furthermore, it has been suggested that the phenotype of HD subjects with African ancestry is the same as that of Caucasians with HD (Wright *et al.,* 1981).

The initial reports available would suggest that HD prevalence in the black population is very low (Hayden *et al.,* 1980). However, there is now emerging evidence of higher frequencies of HD in this population (Baine *et al.,* 2016). Additionally, genetic differences are now emerging between HD subjects with African ancestry and Caucasian ancestry. Firstly, these HD population groups have different haplotypes (Baine *et al.,* 2013; Kay *et al.,* 2018). Secondly, allele sequence diversity within the *HTT* exon 1 repeat has been reported in HD subjects with African ancestry

which has not been found in Caucasians (Levesley *et al.,* 2018). Furthermore, in Chapter 6 we reported the unusual finding of three adult HD subjects with myoclonus, two of whom had generalised seizures. These three subjects were not typical of juvenile onset HD where myoclonus and epilepsy are more common because they did not have long CAG repeat lengths or early age of onset (van Dijk, 1985). Epilepsy in adult onset HD is rare and although this study had a small sample and is therefore not representative of the population, these three cases are unusual (Cloud *et al.,* 2012). Therefore, more research is required to know the actual HDL2 and HD prevalence in the African population, and in light of the emerging genetic differences, re-examining phenotypic differences in this population is needed.

7.6.3 Huntington's disease like 2 and Huntington's disease prevalence study

The prevalence of HDL2 and HD is unknown and epidemiolocal studies for neurodegenerative diseases in Africa are lacking (Lekoubou *et al.,* 2014). A study establishing the prevalence of both diseases is needed to determine the public health implications of the diseases and to assist in developing strategies to provide appropriate services for patients (Krause and Greenberg, 2008). The Agincourt health and socio-demographic surveillance system located in rural northeast South Africa provides an ideal setting for a pilot study of this nature. This is a defined research area with a population of approximately 90 000 people and provides a robust sampling frame and research infrastructure based on an annual update of resident status and vital events (Kahn *et al.,* 2012). Diseases where prevalence was established from this area include stroke (Connor *et al.,* 2007) and epilepsy (Ngugi *et al.,* 2013). Agincourt could therefore provide a useful setting for an HDL2 and HD prevalence study with its established infrastructure and location.

7.6.4 Neurofilament light chains (Nfl)

Recently, Nfl have been established as having potential for detecting early alterations in HD and being more sensitive than other HD biomarkers, like the UHDRS (Byrne *et al.,* 2018). Therefore, it has been proposed that Nfl measurements

be included in HD clinical trials (Wild and Tabrizi, 2017). Nfl is an intracellular structural neuronal protein that is released into the CSF with neuronal damage and can be detected in blood samples; it is not specific to HD. HDL2 has been established as phenotypically, histologically and radiologically very similar to HD and therefore the question arises whether these two diseases may overlap physiologically. Nfl is a biofluid biomarkers that is not specific to HD, whereas others such as HTT are. Therefore, Nfl provide another point of comparison between HDL2 and HD and a possible biomarker of HDL2 that could be explored.

7.6.5 Huntington's disease like 2 brain tissue research

The examination of *post mortem* brain specimens of HDL2 cases is important to establish further histopathological differences and similarities with HD in light of the findings from this research. Specifically, the MRI component findings of this study warrant closer histological examination of the thalamus in HDL2. Furthermore, future research and comparison of the intracellular aggregates of both diseases could expand the understanding of their convergent and divergent disease mechanisms.

It is challenging to obtain HDL2 *post mortem* specimens for several reasons. Thus far, subjects have died far away from centres which can perform the *post mortems.* The brains of donors need to be preserved as soon as possible after death to avoid necrosis and subsequent artifacts. Culturally, the removal of the brain has been frowned upon by many of the families which have been approached regarding *post mortems* and thus far, subjects and families have declined donating tissue. For these reasons this aspect of the research will remain challenging.

7.7 Conclusion

This study has clarified clinical, radiological and haematological features of the HDL2 phenotype. Overall, the outcomes of this study are consistent with the literature regarding the HDL2 pathology and pathogenesis. The current research has found that, whether comparing individuals and groups, HDL2 generally resembles HD phenotypically, which suggests some pathogenic overlap. However, when comparing

HDL2 and HD groups, there are emerging differences which suggest some differing pathogenic processes. These results may not have diagnostic relevance but may have implications for future research and the development of treatments. Treatment for HDL2 is symptomatic and supportive rather than aimed at the underlying cause of the disease. The current treatment strategy for HDL2 is the same as HD since the two diseases are clinically similar (Margolis, 2012). However, potentially promising disease-modifying therapies are under investigation for HD and other neurogenerative disorders that may be relevant for HDL2 (Wild and Tabrizi, 2017). These clinical trials are using well-established HD biomarkers and clinical rating scales to determine the outcomes of these therapies. If similar interventions are to be found for HDL2, sensitive biomarkers and rating scales will need to be established.

The National Center for Advancing Translational Sciences (NCATS)/Office of Rare Diseases Research (ORDR) and the International Rare Diseases Research Consortium (IRDiRC) are developing strategies that will use clinical observations in rare diseases to develop interventions that will benefit patients (Dawkins *et al.,* 2017). Applying similar strategies with the findings from this study, such as using the UHDRS as an assessment tool for HDL2, may provide tools for measuring the outcomes of therapeutic interventions in HDL2 and studying the pathogenesis of HDL2, HD and the other HD phenocopies.

The objective set out in this study, namely, to examine the HDL2 phenotype systematically, was achieved and has clarified many clinical features of the disease. This research has shown that HDL2 is remarkably similar to HD when the individual subject is examined but when the two groups are compared, HDL2 may have greater clinical severity.

Furthermore, this study has found potential clinical and neuroimaging features of HDL2 that may give reference for future HDL2 research. These findings pose questions about the pathogenic similarities and differences between HDL2 and HD. Longitudinal studies that compare HDL2 to HD phenotypically and radiologically may further explain qualitative and quantitative differences between these diseases.

Finally, my hope is that this study, and the publications stemming from it, will draw attention to the plight of people and families living with HDL2. As the world prepares for possible disease-modifying interventions for HD, ensuring that the HDL2 phenotype is better described may open opportunities for similar treatments for those living with HDL2.

8 References

Adjobo-Hermans, M.J.W., Cluitmans, J.C.A. and Bosman, G.J.C.G.M. (2015). Neuroacanthocytosis: Observations, Theories and Perspectives on the Origin and Significance of Acanthocytes, *Tremor and Other Hyperkinetic Movements*, 5, pp. 328.

Albanese, A., Bhatia, K., Bressman, S.B., DeLong, M.R., Fahn, S., Fung, V.S., Hallett, M., Jankovic, J., Jinnah, H.A., Klein, C. and Lang, A.E. (2013). Phenomenology and classification of dystonia: A consensus update. *Movement Disorders*, 28(7), pp. 863–873.

Anderson, D.G., Carmona, S., Naidoo, K., Coetzer, T.L., Carr, J., Rudnicki, D.D., Walker, R.H., Margolis, R.L. and Krause, A. (2017). Absence of Acanthocytosis in Huntington's Disease Like 2: A Prospective Comparison with Huntington's Disease. *Tremor and Other Hyperkinetic Movements,* 7: 512.

Anderson, D.G., Ferreira-Correia, A., Rodrigues, F.B., Aziz, N.A., Carr, J., Wild, E.J., Margolis, R.L. and Krause, A. (2019). Comparison of the Huntington's disease-like 2 and Huntington's disease clinical phenotypes. *Movement Disorders Clinical Practice* 12;6(4) pp. 302-311

Anderson, D.G., Haagensen, M., Ferreira-Correia, A., Pierson, R., Carr, J., Krause, A. and Margolis, R.L. (2019). Emerging differences between Huntington's disease-like 2 and Huntington's disease: A comparison using MRI brain volumetry. *NeuroImage: Clinical*, *21*, pp.101666.

Anderson, D.G., Walker, R.H., Connor, M., Carr, J., Margolis, R.L. and Krause, A. (2017). A Systematic Review of the Huntington Disease Like 2 Phenotype. *Journal of Huntington's Disease*, 6(1), pp. 37–46.

Andresen, J.M., Gayán, J., Djoussé, L., Roberts, S., Brocklebank, D., Cherny, S.S., US-Venezuela Collaborative Research Group, HD MAPS Collaborative Research Group, Cardon, L.R., Gusella, J.F. and MacDonald, M.E. (2006). The Relationship Between CAG Repeat Length and Age of Onset Differs for Huntington's Disease Patients with Juvenile Onset or Adult Onset. *Annals of Human Genetics*, 71(3), pp. 295–301.

Aoki, T.(2017). A comprehensive review of our current understanding of red blood cell (RBC) glycoproteins. *Membranes*, *7*(4), p.56.

Arbez, N., Ratovitski, T., Roby, E., Chighladze, E., Stewart, J.C., Ren, M., Wang, X., Lavery, D.J. and Ross, C.A. (2017). Post-translational modifications clustering within proteolytic domains decrease mutant huntingtin toxicity. *Journal of Biological Chemistry*, 292(47), pp. 19238–19249.

Aretouli, E. and Brandt, J. (2010). Episodic Memory in Dementia: Characteristics of New Learning that Differentiate Alzheimer's, Huntington's and Parkinson's Diseases. *Archives of Clinical Neuropsychology*, 25(5), pp. 396–409.

Arrasate, M. and Finkbeiner, S. (2012). Protein aggregates in Huntington's disease. *Experimental Neurology*, 238(1), pp. 1–11.

Arrasate, M., Mitra, S., Schweitzer, E.S., Segal, M.R. and Finkbeiner, S. *(*2004). Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature*, 431(7010), pp. 805–810.

Baeza, A.V. (2015). Chorea-Acanthocytosis. *GeneReviews® - NCBI Bookshelf*, pp. 1–24.

Baine, F.K., Kay, C., Ketelaar, M.E., Collins, J.A., Semaka, A., Doty, C.N., Krause, A., Greenberg, L.J. and Hayden, M.R. (2013). Huntington disease in the South African population occurs on diverse and ethnically distinct genetic haplotypes. *European Journal of Human Genetics*, 21(10), pp. 1120–1127.

Baine, F.K., Krause, A. and Greenberg, L.J. (2016). The Frequency of Huntington Disease and Huntington Disease Like 2 in the South African Population. *Neuroepidemiology*, 46(3), pp. 198–202.

Bañez-Coronel, M., Ayhan, F., Tarabochia, A.D., Zu, T., Perez, B.A., Tusi, S.K., Pletnikova, O., Borchelt, D.R., Ross, C.A., Margolis, R.L. and Yachnis, A.T. (2015). RAN Translation in Huntington Disease. *Neuron*, 88(4), pp. 667–677.

Bardien, S., Abrahams, F., Soodyall, H., van der Merwe, L., Greenberg, J., Brink, T. and Carr, J. (2007). A South African mixed ancestry family with Huntington disease-like 2: Clinical and genetic features. *Movement Disorders*, 22(14), pp. 2083–2089.

Barkhuizen, M., Rodrigues, F., Anderson, D.G., Wild, E.J. and Kramer, B.W. (2018). Perinatal insults and neurodevelopmental disorders may impact Huntington's disease age of diagnosis. *Parkinsonism & Related Disorders*, 55, pp 55-60

Bates, G.P., Dorsey, R., Gusella, J.F., Hayden, M.R. and Kay, C. (2015). Huntington disease. *Nature Reviews Disease Primers*, 26, pp. 15005–15021.

Benatar, S.R. (2013). The challenges of health disparities in South Africa. *South African Medical Journal*, 103(3), pp. 154–155.

Benjamini, Y. and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57(1), pp. 289–300.

Brignull, H.R., Moore, F.E., Tang, S.J. and Morimoto, R.I. (2006). Polyglutamine Proteins at the Pathogenic Threshold Display Neuron-Specific Aggregation in a Pan-Neuronal Caenorhabditis Elegans Model. *Journal of Neuroscience*, 26(29), pp. 7597–7606.

Byrne, L.M., Rodrigues, F.B., Blennow, K., Durr, A., Leavitt, B.R., Roos, R.A., Scahill, R.I., Tabrizi, S.J., Zetterberg, H., Langbehn, D. and Wild, E.J. (2017). Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's Disease: A retrospective cohort analysis. *Lancet Neurology*, 16(8), pp. 601-609.

Byrne, L.M., Rodrigues, F.B., Johnson, E.B., Wijeratne, P.A., De Vita, E., Alexander, D.C., Palermo, G., Czech, C., Schobel, S., Scahill, R.I. and Heslegrave, A. (2018). Evaluation of mutant huntingtin and neurofilament proteins as potential markers in Huntington's Disease. *Science Translational Medicine*, 10(458), p. 7108.

Castilhos, R.M., Souza, A.F., Furtado, G.V., Gheno, T.C., Silva, A.L., Vargas, F.R., Lima, M.A., Barsottini, O.R.., Pedroso, J.L., Godeiro Jr, C. and Salarini, D. (2013). Huntington Disease and Huntington Disease Like 2 in a case series from Brazil. *Clinical Genetics*, 86(4), pp. 373–377.

Caviness, J.N. and Kurth, M. (1997). Cortical myoclonus in Huntington's disease associated with an enlarged somatosensory evoked potential. *Movement Disorders*, 12(6), pp. 1046–1051.

Cloud, L.J., Rosenblatt, A., Margolis, R.L., Ross, C.A., Pillai, J.A., Corey-Bloom, J., Tully, H.M., Bird, T., Panegyres, P.K., Nichter, C.A. and Higgins Jr, D.S. (2012). Seizures in juvenile Huntington's Disease: Frequency and characterization in a multicenter cohort. *Movement Disorders*, 27(14), pp. 1797–1800.

Coetzer, T.L. and Palek, J. (1986). Partial spectrin deficiency in hereditary pyropoikilocytosis. *Blood*, 67(4), pp. 919–924.

Connor, M.D., Walker, R., Modi, G. and Warlow, C.P. (2007). Burden of stroke in black populations in sub-Saharan Africa. *Lancet Neurology*, 6(3), pp. 269–278.

Cooper-Knock, J., Shaw, P.J. and Kirby, J. (2014). The widening spectrum of C9orf72-related disease: Genotype/phenotype correlations and potential modifiers of clinical phenotype. *Acta Neuropathologica*, 127(3), pp. 333–345.

Coppen, E.M., Jacobs, M., van den Berg-Huysmans, A.A., van der Grond, J. and Roos, R.A. (2018). Grey matter volume loss is associated with specific clinical motor signs in Huntington's Disease. *Parkinsonism & Related Disorders*, 46, pp. 56–61.

Crossman, A.R. (2000). Functional anatomy of movement disorders. *Journal of Anatomy*, 196(4), pp. 519-525.

Danek, A. and Walker, R.H. (2005). Neuroacanthocytosis. *Current Opinion in Neurology*, 18(4), pp. 386-392.

Dawkins, H.J., Draghia-Akli, R., Lasko, P., Lau, L.P., Jonker, A.H., Cutillo, C.M., Rath, A., Boycott, K.M., Baynam, G., Lochmüller, H. and Kaufmann, P. (2017). Progress in Rare Diseases Research 2010-2016: An IRDiRC Perspective. *Clinical and Translational Science*, 11(1), pp. 11–20.

Djousse, L., Knowlton, B., Hayden, M., Almqvist, E.W., Brinkman, R., Ross, C., Margolis, R., Rosenblatt, A., Durr, A., Dode, C. and Morrison, P.J. (2003). Interaction of normal and expanded CAG repeat sizes influences age at onset of Huntington disease, *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 119A(3), pp. 279–282.

Dorsey, E.R. (2012). The Huntington Study Group COHORT Investigators. Characterization of a Large Group of Individuals with Huntington Disease and their Relatives Enrolled in the COHORT Study. *PLoS ONE*, 7(2), pp. e29522–10.

Dorsey, E.R. (2013). Natural History of Huntington Disease, *JAMA Neurology*, 70(12), pp. 1–11.

Dutkowski, J., Kramer, M., Surma, M.A., Balakrishnan, R., Cherry, J.M., Krogan, N.J. and Ideker, T. (2012). A gene ontology inferred from molecular networks. *Nature Biotechnology*, 31(1), pp. 38–45.

Ehrlich, M.E. (2012). Huntington's Disease and the Striatal Medium Spiny Neuron: Cell-Autonomous and Non-Cell-Autonomous Mechanisms of Disease. *Neurotherapeutics*, 9(2), pp. 270–284.

Fahn, S. (2011). Classification of movement disorders. *Movement Disorders*, 26(6), pp. 947–957.

Ferreira-Correia, A. (2019). *The Neurocognitive Profile of Huntington Disease-Like 2: A Comparison with Huntington Disease and Healthy Controls.* (PhD), University of the Witwatersrand, Johannesburg, South Africa.

Fischer, C.A. and Licht, E.A. (2012). The Neuropsychiatric Manifestations of Huntington's Disease Like 2. *The Journal of Neuropsychiatry and Clinical Neurosciences*, 24(4), pp. 489–492.

Fisher, C.A., Sewell, K., Brown, A. and Churchyard, A. (2014). Aggression in Huntington's Disease: A Systematic Review of Rates of Aggression and Treatment Methods, *Journal of Huntington's Disease*, 3(4), pp. 319–332.

Folstein, S.E., Chase, G.A., Wahl, W.E., McDonnell, A.M. and Folstein, M.F. (1987). Huntington disease in Maryland: Clinical aspects of racial variation, *American Journal of Human Genetics*, 41(2), pp. 168–179.

Franciosi, S., Shim, Y., Lau, M., Hayden, M.R. and Leavitt, B.R. (2013). A systematic review and meta-analysis of clinical variables used in Huntington disease research. *Movement Disorders*, 28(14), pp. 1987–1994.

Frank, S. (2013). Treatment of Huntington's Disease. *Neurotherapeutics*, 11(1), pp. 153–160.

Frucht, S.J., Leurgans, S.E., Hallett, M. and Fahn, S. (2002). The Unified Myoclonus Rating Scale. *Advances in Neurology*, 89, pp. 361–376.

Gage, S.H., Smith, G.D., Ware, J.J., Flint, J. and Munafò, M.R. (2016). G = E: What GWAS Can Tell Us about the Environment. In: G. Gibson, ed. *PLOS Genetics*, 12(2), p. e1005765–13.

Goetz, C.G., Tilley, B.C., Shaftman, S.R., Stebbins, G.T., Fahn, S., Martinez-Martin, P., Poewe, W., Sampaio, C., Stern, M.B., Dodel, R. and Dubois, B. (2008). Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. *Movement Disorders*, 23(15), pp. 2129–2170.

Greenstein, P.E., Vonsattel, J.P.G., Margolis, R.L. and Joseph, J.T. (2007). Huntington's Disease like-2 neuropathology. *Movement Disorders*, 22(10), pp. 1416–1423.

Gwet, K.L. (2014). *Handbook of Inter-Rater Reliability, 4th Edition*. USA: Advanced Analytics, LLC.

Harris, G., Andreasen, N.C., Cizadlo, T., Bailey, J.M., Bockholt, H.J., Magnotta, V.A. and Arndt, S. (1999). Improving tissue classification in MRI: A three-dimensional multispectral discriminant analysis method with automated training class selection. *Journal of Computer Assisted Tomography*, 23(1), pp. 144–154.

Harris, P.A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N. and Conde, J.G. (2009). Research electronic data capture (REDCap) — A metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics*, 42(2), pp. 377–381.

Hasegawa, A., Ikeuchi, T., Koike, R., Matsubara, N., Tsuchiya, M., Nozaki, H., Homma, A., Idezuka, J., Nishizawa, M. and Onodera, O. (2010). Long-term disability and prognosis in Dentatorubral-Pallidoluysian atrophy: A correlation with CAG repeat length. *Movement Disorders*, 25(11), pp. 1694–1700.

Hayden, M.R. and Beighton, P.(1977). Huntington's chorea in the Cape coloured community of South Africa. *South African Medical Journal*, *52*(22), pp. 886-888.

Hayden, M.R., MacGregor, J.M. and Beighton, P.M. (1980). The Prevalence of Huntington's Chorea in South Africa. *South African Medical Journal*, 58(5), pp. 193–196.

Hayflick, S.J., Westaway, S.K., Levinson, B., Zhou, B., Johnson, M.A., Ching, K.H. and Gitschier, J. (2003). Genetic, clinical and radiographic delineation of Hallervorden-Spatz syndrome. *The New England Journal of Medicine*, 348(1), pp. 33–40.

Heemskerk, A.W. and Roos, R.A.C. (2010). Dysphagia in Huntington's Disease: A Review. *Dysphagia*, 26(1), pp. 62–66.

Heldman, D.A., Giuffrida, J.P., Chen, R., Payne, M., Mazzella, F., Duker, A.P., Sahay, A., Kim, S.J., Revilla, F.J. and Espay, A.J. (2011). The modified bradykinesia rating scale for Parkinson's disease: Reliability and comparison with kinematic measures. *Movement Disorders*, 26(10), pp. 1859–1863.

Henley, S.M., Bates, G.P. and Tabrizi, S.J. (2005). Biomarkers for neurodegenerative diseases. *Current Opinion in Neurology*, 18(6), pp. 698–705.

Hoffman, R., Benz Jr, E.J., Silberstein, L.E., Heslop, H., Anastasi, J. and Weitz, J. (2013). *Hematology: Basic principles and practice, 6th Edition*. Chapter 43, P Gallagher, pp592-614

Hogarth, P., Kayson, E., Kieburtz, K., Marder, K., Oakes, D., Rosas, D., Shoulson, I., Wexler, N.S., Young, A.B., Zhao, H. and United States–Venezuela Huntington's Disease Collaborative Research Group.. (2005). Interrater agreement in the assessment of motor manifestations of Huntington's Disease. *Movement Disorders*, 20(3), pp. 293–297.

Holmes, S.E., O'Hearn, E., Rosenblatt, A., Callahan, C., Hwang, H.S., Ingersoll-Ashworth, R.G., Fleisher, A., Stevanin, G., Brice, A., Potter, N.T. and Ross, C.A. (2001). A repeat expansion in the gene encoding junctophilin-3 is associated with Huntington disease-Like 2. *Nature Genetics*, 29(4), pp. 377–378.

Huntington, G. (1872). On chorea. *Medical and Surgical Reporter of Philadelphia*, 26, pp. 317–321.

Huntington Study Group. (1996). Unified Huntington's Disease Rating Scale: Reliability and consistency. *Movement Disorders*, 11(2), pp. 136–142.

Huntington Study Group PHAROS Investigators. (2006). At risk for Huntington disease: The PHAROS (Prospective Huntington At Risk Observational Study) cohort enrolled. *Archives of Neurology*, 63(7), pp. 991–996.

Jankovic J. (2008). Parkinson's disease: Clinical features and diagnosis. *Journal of Neurology, Neurosurgery & Psychiatry*, 79(4), pp. 368–376.

Jung, H.H., Danek, A. and Walker, R.H. (2011). Neuroacanthocytosis Syndromes. *Orphanet Journal of Rare Diseases*, 6(1), p. 68.

Kahn, K., Collinson, M.A., Gómez-Olivé, F.X., Mokoena, O., Twine, R., Mee, P., Afolabi, S.A., Clark, B.D., Kabudula, C.W., Khosa, A. and Khoza, S. (2012). Profile: Agincourt Health and Socio-demographic Surveillance System. *International Journal of Epidemiology*, 41(4), pp. 988–1001.

Kambouris, M., Bohlega, S., Al-Tahan, A. and Meyer, B.F. (2000). Localization of the gene for a novel autosomal recessive neurodegenerative Huntington-like disorder to 4p15.3. *American Journal of Human Genetics*, 66(2), pp. 445–452.

Kassubek, J., Juengling, F.D., Ecker, D. and Landwehrmeyer, G.B. (2004). Thalamic Atrophy in Huntington's Disease Co-varies with Cognitive Performance: A Morphometric MRI Analysis. *Cerebral Cortex*, 15(6), pp. 846–853.

Kay, C., Collins, J.A., Wright, G.E., Baine, F., Miedzybrodzka, Z., Aminkeng, F., Semaka, A.J., McDonald, C., Davidson, M., Madore, S.J. and Gordon, E.S. (2018). The molecular epidemiology of Huntington disease is related to intermediate allele

frequency and haplotype in the general population. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 177(3), pp. 346–357.

Kay, M.M. (2004). Band 3 and its alterations in health and disease. *Cellular and Molecular Biology* (*Noisy-le-Grand, France)*, 50(2), pp. 117–138.

Klempír, J., Klempírová, O., Spacková, N., Zidovská, J. and Roth, J. (2006). Unified Huntington's Disease Rating Scale: Clinical practice and a critical approach. *Functional Neurology*, 21(4), pp. 217–221.

Klintworth, G.K. (1962). Huntington's chorea in South Africa: A preliminary communication drawing attention to its frequent occurrence. *South African Medical Journal*, 36(10), pp. 896-898.

Koide, R., Ikeuchi, T., Onodera, O., Tanaka, H., Igarashi, S., Endo, K., Takahashi, H., Kondo, R., Ishikawa, A., Hayashi, T. and Saito, M. (1994). Unstable expansion of CAG repeat in hereditary Dentatorubral–Pallidoluysian atrophy (DRPLA). *Nature Genetics*, 6(1), pp. 9–13.

Krause, A. and Greenberg, J. (2008). Genetic testing for Huntington's disease in South Africa. *South African Medical Journal*, 98(3), pp. 193–194.

Krause, A., Mitchell, C., Essop, F., Tager, S., Temlett, J., Stevanin, G., Ross, C., Rudnicki, D. and Margolis, R. (2015). Junctophilin 3 (JPH3) expansion mutations causing Huntington disease Like 2 (HDL2) are common in South African patients with African ancestry and a Huntington disease phenotype. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The official publication of the International Society of Psychiatric Genetics*, 168(7), pp. 573–585.

Krause, A., Temlett, J., Van der Meyden, K., Ross, C.A., Callahan, C. and Margolis, R.L. (2002). CAG/CTG repeat expansions at the HDL2 locus are a common cause of Huntington disease in black South Africans. *American Journal of Human Genetics*, 71(4), pp. 528–528.

Krench, M., Cho, R.W. and Littleton, J.T., (2016). A Drosophila model of Huntington disease-like 2 exhibits nuclear toxicity and distinct pathogenic mechanisms from Huntington disease. *Human molecular genetics*, *25*(15), pp. 3164–3177.

Kremer, B. *et al.* (1994). A Worldwide Study of the Huntington's Disease Mutation: The Sensitivity and Specificity of Measuring CAG Repeats. *New England Journal of Medicine*, 330(20), pp. 1401–1406.

Landis, J.R. and Koch, G.G. (1977). The Measurement of Observer Agreement for Categorical Data. *Biometrics*, 33(1), p. 159.

Landstrom, A.P., Beavers, D.L. and Wehrens, X.H.T. (2014). The junctophilin family of proteins: From bench to bedside. *Trends in Molecular Medicine*, 20(6), pp. 353–362.

Langbehn, D.R., Brinkman, R.R., Falush, D., Paulsen, J.S., Hayden, M.R. and an International Huntington's Disease Collaborative Group. (2004). A new model for

prediction of the age of onset and penetrance for Huntington's Disease based on CAG length. *Clinical Genetics*, 65(4), pp. 267–277.

Langbehn, D.R., Hayden, M.R., Paulsen, J.S. and PREDICT-HD Investigators of the Huntington Study Group. (2009). CAG-repeat length and the age of onset in Huntington disease (HD): A review and validation study of statistical approaches. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 153(2), pp. 397-408.

Lasker, A.G. and Zee, D.S. (1997). Ocular motor abnormalities in Huntington's disease. *Vision Research*, 37(24), pp. 3639–3645.

Lawerman, T.F., Brandsma, R., Van Geffen, J.T., Lunsing, R.J., Burger, H., Tijssen, M.A., De Vries, J.J., De Koning, T.J. and Sival, D.A. (2015). Reliability of phenotypic early-onset ataxia assessment: A pilot study. *Developmental Medicine & Child Neurology*, 58(1), pp. 70–76.

Lee, W.H., Lee, C.C., Shyu, W.C., Chong, P.N. and Lin, S.Z. (2005). Hyperintense putaminal rim sign is not a hallmark of multiple system atrophy at 3T. *American Journal of Neuroradiology*, 26(9), pp. 2238–2242.

Lekoubou, A., Echouffo-Tcheugui, J.B. and Kengne, A.P. (2014). Epidemiology of neurodegenerative diseases in sub-Saharan Africa: A systematic review. *BMC Public Health*, 14(1), p. 653.

Levesley, J., Baine, F., Ciosi, M., Maxwell, A., Monckton, D.G. and Krause, A. (2018). C03 A CIS-acting modifier of age at diagnosis of Huntington disease in black South African patients. *Journal of Neurology, Neurosurgery & Psychiatry*, 89(Suppl1). A27-A28

Li, S.H. and Li, X.J. (2004). Huntingtin-protein interactions and the pathogenesis of Huntington's disease. *Trends in Genetics*, 20(3), pp. 146–154.

Li, W., Serpell, L.C., Carter, W.J., Rubinsztein, D.C. and Huntington, J.A. (2006). Expression and characterization of full-length human huntingtin, an elongated HEAT repeat protein. *The Journal of Biological Chemistry*, 281(23), pp. 15916–15922.

Liou, S. (2010). *Huntington's Outreach Project for Education at Stanford*. [Online]. Available from: http://web.stanford.edu/group/hopes/cgi-bin/hopes_test/population-genetics-and-hd/ [Accessed October 16, 2018].

Louis, E.D., Lee, P., Quinn, L. and Marder, K. (1999). Dystonia in Huntington's disease: Prevalence and clinical characteristics. *Movement Disorders*, 14(1), pp. 95–101.

MacDonald, M. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's Disease chromosomes. *Cell*, 72(6), pp. 971–983.

Magazi, D.S., Krause, A., Bonev, V., Moagi, M., Iqbal, Z., Dludla, M. and Van der Meyden, C.H. (2008). Huntington's Disease: Genetic heterogeneity in black African patients. *South African Medical Journal,* 98(3), pp. 200–203.

Mahner, M. and Kary, M. (1997). What exactly are genomes, genotypes and phenotypes? And what about phenomes? *Journal of Theoretical Biology*, 186(1), pp. 55–63.

Mann, C.J. (2003). Observational research methods. Research design II: Cohort, cross sectional and case-control studies. *Emergency Medicine Journal*, 20(1), pp. 54–60.

Margolis, R.L. (2012). Huntington Disease Like 2. *GeneReviews® - NCBI Bookshelf*, pp. 1–14.

Margolis, R.L., Holmes, S.E., Rosenblatt, A., Gourley, L., O'Hearn, E., Ross, C.A., Seltzer, W.K., Walker, R.H., Ashizawa, T., Rasmussen, A. and Hayden, M. (2004). Huntington's Disease-Like 2 (HDL2) in North America and Japan. *Annals of Neurology*, 56(5), pp. 670–674.

Margolis, R.L., O'Hearn, E., Rosenblatt, A., Willour, V., Holmes, S.E., Franz, M.L., Callahan, C., Hwang, H.S., Troncoso, J.C. and Ross, C.A.. (2001). A disorder similar to Huntington's disease is associated with a novel CAG repeat expansion. *Annals of Neurology*, 50(3), pp. 373–380.

Margolis, R.L. and Ross, C.A. (2001). Expansion explosion: New clues to the pathogenesis of repeat expansion neurodegenerative diseases. *Trends in Molecular Medicine*, 7(11), pp. 479–482.

Margolis, R.L., Rudnicki, D.D. and Holmes, S.E. (2005). Huntington's Disease like-2: Review and update. *Acta Neurologica Taiwanica*, 14(1) pp.1-8

Mariani, L.L., Tesson, C., Charles, P., Cazeneuve, C., Hahn, V., Youssov, K., Freeman, L., Grabli, D., Roze, E., Noël, S. and Peuvion, J.N. (2016). Expanding the Spectrum of Genes Involved in Huntington Disease Using a Combined Clinical and Genetic Approach. *JAMA Neurology*, *73*(9), pp.1105-1114

Martí, E. (2016). RNA toxicity induced by expanded CAG repeats in Huntington's Disease. *Brain Pathology*, 26(6), pp. 779–786.

McColgan, P. and Tabrizi, S.J. (2017). Huntington's Disease: A clinical review. *European Journal of Neurology*, 139, *25*(1), pp.24-34

McLaren, Z.M., Ardington, C. and Leibbrandt, M. (2014). Distance decay and persistent health care disparities in South Africa. *BMC Health Services Research*, 14(1), p. 541.

Mencacci, N.E. and Carecchio, M. (2016). Recent advances in genetics of chorea. *Current Opinion in Neurology*, 29(4), pp. 486–495.

Mestre, T.A., Forjaz, M.J., Mahlknecht, P., Cardoso, F., Ferreira, J.J., Reilmann, R., Sampaio, C., Goetz, C.G., Cubo, E., Martinez-Martin, P. and Stebbins, G.T. (2018). Rating Scales for Motor Symptoms and Signs in Huntington's Disease: Critique and Recommendations. *Movement Disorders Clinical Practice*, 5(2), pp. 111–117.

Montgomery, Jr. E.B., and Gale, J.T. (2008). Mechanisms of action of deep brain stimulation (DBS). *Neuroscience & Biobehavioral Reviews*, 32(3), pp. 388–407.

Moore, R.C., Xiang, F., Monaghan, J., Han, D., Zhang, Z., Edström, L., Anvret, M. and Prusiner, S.B. (2001). Huntington disease phenocopy is a familial prion disease. *American Journal of Human Genetics*, 69(6), pp. 1385–1388.

Moss, D.J.H., Poulter, M., Beck, J., Hehir, J., Polke, J.M., Campbell, T., Adamson, G., Mudanohwo, E., McColgan, P., Haworth, A. and Wild, E.J. (2014). C9orf72 expansions are the most common genetic cause of Huntington disease phenocopies. *Neurology*, 82(4), pp. 292–299.

Moulton, C.D., Hopkins, C.W.P. and Bevan-Jones, W.R. (2014). Systematic review of pharmacological treatments for depressive symptoms in Huntington's Disease. *Movement Disorders*, 29(12), pp. 1556–1561.

Nance, M.A. and Group, U.H.D.G.T. (1997). Genetic testing of children at risk for Huntington disease. *Neurology*, 49(4), pp. 1048–1053.

Ngugi, A.K., Bottomley, C., Kleinschmidt, I., Wagner, R.G., Kakooza-Mwesige, A., Ae-Ngibise, K., Owusu-Agyei, S., Masanja, H., Kamuyu, G., Odhiambo, R. and Chengo, E. (2013). Prevalence of active convulsive epilepsy in sub-Saharan Africa and associated risk factors: Cross-sectional and case-control studies. *Lancet Neurology*, 12(3), pp. 253–263.

Nishi, M., Mizushima, A., Nakagawara, K.I. and Takeshima, H. (2000). Characterization of Human Junctophilin Subtype Genes. *Biochemical and Biophysical Research Communications*, 273(3), pp. 920–927.
Nishi, M., Sakagami, H., Komazaki, S., Kondo, H. and Takeshima, H. (2003). Co-expression of junctophilin type 3 and type 4 in the brain. *Brain Research. Molecular Brain Research*, 118(1-2), pp. 102–110.

Ocampo, C., Daimari, R. and Oyekunle, A.A. (2018). Huntington's Disease-Like 2 with an expansion mutation of the Junctophilin-3 gene: First reported case from Botswana. *Journal of Clinical Neuroscience*, 47 (1), pp. 126–127.

Orgogozo, V., Morizot, B. and Martin, A. (2015). The differential view of genotype-phenotype relationships. *Frontiers in Genetics*, 6, p. 179.

Orr, H.T. (2012). Polyglutamine neurodegeneration: expanded glutamines enhance native functions. *Current Opinion in Genetics & Development*, 22(3), pp. 251–255.

Orr, H.T. and Zoghbi, H.Y. (2007). Trinucleotide Repeat Disorders. *Annual Review of Neuroscience*, 30(1), pp. 575–621.

Papoutsi, M., Labuschagne, I., Tabrizi, S.J. and Stout, J.C. (2014). The cognitive burden in Huntington's disease: Pathology, phenotype, and mechanisms of compensation. *Movement Disorders*, 29(5), pp. 673–683.

Paradisi, I., Ikonomu, V. and Arias, S. (2012). Huntington disease-like 2 (HDL2) in Venezuela: Frequency and ethnic origin. *Journal of Human Genetics*, 58(1), pp. 3–6.

Paulsen, J.S., Langbehn, D.R., Stout, J.C., Aylward, E., Ross, C.A., Nance, M., Guttman, M., Johnson, S., MacDonald, M., Beglinger, L.J. and Duff, K. (2008). Detection of Huntington's disease decades before diagnosis: The Predict-HD study. *Journal of Neurology, Neurosurgery & Psychiatry*, 79(8), pp. 874–880.

Paulsen, J.S., Long, J.D., Ross, C.A., Harrington, D.L., Erwin, C.J., Williams, J.K., Westervelt, H.J., Johnson, H.J., Aylward, E.H., Zhang, Y. and Bockholt, H.J. (2014). Prediction of manifest Huntington's disease with clinical and imaging measures: A prospective observational study. *The Lancet Neurology*, 13(12), pp. 1193–1201.

Paulson, H. (2018). Repeat expansion diseases. *Handbook of Clinical Neurology*, 147, pp. 105–123. Elsevier

Penney Jr, J.B., Young, A.B., Shoulson, I., Starosta-Rubenstein, S., Snodgrass, S.R., Sanchez-Ramos, J., Ramos-Arroyo, M., Gomez, F., Penchaszadeh, G., Alvir, J. and Esteves, J. (1990). Huntington's disease in Venezuela: 7 years of follow-up on symptomatic and asymptomatic individuals. *Movement Disorders*, 5(2), pp. 93–99.

Piccioni, F., Simeoni, S., Andriola, I., Armatura, E., Bassanini, S., Pozzi, P. and Poletti, A. (2001). Polyglutamine tract expansion of the androgen receptor in a motoneuronal model of spinal and bulbar muscular atrophy. *Brain Research Bulletin*, 56(3-4), pp. 215–220.

Pierson, R., Johnson, H., Harris, G., Keefe, H., Paulsen, J.S., Andreasen, N.C. and Magnotta, V.A. (2011). Fully automated analysis using BRAINS: AutoWorkup. *Neuroimage*, 54(1), pp. 328–336.

Plotkin, J.L. and Surmeier, D.J. (2015). Corticostriatal synaptic adaptations in Huntington's disease. *Current Opinion in Neurobiology*, 33, pp. 53–62.

Podsiadlo, D. and Richardson, S. (2015). The Timed ‘Up & Go’: A Test of Basic Functional Mobility for Frail Elderly Persons. *Journal of the American Geriatrics Society*, 39(2), pp. 142–148.

Powell, S., Magnotta, V.A., Johnson, H., Jammalamadaka, V.K., Pierson, R. and Andreasen, N.C. (2008). Registration and machine learning-based automated segmentation of subcortical and cerebellar brain structures. *Neuroimage*, 39(1), pp. 238–247.

Quarrell, O., O'Donovan, K.L., Bandmann, O. and Strong, M. (2012). The Prevalence of Juvenile Huntington's Disease: A Review of the Literature and Meta-Analysis. *PLoS Currents*, 4, p. e4f8606b742ef3–17.

Rawlins, M.D., Wexler, N.S., Wexler, A.R., Tabrizi, S.J., Douglas, I., Evans, S.J. and Smeeth, L. (2016). The Prevalence of Huntington's Disease. *Neuroepidemiology*, 46(2), pp. 144–153.

Reilmann, R., Bohlen, S., Saß, C., Bechtel, N., Jauffret, C., Sturrock, A., Coleman, A., Read, J., Say, M., t’Hart, E. and van den Bogaard, S. (2013). Q-motor – Quantitative motor assessments: Potential novel endpoints for clinical trials in pre-

manifest and symptomatic Huntington's disease – 36 months longitudinal results from the multicenter TRACK-HD study. *Basal Ganglia*, 3(1), pp. 67–68.

Rodrigues, G.G.R., Walker, R.H., Brice, A., Cazeneuve, C., Russaouen, O., Teive, H.A., Munhoz, R.P., Becker, N., Raskin, S., Werneck, L.C. and Junior, W.M. (2008). Huntington's disease-like 2 in Brazil-Report of 4 patients. *Movement Disorders*, 23(15), pp. 2244–2247.

Rodrigues, G.R., Walker, R.H., Bader, B., Danek, A., Brice, A., Cazeneuve, C., Russaouen, O., Lopes-Cendes, I., Marques Jr, W. and Tumas, V. (2011). Clinical and genetic analysis of 29 Brazilian patients with Huntington's disease-like phenotype. *Arquivos de Neuro-psiquiatria*, 69(3), pp. 419–423.

Rodriguez-Revenga, L., Gómez-Ansón, B., Moreno, E.G., Pagonabarraga, J. and Mila, M. (2012). Neuroimaging in Fragile X-Associated Tremor/Ataxia. *Neuroimaging - Clinical Applications*, 1(19), pp. 423-440.

Romo, L., Mohn, E.S. and Aronin, N. (2018). A Fresh Look at Huntingtin mRNA Processing in Huntington's Disease. *Journal of Huntington's Disease*, 7(2), pp. 101–108.

Roos, R.A. (2010). Huntington's disease: A clinical review. *Orphanet Journal of Rare Diseases*, 5(1), p. 40.

Rosas, H.D., Koroshetz, W.J., Chen, Y.I., Skeuse, C., Vangel, M., Cudkowicz, M.E., Caplan, K., Marek, K., Seidman, L.J., Makris, N. and Jenkins, B.G. (2003). Evidence for more widespread cerebral pathology in early HD: An MRI-based morphometric analysis. *Neurology*, 60(10), pp. 1615–1620.

Rosenblatt, A., Kumar, B.V., Mo, A., Welsh, C.S., Margolis, R.L. and Ross, C.A. (2011). Age, CAG repeat length and clinical progression in Huntington's disease. *Movement Disorders*, 27(2), pp. 272–276.

Rosenblatt, A., Liang, K.Y., Zhou, H., Abbott, M.H., Gourley, L.M., Margolis, R.L., Brandt, J. and Ross, C.A.. (2006). The association of CAG repeat length with clinical progression in Huntington disease. *Neurology*, 66(7), pp. 1016–1020.

Ross, C.A. (2003). Diagnosis of Huntington disease. *Clinical Chemistry*, 49(10), pp. 1726–1732.

Ross, C.A. and Tabrizi, S.J. (2011). Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet neurology*, 10(1), pp. 83–98.

Ross, C.A., Aylward, E.H., Wild, E.J., Langbehn, D.R., Long, J.D., Warner, J.H., Scahill, R.I., Leavitt, B.R., Stout, J.C., Paulsen, J.S. and Reilmann, R. (2014). Huntington disease: Natural history, biomarkers and prospects for therapeutics. *Nature Reviews Neurology*, 10(4), pp. 204–216.

Rudnicki, D.D., Holmes, S.E., Lin, M.W., Thornton, C.A., Ross, C.A. and Margolis, R.L. (2007). Huntington's disease-like 2 is associated with CUG repeat-containing RNA foci. *Annals of Neurology*, 61(3), pp. 272–282.

Rudnicki, D.D., Pletnikova, O., Vonsattel, J.P.G., Ross, C.A. and Margolis, R.L. (2008). A comparison of Huntington disease and Huntington disease-like 2 neuropathology. *Journal of Neuropathology & Experimental Neurology*, 67(4), pp. 366–374.

Rüb, U., Vonsattel, J.P.G., Heinsen, H. and Korf, H.W. (2015). The Neuropathology of Huntington´s disease: Classical findings, recent developments and correlation to functional neuroanatomy. *Advances in Anatomy, Embryology and Cell Biology*, 217, pp. 1–146.

Sacktor, N., Skolasky, R.L., Seaberg, E., Munro, C., Becker, J.T., Martin, E., Ragin, A., Levine, A. and Miller, E.. (2016). Prevalence of HIV-associated neurocognitive disorders in the Multicenter AIDS Cohort Study. *Neurology*, 86(4), pp. 334–340.

Samuels, B.L. and Gelfand, M. (1978). Huntington's chorea in a black Rhodesian family. *South African Medical Journal*, 54(16), pp. 648–651.

Santos, C., Wanderley, H., Vedolin, L., Pena, S.D.J., Jardim, L. and Sequeiros, J. (2008). Huntington disease-like 2: The first patient with apparent European ancestry. *Clinical Genetics*, 73(5), pp. 480–485.

Saudou, F. and Humbert, S. (2016). The Biology of Huntingtin. *Neuron*, 89(5), pp. 910–926.

Scarpina, F. and Tagini, S. (2017). The Stroop Color and Word Test. *Frontiers in Psychology*, 8, pp. 29–8.

Schneider, S.A. and Bird, T. (2016). Huntington's Disease, Huntington's Disease Look-Alikes and Benign Hereditary Chorea: What's New? *Movement Disorders Clinical Practice*, 3(4), pp. 342–354.

Schneider, S.A., Marshall, K.E., Xiao, J. and LeDoux, M.S. (2012). JPH3 repeat expansions cause a progressive akinetic-rigid syndrome with severe dementia and putaminal rim in a five-generation African-American family. *Neurogenetics*, 13(2), pp. 133–140.

Schultz, J.L., Nopoulos, P.C. and Gonzalez-Alegre, P. (2018). Human Immunodeficiency Virus Infection in Huntington's Disease is Associated with an Earlier Age of Symptom Onset. *Journal of Huntington's Disease*, 7(2), pp. 163–166.

Scrimgeour, E.M. and Pfumojena, J.W. (1992). Huntington disease in black Zimbabwean families living near the Mozambique border. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 44(6), pp. 762–766.

Seixas, A.I., Holmes, S.E., Takeshima, H., Pavlovich, A., Sachs, N., Pruitt, J.L., Silveira, I., Ross, C.A., Margolis, R.L. and Rudnicki, D.D.(2012). Loss of junctophilin-3 contributes to Huntington disease-like 2 pathogenesis. *Annals of Neurology*, 71(2), pp. 245–257.

Shannon, K.M. and Fraint, A. (2015). Therapeutic advances in Huntington's Disease. *Movement Disorders*, 30(11), pp. 1539–1546.

Shao, J. and Diamond, M.I. (2007). Polyglutamine diseases: Emerging concepts in pathogenesis and therapy. *Human Molecular Genetics*, 16(R2), pp. R115–R123.

Shoulson, I. and Fahn, S. (1979). Huntington disease: Clinical care and evaluation. *Neurology*, 29(1), pp. 1-1.

Siegl, C., Hamminger, P., Jank, H., Ahting, U., Bader, B., Danek, A., Gregory, A., Hartig, M., Hayflick, S., Hermann, A. and Prokisch, H.(2013). Alterations of red cell membrane properties in neuroacanthocytosis. *PLoS ONE*, 8(10), p. e76715–13.

Sizer, E.B., Haw, T., Wessels, T.M., Kromberg, J.G. and Krause, A.(2012). The utilization and outcome of diagnostic, predictive and prenatal genetic testing for Huntington disease in Johannesburg, South Africa. *Genetic Testing and Molecular Biomarkers*, 16(1), pp. 58–62.

Statistics South Africa (2017). Mid-year population estimates, 2017. *Pretoria: Statistics South Africa*

Stevanin, G. and Brice, A. (2008). Spinocerebellar ataxia 17 (SCA17) and Huntington's disease-like 4 (HDL4). *The Cerebellum*, 7(2), pp. 170–178.

Stevanin, G., Camuzat, A., Holmes, S.E., Julien, C., Sahloul, R., Dode, C., Hahn-Barma, V., Ross, C.A., Margolis, R.L., Durr, A. and Brice, A. (2002). CAG/CTG repeat expansions at the Huntington's disease-like 2 locus are rare in Huntington's disease patients, 58(6), pp. 965–967.

Stevanin, G., Durr, A. and Brice, A. (2000). Clinical and molecular advances in autosomal dominant cerebellar ataxias: From genotype to phenotype and physiopathology. *European Journal of Human Genetics*, 8(1), pp. 4–18.

Stevanin, G., Fujigasaki, H., Lebre, A.S., Camuzat, A., Jeannequin, C., Dodé, C., Takahashi, J., Sân, C., Bellance, R., Brice, A. and Durr, A. (2003). Huntington's disease-like phenotype due to trinucleotide repeat expansions in the TBP and JPH3 genes. *Brain*, 126(7), pp. 1599–1603.

Stevenson, V.L. and Hardie, R.J. (2001). Acanthocytosis and neurological disorders. *Journal of neurology*, 248(2), pp. 87–94.

Storch, A., Kornhass, M. and Schwarz, J. (2005). Testing for acanthocytosis. *Journal of Neurology*, 252(1), pp. 84–90.

Strasak, A.M., Zaman, Q., Pfeiffer, K.P., Göbel, G. and Ulmer, H.(2007). Statistical errors in medical research-a review of common pitfalls. *Swiss Medical Weekly*, 137(3-4), pp. 44–49.

Tabrizi, S.J., Langbehn, D.R., Leavitt, B.R., Roos, R.A., Durr, A., Craufurd, D., Kennard, C., Hicks, S.L., Fox, N.C., Scahill, R.I. and Borowsky, B. (2009). Biological and clinical manifestations of Huntington's disease in the longitudinal TRACK-HD study: Cross-sectional analysis of baseline data. *Lancet Neurology*, 8(9), pp. 791–801.

Tabrizi, S.J., Scahill, R.I., Owen, G., Durr, A., Leavitt, B.R., Roos, R.A., Borowsky, B., Landwehrmeyer, B., Frost, C., Johnson, H. and Craufurd, D. (2013). Predictors of phenotypic progression and disease onset in premanifest and early-stage Huntington's disease in the TRACK-HD study: Analysis of 36-month observational data. *Lancet Neurology*, 12(7), pp. 637–649.

Takeshima, H., Komazaki, S., Nishi, M., Iino, M. and Kangawa, K. (2000). Junctophilins: A novel family of junctional membrane complex proteins. *Molecular Cell*, 6(1), pp. 11–22.

Teive, H.A.G., Becker, N., Munhoz, R.P., Raskin, S., Werneck, L.C., Cazeneuve, C., Durr, A., Russaouen, O. and Brice, A.(2007). Huntington's disease-like 2: The first case report in Latin America in a patient without African ethnic origin. *Movement Disorders*, 16(22), p. S27.

Telenius, H., Kremer, B., Goldberg, Y.P., Theilmann, J., Andrew, S.E., Zeisler, J., Adam, S., Greenberg, C., Ives, E.J., Clarke, L.A. and Hayden, M.R. (1994). Somatic and gonadal mosaicism of the Huntington disease gene CAG repeat in brain and sperm. *Nature Genetics*, 6(4), pp. 409–414.

Tesic, T., Boban, J., Bjelan, M., Todorovic, A., Kozic, D. and Brkic, S., 2018. Basal ganglia shrinkage without remarkable hippocampal atrophy in chronic aviremic HIV-positive patients. *Journal of neurovirology*, *24*(4), pp.478-487.

Tha, K.K., Terae, S., Tsukahara, A., Soma, H., Morita, R., Yabe, I., Ito, Y.M., Sasaki, H. and Shirato, H.(2012). Hyperintense putaminal rim at 1.5 T: Prevalence in normal subjects and distinguishing features from multiple system atrophy. *BMC Neurology*, 12(1), p. 39.

Thompson, J.C., Harris, J., Sollom, A.C., Stopford, C.L., Howard, E., Snowden, J.S. and Craufurd, D. (2012). Longitudinal Evaluation of Neuropsychiatric Symptoms in Huntington's Disease. *The Journal of Neuropsychiatry and Clinical Neurosciences*, 24(1), pp. 53–60.

Todd, P.K., Oh, S.Y., Krans, A., He, F., Sellier, C., Frazer, M., Renoux, A.J., Chen, K.C., Scaglione, K.M., Basrur, V. and Elenitoba-Johnson, K. (2013). CGG Repeat-Associated Translation Mediates Neurodegeneration in Fragile X Tremor Ataxia Syndrome. *Neuron*, 78(3), pp. 440–455.

Tse, W., Cersosimo, M.G., Gracies, J.M., Morgello, S., Olanow, C.W. and Koller, W. (2004). Movement disorders and AIDS: A review. *Parkinsonism & Related Disorders*, 10(6), pp. 323–334.

van den Bogaard, S.J., Dumas, E.M., Acharya, T.P., Johnson, H., Langbehn, D.R., Scahill, R.I., Tabrizi, S.J., van Buchem, M.A., van der Grond, J. and Roos, R.A. (2011). Early atrophy of pallidum and accumbens nucleus in Huntington's disease. *Journal of Neurology*, 258(3), pp. 412–420.

van der Burg, J.M., Björkqvist, M. and Brundin, P. (2009). Beyond the brain: Widespread pathology in Huntington's disease. *The Lancet Neurology*, 8(8), pp. 765–774.

van Dijk, J.G. (1985). Juvenile Huntington's disease. *Clinical Neurology and Neurosurgery*, 87(2), p. 150.

van Vugt, J.P.P., van Hilten, B.J. and Roos, R.A.C. (1996). Hypokinesia in Huntington's disease. *Movement Disorders*, 11(4), pp. 384–388.

Vasconcellos, L.F.R., Macêdo, P.J.O.M., Franck, J.B., Tumas, V., Marques, J.W. and Spitz, M. (2017). Huntington's Disease like 2 presenting with isolated Parkinsonism. *Journal of the Neurological Sciences*, 373, pp. 105–106.

Vonsattel, J.P.G. (2007). Huntington disease models and human neuropathology: Similarities and differences. *Acta Neuropathologica*, 115(1), pp. 55–69.

Vonsattel, J.P.G. and DiFiglia, M. (1998). Huntington disease. *Journal Of Neuropathology And Experimental Neurology*, *57*(5), pp.369-377.

Waddington, C.H. (1942). Canalization of Development and the Inheritance of Acquired Characters. *Nature*, 150(3811), pp. 563–565.

Wahlin, T.B. and Byrne, G.J. (2012). Cognition in Huntington's disease. *Huntington's disease — Core Concepts and Current Advances,* 1(7), pp174-200

Walker, F.O. (2007). Huntington's disease. *The Lancet*, 369(9557), pp. 218–228.

Walker, R.H., Gatto, E.M., Bustamante, M.L., Bernal-Pacheco, O., Cardoso, F., Castilhos, R.M., Chana, P., Cornejo-Olivas, M., Bellmann, I.E., Jardim, L.B. and López-Castellanos, R. (2018). Huntington's disease-like disorders in Latin America and the Caribbean. *Parkinsonism & Related Disorders*, pp. 1–12.

Walker, R.H., Jankovic, J., O'Hearn, E. and Margolis, R.L. (2003). Phenotypic features of Huntington's disease-like 2. *Movement Disorders*, 18(12), pp. 1527–1530.

Walker, R.H., Jung, H.H., Dobson-Stone, C., Rampoldi, L., Sano, A., Tison, F. and Danek, A. (2007). Neurologic phenotypes associated with acanthocytosis. *Neurology*, 68(2), pp. 92–98.

Walker, R.H., Morgello, S., Davidoff–Feldman, B., Melnick, A., Walsh, M.J., Shashidharan, P. and Brin, M.F. (2002). Autosomal dominant chorea-acanthocytosis with polyglutamine-containing neuronal inclusions. *Neurology*, 58(7), pp. 1031–1037.

Walker, R.H., Rasmussen, A., Rudnicki, D., Holmes, S.E., Alonso, E., Matsuura, T., Ashizawa, T., Davidoff-Feldman, B. and Margolis, R.L. (2003). Huntington's disease-like 2 can present as chorea-acanthocytosis. *Neurology*, 61(7), pp. 1002–1004.

Watts, A.D. and Shuttleworth-Edwards, A.B. (2016). Neuropsychology in South Africa: Confronting the challenges of specialist practice in a culturally diverse developing country. *The Clinical Neuropsychologist*, 30(8), pp. 1305–1324.

Weiner, M.F., Hynan, L.S., Rossetti, H. and Falkowski, J. (2011). Luria's three-step test: What is it and what does it tell us? *International Psychogeriatrics*, 23(10), pp. 1602–1606.

Weir, D.W., Sturrock, A. and Leavitt, B.R. (2011). Development of biomarkers for Huntington's disease. *The Lancet Neurology*, 10(6), pp. 573–590.

Wexler, A. (2013). A Brief Prehistory of Huntington's Disease. *Journal of Huntington's Disease*, 2(3), pp. 231–237.

Wexler, N.S. (2004). Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. *Proceedings of the National Academy of Sciences*, *101*(10), pp. 3498–3503.

Weyer, A., Abele, M., Schmitz-Hübsch, T., Schoch, B., Frings, M., Timmann, D. and Klockgether, T. (2007). Reliability and validity of the scale for the assessment and rating of ataxia: A study in 64 ataxia patients. *Movement Disorders*, 22(11), pp. 1633–1637.

Wijemanne, S. and Jankovic J. (2015). Dopa-responsive dystonia — Clinical and genetic heterogeneity. *Nature Reviews Neurology*, 11(7), pp. 414–424.

Wilburn, B., Rudnicki, D.D., Zhao, J., Weitz, T.M., Cheng, Y., Gu, X., Greiner, E., Park, C.S., Wang, N., Sopher, B.L. and La Spada, A.R. (2011). An Antisense CAG Repeat Transcript at JPH3 Locus Mediates Expanded Polyglutamine Protein Toxicity in Huntington's Disease-like 2 Mice. *Neuron*, 70(3), pp. 427–440.

Wild, E.J. and Tabrizi, S.J. (2007). Huntington's disease phenocopy syndromes. *Current Opinion in Neurology*, 20(6), pp. 681–687.

Wild, E.J. and Tabrizi, S.J. (2017). Therapies targeting DNA and RNA in Huntington's disease. *The Lancet Neurology*, 16(10), pp. 837–847.

Wild, E.J., Mudanohwo, E.E., Sweeney, M.G., Schneider, S.A., Beck, J., Bhatia, K.P., Rossor, M.N., Davis, M.B. and Tabrizi, S.J. (2008). Huntington's disease phenocopies are clinically and genetically heterogeneous. *Movement Disorders*, 23(5), pp. 716–720.

Winder, J.Y., Achterberg, W.P., Marinus, J., Gardiner, S.L. and Roos, R.A. (2018). Assessment Scales for Patients with Advanced Huntington's Disease: Comparison of the UHDRS and UHDRS-FAP. *Movement Disorders Clinical Practice*, 5(5), pp. 527–533.

Winder, J.Y., Roos, R.A., Burgunder, J.M., Marinus, J. and Reilmann, R. (2018). Interrater Reliability of the Unified Huntington's Disease Rating Scale-Total Motor Score Certification. *Movement Disorders Clinical Practice*, 5(3), pp. 290–295.

Wright, H.H., Still, C.N. and Abramson, R.K. (1981). Huntington's Disease in Black Kindreds in South Carolina. *Archives of Neurology*, 38(7), pp. 412–414.

Yanai, A., Huang, K., Kang, R., Singaraja, R.R., Arstikaitis, P., Gan, L., Orban, P.C., Mullard, A., Cowan, C.M., Raymond, L.A. and Drisdel, R.C. (2006). Palmitoylation of huntingtin by HIP14 is essential for its trafficking and function. *Nature Neuroscience*, 9(6), pp. 824–831.

Young, A.B., Shoulson, I., Penney, J.B., Starosta-Rubinstein, S., Gomez, F., Travers, H., Ramos-Arroyo, M.A., Snodgrass, S.R., Bonilla, E., Moreno, H. and Wexler, N.S. (1986). Huntington's disease in Venezuela: Neurologic features and functional decline. *Neurology*, 36(2), pp. 244–249.

Yu, M.K., Kramer, M., Dutkowski, J., Srivas, R., Licon, K., Kreisberg, J.F., Ng, C.T., Krogan, N., Sharan, R. and Ideker, T. (2016). Translation of Genotype to Phenotype by a Hierarchy of Cell Subsystems. *Cell Systems*, 2(2), pp. 77–88.

Zori, R.T. and Williams, C.A. (1991). Phenocopy versus genocopy. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 40(2), pp. 248–249.

9 Appendices

9.1 Appendix 1: Informed consent and participant information sheets

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Information and consent form

***Title*: The Phenotype of Huntington Disease Like 2**

***Investigators*: Dr David Anderson & Ms. Aline Ferreira Correia**

Department Human Genetics

&

The University of the Witwatersrand Donald Gordon Medical Centre

Good Day

We are Dr. Dave Anderson, a neurologist specializing in movement disorders and Ms. Aline Ferreira Correia, a neuropsychologist. We are currently doing PhDs in Huntington disease.

Introduction

We are asking you to take part in this study because you have been diagnosed with Huntington Disease (HD) or a disease believed to be similar to it called Huntington Disease Like 2 (HDL2). In this form we will explain what this study involves and why this study is being performed.

Those with HD may experience difficulty thinking and making choices as well as mood problems and involuntary movements that start in adulthood. HD and HDL2 are genetic diseases that affect the brain. The cells of the human body carry genetic material in the form of long molecules called DNA, which is then copied and passed on the next generation. Each DNA molecule contains thousands of coded instructions called genes that make the cells work normally. The abnormal genes for HD and HDL2 have been identified and are not the same. We have discovered that

other genetic factors outside the 'disease gene' can affect when the disease starts and how the disease it looks.

Generally, HD and HDL2 have been thought to be clinically identical, that is, it is impossible to tell them apart on the basis of a neurological examination. However, there is some information to suggest this may not be the case. If we were able to tell the difference between the two it might suggest differences in the disease pathways underlying the conditions and this might help us understand the processes causing them and where to focus on treating them.

In order to see if there are any differences between HD and HDL2 we will be doing detailed physical examinations of patients with HD and HDL2 and comparing them. We will also compare their brain in the form of MRI scans and lastly, we will do blood tests to compare their DNA. And what their blood cells look like.

What will the study involve?

The HDL2 Phenotype study will involve a visit to Area 256 at the Charlotte Maxeke Johannesburg Academic Hospital. This visit will last 3-4 hours. You will then be taken to the University of the Witwatersrand Donald Gordan Medical Centre (WDGMC) where you will be given lunch and the MRI scan will be done. This will take another hour.

What will happen during the study?

If you have consented to participate in this study, you will receive a unique identification number at the beginning of your visit. This number will replace your name and all your personal and identifying data. This number will be used to identify you afterwards and only the local research staff will know who you are.

1. We will record all your unique information (name, date of birth, home address etc) and assign you a study number.
2. We will ask you questions about yourself, your past medical history, medications and other members of your family that may be affected by similar symptoms.
3. A full neurological examination will be performed as part of the Unified Huntington's Disease Rating Scale (UHDRS). Part of this will be videoed for analysis later.

evaluation does reveal something important we will inform you as soon as possible and assist as the need arises.
The questionnaires, examinations and UHDRS pose no more risk to you than a regular physical examination. These will take longer than usual. The blood sample is also a standard test that you may have had before. You can have some discomfort from the needle and bruising. The MRI scan takes 30-40 minutes. It is a safe procedure that allows us to see the structure of the brain. You need to lie in a tunnel and keep still for the duration of the test. Some people feel uncomfortable in closed spaces and do not tolerate the test. We can work around this if you think this is going to be a problem. The cognitive testing might elicit distress if some of the tasks are perceived as difficult, however, the psychologist will monitor how you are feeling and will refer you to a free counseling service if needed[1]. All these tests are paid for by the study.

What are the benefits of the study?
You will not receive any direct benefits from undergoing these evaluations. What this study will do is provide important information about HDL2.

What are the financial implications?
Participating in this study will not cost you anything. All tests will be paid for.
Although you will not be paid for participating in the study, we will offer you R100 for travel costs incurred on the day and a sandwich will be provided for you.

Do I have to participate?
You are not obligated to participate in the study. Should you choose not to participate it will not affect your access to the HD clinic or the treatment you receive. If you decide that you would like to join the study a consent form will need to be signed by you or your legal guardian. Once it is signed you are still free to withdraw at any time without giving a reason and your information will not be used in the study.

You may be withdrawn from the study if you do not follow the directions of the study or your medical condition changes so that staying in the study may risk your health or the research.

Questions?
Should you have questions regarding the study please contact the project co ordinator, Dr Anderson at 011-726-8741.

If there are any complaints, you have regarding the way the study is being conducted The Human Research Ethics Committee (HREC) of the University of the Witwatersrand can also be contacted with any concerns. Prof Cleaton Jones is the chairman 011 717 2301 (Monday-Wednesday-Friday mornings) alternatively the Jennifer Palmer can be contacted. 011 274-9278 (E Mail:jpalmer@witshealth.co.za)

Informed Consent to participate in the HDL2 Phenotype study for patients over 18 years of age

This study will be conducted according to the Declaration of Helsinki (2008) and the South African Good Clinical Practice (SA GCP) Guidelines. These ensure, among other things the protection of individuals participating in research studies.

I _____ hereby declare that I have read and understood the information document for the research report entitled The Phenotype of Huntington Disease Like 2

I _____

1. Have read (or been read to me) and understood the information in this consent form. ☐
2. Have been encouraged to ask questions and am satisfied with the responses to my questions. ☐
3. Have been told I will receive a dated and signed copy of this consent form. ☐
4. Understand all the data collected will be kept confidential and that the results are to be used for scientific objectives. ☐
5. Understand that my participation in this study is voluntary and I am free to withdraw at any time and that this will not affect the care and treatment I receive. ☐
6. Understand that I am not waving my legal rights as a result of signing this consent form. ☐
7. Understand that there is no guarantee that this study will provide any benefits to me. ☐
8. Do consent to participate in this study by signing this consent form. ☐
9. Agree to be examined and videoed as has been explained. ☐
10. Agree to being interviewed as has been explained. ☐
11. Agree to my blood being taken as has been explained. ☐
12. Agree to have an MRI scan as has been explained. ☐

_____	_____	_____
Signature of research Subject	Name Printed	Date
_____	_____	_____
Signature of Witness	Name Printed	Date
_____	_____	_____
Signature of Witness	Name Printed	Date
_____	_____	_____
Translator	Name Printed	Date
_____	Dr David Anderson	_____
Signature of Principal Investigator	Name Printed	Date

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Video Information Sheet

***Title*: The Phenotype of Huntington Disease type 2**

***Investigator*: Dr David Anderson**

Department Human Genetics

&

The University of the Witwatersrand Donald Gordon Medical Centre

Good Day

My name is Dr Dave Anderson I am a neurologist specializing in movement disorders and I am currently doing a PhD in Huntington disease.

Introduction

We are asking you to take part in this study because you have been diagnosed with Huntington Disease (HD) or a disease believed to be similar to it called Huntington Disease Like 2 (HDL2). In this form we will explain what this study involves and why this study is being performed.

Those with HD may experience difficulty thinking and making choices as well as mood problems and involuntary movements that start in adulthood. HD and HDL2 are genetic diseases that affect the brain. The cells of the human body carry genetic material in the form of long molecules called DNA, which is then copied and passed on to the next generation. Each DNA molecule contains thousands of coded instructions called genes that make the cells work normally. The abnormal genes for HD and HDL2 have been identified and are not the same. We have discovered that other genetic factors outside the 'disease gene' can affect when the disease starts and how the disease looks.

Generally HD and HDL2 have been thought to be clinically identical, that is, it is impossible to tell them apart on the basis of a neurological examination. However there is some information to suggest this may not be the case. If we were able to tell the difference between the two it might suggest differences in the disease pathways underlying the conditions and this might help us understand that processes causing the them and where to focus on treating them.
In order to see if there are any differences between HD and HDL2 we will be doing detailed physical examinations of patients with HD and HDL2 and comparing them. We will also compare their brain in the form of MRI scans and lastly, we will do blood tests to compare their DNA and what their blood cells look like.

Why is video needed?

HD and HDL2 are movement disorders. In order to understand the differences, we would like to video your movements and compare them and study the differences. This will allow make it easier for other doctors to tell the differences when faced with similar cases in future.

What will happen to the videos once taken?

If you have consented to participate in this study, you will receive a unique identification number at the beginning of the your visit. This number will replace your name and all your personal and identifying data. This number will be used to identify you afterwards and only the local research staff will know who you are. This number will be assigned to you video.

8. Your video will be stored buy Dr Anderson on a hard drive and kept in a safe.
9. Your video will not be placed on the internet.
10. Your videos will be kept for 10 years.

Who will see the videos?

We would like to use the video s in two circumstances

1. For the study Doctors will watch the videos comparing the two diseases.
2. We would like to ask your permission to show your videos at meetings or congresses. These are places where doctor and medical professionals can learn about HD and HDL2.

Questions?

Should you have questions regarding the study please contact the project co ordinator, Dr Anderson at 011-726-8741.

If there are any complaints, you have regarding the way the study is being conducted The Human Research Ethics Committee (HREC) of the University of the Witwatersrand can also be contacted with any concerns. Prof Cleaton Jones is the chairman 011 717 2301 (Monday-Wednesday-Friday mornings) alternatively the Jennifer Palmer can be contacted. 011 274-9278 (E Mail: jpalmer@witshealth.co.za)

Informed Consent to participate in the HLD2 Phenotype study for Video in affected patients

This study will be conducted according to the Declaration of Helsinki (2008) and the South African Good Clinical Practice (SA GCP) Guidelines. These ensure, among other things the protection of individuals participating in research studies.

I _____ hereby declare that I have read and understood the information document regarding the storage and use of videos for the research report entitled The Phenotype of Huntington Disease Like 2

I _____,

1. Have read (or been read to me) and understood the information in this consent form. YES/NO
2. Have been encouraged to ask questions and am satisfied with the responses to my questions. YES/NO
3. Have been told I will receive a dated and signed copy of this consent form. YES/NO
4. Understand all the data collected will be kept confidential and that the results are to be used for scientific objectives. YES/NO
5. Understand that my participation in this study is voluntary and I am free to withdraw at any time and that this will not affect the care and treatment I receive. YES/NO
6. Understand that I am not waving my legal rights as a result of signing this consent form. YES/NO
7. Understand that it is unlikely that this study will provide any benefits to me- YES/NO

8. I acknowledge that my videos will be kept sake and will not appear on the internet
9. Do consent to my videos being used in this study by signing this consent form- YES/NO
10. Do consent to my videos being used at academic meetings by signing this consent form- YES/NO

Signature of research Subject	Name Printed	Date
Signature of Witness	Name Printed	Date
Translator	Name Printed	Date
Signature of Principal Investigator	Dr David Anderson Name Printed	Date

9.2 Appendix 2: Datasheets

1. Participant demographics
2. Genetic data
3. General medical history
4. Neurological history
5. General neurological examination
6. UHDRS
7. MoCA
8. STROOP
9. SARA
10. Myoclonus
11. Timed get up and go
12. Haematology
13. Radiology

Participant Demographics

Participant ID	_____
As a participant in this study, please answer the questions below. Thank you!	
First Name	_____
Surname	_____
ID Number	_____
Date of birth	_____
Visit date	_____
Age	_____
Initials	_____
Home number	_____ (Include Area Code)
Cellphone number	_____
Email address	_____
Home address	_____
Gender	◯ Male ◯ Female

Contact information	
Contact name	_____
Contact relationship	_____
Contact telephone - home	_____ (Include Area Code)
Contact telephone - cellphone	_____
Home address	_____

Demographic data of participant (Affected/Control)	
Race	◯ Black ◯ Indian ◯ Mixed ◯ White
Home Language	☐ Afrikaans ☐ English ☐ Ndebele ☐ Sepedi ☐ Venda ☐ Xhosa ☐ Southern Sotho ☐ Zulu ☐ Swazi ☐ Tsonga ☐ Tswana ☐ Other
Other home language	_____
Home Language: mother	☐ Afrikaans ☐ English ☐ Ndebele ☐ Sepedi ☐ Venda ☐ Xhosa ☐ Southern Sotho ☐ Zulu ☐ Swazi ☐ Tsonga ☐ Tswana ☐ Other
Other home language mother	_____

Home Language: father	☐ Afrikaans ☐ English ☐ Ndebele ☐ Sepedi ☐ Venda ☐ Xhosa ☐ Southern Sotho ☐ Zulu ☐ Swazi ☐ Tsonga ☐ Tswana ☐ Other
Other home language father	_____
Occupation	_____
Previous occupation	_____
Place of birth	_____

Genetics

Participant ID	_____
Genetic Information of patient	
Date of Study Participation	_____
Lab Report Number	_____
HC number	_____
Disease type	◯ HD ◯ HDL2
Date of Genetic test	_____
Repeat number on (abnormal) allele I	_____
Repeat number on (normal) allele II	_____
Age of onset (years)	_____
Age at diagnosis	_____
Disease duration (years)	_____
Was test?	◯ Predictive ◯ Diagnostic
First symptoms	☐ Psychiatric ☐ Cognitive ☐ Movement ☐ Other
Other first symptoms	_____

Affected family member 3 1st symptom	◯ Psychiatric ◯ Cognitive ◯ Movement
Affected family member 4 Name	_____
Affected family member 4 Contact number	_____
Included in current study?	◯ Yes ◯ No
Patient number for family member 4 included in study	_____
Affected family member 4 Age of onset	_____
Affected family member 4 1st symptom	◯ Psychiatric ◯ Cognitive ◯ Movement
Affected family member 5 Name	_____
Affected family member 5 Contact number	_____
Included in current study?	◯ Yes ◯ No
Patient number for family member 5 included in study	_____
Affected family member 5 Age of onset	_____
Affected family member 5 1st symptom	◯ Psychiatric ◯ Cognitive ◯ Movement
Pedigree	

General Medical History

Participant ID	_____
Do you drink alcohol?	◯ Yes ◯ No
Amount per week	_____
If you quit, when?	_____
Do you use tobacco?	◯ Yes ◯ No
Type	☐ Cigarettes ☐ Cigars ☐ Snuff ☐ Pipe
If you quit, when?	_____
Do you drink caffeine?	◯ Yes ◯ No
Type	☐ Tea ☐ Coffee ☐ Cola ☐ Energy drinks
Amount per day	_____
If you quit, when?	_____
Recreational drug use?	◯ Yes ◯ No
Type of drugs	☐ Cannabis (dagga) ☐ Cocaine ☐ Ecstasy ☐ LSD ☐ TIK ☐ other
Number per week	_____
If you quit, when?	_____

Do you exercise?	◯ Yes ◯ No
No times per week	_____
Duration of session	_____
Heart disease	☐ Murmur ☐ Angina/Coronary ☐ Congestive heart failure ☐ Rheumatic fever ☐ Valve replacement ☐ Irregular heartbeat ☐ Heart attack ☐ High blood pressure
Infectious disease	☐ AIDS/HIV positive ☐ Tuberculosis ☐ Sexually transmitted disease
CD4 count	_____
Musculoskeletal	☐ Rheumatoid arthritis ☐ Gout ☐ Osteoarthritis ☐ Fibromyalgia
Gynaecological	☐ Abnormal pap ☐ Endometriosis ☐ Fibroids ☐ Ovarian cysts ☐ Irregular bleeding
Respiratory	☐ Asthma ☐ Allergies/hay fever ☐ Emphysema/COPD ☐ Chronic bronchitis ☐ Pneumonia ☐ Asbestos exposure ☐ Sleep apnea
Gastrointestinal	☐ Ulcers ☐ Colon polyps ☐ Gallstones ☐ Hiatal hernia ☐ Hepatitis ☐ Hemmorrhoids(piles) ☐ Irritable bowel ☐ Colitis ☐ Diverticulosis ☐ Gastrointestinal bleeding
Type	◯ Bloody Vomit (haemoptisis) ◯ Bloody Stools (dark blood) ◯ Bloody Stools (bright blood)

Kidney/bladder	☐ Stones ☐ Prostate disorder ☐ Incontinence ☐ Infection
Metabolic/nutrition	☐ Diabetes ☐ High cholesterol ☐ Anaemia ☐ Thyroid problem ☐ Bleeding disorder
Cancer	◯ Yes ◯ No
Location	_____

Participant hospitalisations in last 10 years

Hospitalisation 1	◯ Yes ◯ No
Date	_____
Place	_____
Reason	☐ Psychiatric ☐ Medical ☐ Surgical ☐ Trauma
Hospitalisation 2	◯ Yes ◯ No
Date	_____
Place	_____
Reason	☐ Psychiatric ☐ Medical ☐ Surgical ☐ Trauma
Hospitalisation 3	◯ Yes ◯ No
Date	_____
Place	_____

Reason	☐ Psychiatric ☐ Medical ☐ Surgical ☐ Trauma
Is the participant currently on medication?	◯ Yes ◯ No
Number of medications	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5
Medication 1	_____ (Medication Name)
Dose	_____
Times/day	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ as required
Is this psychiatric medication?	☐ Antipsychotic ☐ Antidepressant
Medication 2	_____ (Medication Name)
Dose	_____
Times/day	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ as required
Is this psychiatric medication?	☐ Antipsychotic ☐ Antidepressant
Medication 3	_____ (Medication Name)
Dose	_____

Times/day	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ as required
is this psychiatric medication	☐ Antipsychotic ☐ Antidepressant
Medication 4	_____ (Medication Name)
Dose	_____
Times/day	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ as required
Is this psychiatric medication?	☐ Antipsychotic ☐ Antidepressant
Medication 5	_____ (medication Name)
Dose	_____
Times/day	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ as required
Is this psychiatric medication?	☐ Antipsychotic ☐ Antidepressant

History of previous medication currently not being taken

Has the patient ever been on psychiatric medication before?	◯ Yes ◯ No
The types of psychiatric medications?	☐ Antipsychotic ☐ Antidepressant
Name of medication names	_____ (Medication Name)

Name of medication names

_____
(Medication Name)

Name of medication names

_____
(Medication Name)

Neurological History

Participant ID	_____
Seizures: has the patient ever had any seizures?	◯ Yes ◯ No
Age of onset	_____
Type	☐ Generalised ☐ Myoclonic ☐ Dialeptic
Date of last seizure	_____
Myoclonus: does the patient have sudden jerking of the body?	◯ Yes ◯ No
Age of onset	_____
Frequency	◯ Daily ◯ Monthly ◯ Rarely
Head injury: Has the patient had a head injury?	◯ Yes ◯ No
When?	_____
Due to:	◯ Falls ◯ Motor vehicle accident ◯ Other
Loss of consciousness?	◯ Nil ◯ < 10min ◯ >10min/required hospitalisation
Falls: Has the patient fallen due to gait difficulties?	◯ Yes ◯ No
When did the falls begin? (years)	_____ (If before 1 year, enter 0)
Frequency of falls	◯ Daily ◯ >1/week ◯ >1/month ◯ < 1/month ◯ rarely

Does the patient have any symptoms of HD/HDL2, subjectively?	◯ Yes ◯ No
Which was the first subjective symptom felt by the patient	◯ Psychiatric ◯ Movement ◯ Cognitive ◯ Other
When was this first noticed (in years)?	_____
Does the patient's family/friend feel that the patient has any symptoms of HD/HDL2, subjectively?	◯ Yes ◯ No
Which was the first symptom seen by the family\friend?	◯ Psychiatric ◯ Movement ◯ Cognitive ◯ Other
When was this first observed (in years)?	_____

General Neurological Examination

Participant ID	_____
Weight	_____ (kg)
Can the patient smell?	◯ Yes ◯ No
Vision	☐ Normal ☐ Abmormal
Motor facial	◯ Normal ◯ Abnormal
Abnormal facial motor function	◯ Right Upper Motor ◯ Left Upper Motor ◯ Both Right and Left Upper Motor ◯ Right Lower Motor ◯ Left Lower Motor ◯ Both Right and Left Lower Motor
Upper limbs	◯ Normal ◯ Abnormal
Abnormal upper limb examination	◯ Symmetrical ◯ Asymmetrical
Asymmetrical upper limb examination	◯ Right affected ◯ Left affected
Upper limb tone	◯ Normal ◯ Abnormal
Abnormal upper limb tone	◯ Increased ◯ Decreased
Increased upper limb tone	◯ Spastic ◯ Rigid ◯ Paratonia
Upper limb power	◯ Normal ◯ Decreased
Decreased upper limb power	◯ Distal ◯ Proximal ◯ Both

Upper limb reflexes	◯ 0 ◯ 1 ◯ 2 ◯ 3 ◯ 4 (Description 0=Absent 1=hypoactive but present 2="Normal" 3=Hyperactive without clonus 4=Hyperactive with clonus)
Lower limbs	◯ Normal ◯ Abnormal
Abnormal lower limb examination	◯ Symmetrical ◯ Asymmetrical
Asymmetrical lower limb examination	◯ Right affected ◯ Left affected
Lower limb tone	◯ Normal ◯ Abnormal
Abnormal lower limb tone	◯ Increased ◯ Decreased
Increased lower limb tone	◯ Spastic ◯ Rigid ◯ Paratonia
Lower limb power	◯ Normal ◯ Decreased
Decreased lower limb power	◯ Proximal ◯ Distal ◯ Both
Lower limb reflexes	◯ 0 ◯ 1 ◯ 2 ◯ 3 ◯ 4 (Description 0=Absent 1=hypoactive but present 2="Normal" 3=Hyperactive without clonus 4=Hyperactive with clonus)
Plantar response	◯ Up going ◯ Down going ◯ Equivocal

UHDRS

Participant ID	_____
Ocular pursuit (horizontal)	◯ normal ◯ jerky ◯ interrupted pursuits with full range ◯ incomplete range ◯ cannot pursue (Slow finger right and left)
Ocular pursuit (vertical)	◯ normal ◯ jerky ◯ interrupted pursuits with full range ◯ incomplete range ◯ cannot pursue (Slow finger up and down)
Saccade initiation (horizontal)	◯ normal ◯ increased latency only ◯ suppressible blinks/had movements to initiate ◯ insuppressible head movements ◯ cannot initiate saccades (Fast finger to hand)
Saccade initiation (vertical)	◯ normal ◯ increased latency only ◯ suppressible blinks/had movements to initiate ◯ insuppressible head movements ◯ cannot initiate saccades
Saccade velocity (horizontal)	◯ normal ◯ mild slowing ◯ moderate slowing ◯ Severe slowing but full range ◯ Incomplete range (Fast finger left to right)
Saccade velocity (vertical)	◯ normal ◯ mild slowing ◯ moderate slowing ◯ Severe slowing but full range ◯ Incomplete range (Fast finger up and down)
Dysarthria	◯ normal ◯ unclear no need to repeat ◯ must repeat to be understood ◯ mostly incomprehensible ◯ mute (Days of the week)
Tongue protrusion	◯ can fully protrude tongue for >10 sec ◯ cannot protrude tongue for >10 sec ◯ cannot keep fully protruded for 5 sec ◯ cannot fully protrude tongue ◯ cannot protrude tongue beyond lips

Rigidity Arms (R)	◯ absent ◯ slight or present with activation ◯ mild to moderate ◯ severe but full range of movement ◯ severe, limited range of movement
Rigidity Arms (L)	◯ absent ◯ slight or present with activation ◯ mild to moderate ◯ severe but full range of movement ◯ severe, limited range of movement
Finger tap (right)	◯ normal(>15taps/5 sec.) ◯ mild slowing and or reduction in amplitude (11-14/5 sec.) ◯ moderately impaired. Definite and early fatiguing. May have occasional arrests in movement (7-10/5 sec.) ◯ severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movements (3-6/5 sec.) ◯ can barely perform the task (0-2/5 sec.)
Finger tap (left)	◯ normal(>15taps/5 sec.) ◯ mild slowing and or reduction in amplitude (11-14/5 sec.) ◯ moderately impaired. Definite and early fatiguing. May have occasional arrests in movement (7-10/5 sec.) ◯ severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movements (3-6/5 sec.) ◯ can barely perform the task (0-2/5 sec.)
Pronate/Supinate Hand (R)	◯ normal ◯ mild slowing and/or irregular ◯ moderate slowing and irregular ◯ severe slowing and irregular ◯ cannot perform
Pronate/Supinate Hand (L)	◯ normal ◯ mild slowing and/or irregular ◯ moderate slowing and irregular ◯ severe slowing and irregular ◯ cannot perform
Luria (fist-hand-palm-test)	◯ > 4 in 10 seconds, no cue ◯ < 4 in 10 seconds, no cue ◯ > 4 in 10 seconds, with cues ◯ < 4 in 10 seconds, with cues ◯ Unable to perform task (Instructions-"Look carefully at what I?m doing." The examiner, seated in front of the patient, performs alone three times with his left hand the series of "fist-edge-palm." "Now, with your right hand do the same series, first with me, then alone." The examiner performs the series three times with the patient, then says to him/her: "Now, do it on your own.")

Maximal Dystonia (trunk)	◯ absent ◯ slight/intermittent ◯ mild/common or moderate/intermittent ◯ moderate/common ◯ marked/prolonged
Maximal Dystonia (RUL)	◯ absent ◯ slight/intermittent ◯ mild/common or moderate/intermittent ◯ moderate/common ◯ marked/prolonged (Right upper limb seated for 60sec)
Maximal Dystonia (LUL)	◯ absent ◯ slight/intermittent ◯ mild/common or moderate/intermittent ◯ moderate/common ◯ marked/prolonged (Left upper limb seated for 60sec)
Maximal Dystonia (RLL)	◯ absent ◯ slight/intermittent ◯ mild/common or moderate/intermittent ◯ moderate/common ◯ marked/prolonged (Right lower limb seated 60sec)
Maximal Dystonia (LLL)	◯ absent ◯ slight/intermittent ◯ mild/common or moderate/intermittent ◯ moderate/common ◯ marked/prolonged (Left lower limb seated for 60 seconds)
Maximal Chorea (face)	◯ absent ◯ slight/intermittent ◯ mild/common or moderate/intermittent ◯ moderate/common ◯ marked/prolonged (Face seated for 60 sec)
Maximal Chorea (BOL)	◯ absent ◯ slight/intermittent ◯ mild/common or moderate/intermittent ◯ moderate/common ◯ marked/prolonged (Bucco orro lingual 60sec)
Maximal Chorea (trunk)	◯ absent ◯ slight/intermittent ◯ mild/common or moderate/intermittent ◯ moderate/common ◯ marked/prolonged (Trunk (no back support) 60 sec)

Behavioural Assessment: severity

	absent	slight/questionable	mild	moderate	severe
Sad/mood	◯	◯	◯	◯	◯
Low self-esteem/guilt	◯	◯	◯	◯	◯
Anxiety	◯	◯	◯	◯	◯
Suicidal thoughts	◯	◯	◯	◯	◯
Disruptive or aggressive behaviour	◯	◯	◯	◯	◯
Irritable behaviour	◯	◯	◯	◯	◯
Obsessions	◯	◯	◯	◯	◯
Compulsions	◯	◯	◯	◯	◯
Delusions	◯	◯	◯	◯	◯
Hallucinations	◯	◯	◯	◯	◯

Behavioural Assessment: Severity Total _____

Behavioural assessment: Frequency

	almost never	seldom	sometimes	frequently	almost always
Sad/mood	◯	◯	◯	◯	◯
Low self-esteem/guilt	◯	◯	◯	◯	◯
Anxiety	◯	◯	◯	◯	◯
Suicidal thoughts	◯	◯	◯	◯	◯
Disruptive or aggressive behaviour	◯	◯	◯	◯	◯
Irritable behaviour	◯	◯	◯	◯	◯
Obsessions	◯	◯	◯	◯	◯
Compulsions	◯	◯	◯	◯	◯
Delusions	◯	◯	◯	◯	◯
Hallucinations	◯	◯	◯	◯	◯

Behavioural Assessment: Frequency Total _____

Does the investigator believe the subject is confused?
◯ Yes
◯ No

Does the investigator believe the subject is demented?
◯ Yes
◯ No

Does the investigator believe the subject is depressed?
◯ Yes
◯ No

Does the subject require pharmacotherapy for depression?
◯ Yes
◯ No

Could subject engage in gainful employment in his/her accustomed work?	◯ Yes ◯ No
Could subject engage in any kind of gainful employment?	◯ Yes ◯ No
Could subject engage in any kind of volunteer or non-gainful work?	◯ Yes ◯ No
Could subject manage his/her finances (monthly) without any help?	◯ Yes ◯ No
Could subject shop for groceries without help?	◯ Yes ◯ No
Could subject handle money as a purchaser in a simple cash (store) transaction?	◯ Yes ◯ No
Could subject supervise children without help?	◯ Yes ◯ No
Could subject operate an automobile safely and independently?	◯ Yes ◯ No
Could subject do his/her own housework without help?	◯ Yes ◯ No
Could subject do his/her own laundry (wash/dry) without help?	◯ Yes ◯ No
Could subject prepare his/her own meals without help?	◯ Yes ◯ No
Could subject use the telephone without help?	◯ Yes ◯ No
Could subject take his/her own medications without help?	◯ Yes ◯ No
Could subject feed himself/herself without help?	◯ Yes ◯ No
Could subject dress himself/herself without help?	◯ Yes ◯ No
Could subject bathe himself/herself without help?	◯ Yes ◯ No
Could subject use public transportation to get places without help?	◯ Yes ◯ No
Could subject walk to places in his/her neighborhood without help?	◯ Yes ◯ No

Could subject walk without falling?	◯ Yes ◯ No
Could subject walk without help?	◯ Yes ◯ No
Could subject comb hair without help?	◯ Yes ◯ No
Could subject transfer between chairs without help?	◯ Yes ◯ No
Could subject get in and out of bed without help?	◯ Yes ◯ No
Could subject use toilet/commode without help?	◯ Yes ◯ No
Could subject's care still be provided at home?	◯ Yes ◯ No
Independence scale (indicate the most accurate level of subject's independence)	◯ no special care needed ◯ no physical care needed if difficult tasks are avoided ◯ pre-disease level of employment changes or ends; cannot perform household chores to pre-disease level, may need help with finances ◯ self-care maintained for bathing, limited household duties (cooking and use of knives), driving terminates; unable to manage finances ◯ needs minor assistance in dressing, toileting, bathing; food must be cut for patient ◯ 24-hour supervision appropriate; assistance required for bathing; eating, toileting ◯ chronic care facility needed; limited self-feeding, liquefied diet ◯ patient provides minimal assistance in own feeding, bathing, and toileting ◯ no speech, must be fed ◯ tube fed, total bed care
Functional capacity: Occupation	◯ unable ◯ marginal work only ◯ reduced capacity for usual job
Functional capacity: Domestic chores	◯ unable ◯ impaired ◯ normal
Functional capacity: ADL	◯ total care ◯ gross tasks only ◯ minimal impairment ◯ normal
Functional capacity: Care level	◯ full time skilled nursing ◯ home or chronic care ◯ home ◯ normal

Functional capacity: Finances	◯ unable ◯ major assistance ◯ slight assistance ◯ normal
Functional capacity total	_____

Moca

Participant ID	_____
Visuospatial Executive Trail Making	_____
Visuospatial Executive Cube	_____
Visuospatial Executive Clock	☐ Contour ☐ Numbers ☐ Hands
Visuospatial Executive Clock total	_____
Visuospatial Executive Total Score	_____
Naming	☐ Lion ☐ Rhino ☐ Camel
Naming Total	_____
Memory Trial 1	☐ Face ☐ Velvet ☐ Church ☐ Daisy ☐ Red
Memory trial 2	☐ Face ☐ Velvet ☐ Church ☐ Daisy ☐ Red
Attention Digits 1	◯ 0 ◯ 1
Attention Digits 2	◯ 0 ◯ 1
Attention Digits Total	◯ 0 ◯ 1 ◯ 2
Attention Tapping	◯ 0 errors ◯ 1 error ◯ 2 errors ◯ more than 2 errors

Attention Tapping Total	◯ 0 ◯ 1
Attention Serial Substraction	☐ 93 ☐ 86 ☐ 79 ☐ 72 ☐ 65
Attention Serial Substraction Total	☐ 0 ☐ 1 ☐ 2 ☐ 3
Repeat sentence 1	◯ 0 ◯ 1
Repeat sentence 2	◯ 0 ◯ 1
Repetition total	◯ 0 ◯ 1 ◯ 2
Fluency Total	◯ 0 ◯ 1
Abstraction	☐ train bicycle ☐ watch ruler
Abstraction Total	☐ 0 ☐ 1 ☐ 2
Delayed Recall	☐ Face ☐ Velvet ☐ Church ☐ Daisy ☐ Red
Delayed Recall Total	◯ 0 ◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5
Orientation	☐ Date ☐ Month ☐ Year ☐ Day ☐ Place ☐ City

Orientation Total	◯ 0 ◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6
Extra point for low education	◯ 0 ◯ 1
MOCA TOTAL	_____

Stroop Colour And Word Test Adult Version

Participant ID	_____
Word Score (w) raw score	_____
Word Score (W) age/ed predicted	_____
Word Score (W) residual	_____
Word Score (W) T scores	_____
Colour Score (C) raw score	_____
Colour Score (C) age/ed predicted	_____
Colour Score (C) residual	_____
Colour Score (C) T scores	_____
Colour-Word Scores (CW) raw score	_____
Colour-Word Scores (CW) age/ed predicted	_____
Colour-Word Scores (CW) residual	_____
Colour-Word Scores (CW) T scores	_____
CW-predicted=interference T score	_____

Symbol Digit Modality Test

Participant ID	_____
Written score	_____
Oral score	_____
Speed and accuracy index	_____

SARA

Participant ID	_____
Gait: Proband is asked (1) to walk at a safe distance parallel to a wall including a half-turn (turn around to face the opposite direction of gait) and (2) to walk in tandem (heels to toes) without support.	◯ normal, no difficulties in walking, turning and walking tandem (up to one misstep allowed) ◯ slight difficulties, only visible when walking 10 consecutive steps in tandem ◯ clearly abnormal, tandem walking >10 steps not possible ◯ considerable staggering, difficulties in half-turn, but without support ◯ marked staggering, intermittent support of the wall required ◯ severe staggering, permanent support of one stick or light support by one arm required ◯ walking > 10 m only with strong support (two special sticks or stroller or accompanying person) ◯ walking < 10 m only with strong support (two special sticks or stroller or accompanying person) ◯ unable to walk, even supported
Stance Proband is asked to stand (1) in natural position, (2) with feet together in parallel (big toes touching each other) and (3) in tandem (both feet on one line, no space between heel and toe). Proband does not wear shoes, eyes are open. For each condition, three trials are allowed. Best trial is rated.	◯ normal, able to stand in tandem for > 10 s ◯ able to stand with feet together without sway, but not in tandem for > 10s ◯ able to stand with feet together for > 10 s, but only with sway ◯ able to stand for > 10 s without support in natural position, but not with feet together ◯ able to stand for >10 s in natural position only with intermittent support ◯ able to stand >10 s in natural position only with constant support of one arm ◯ unable to stand for >10 s even with constant support of one arm
Sitting Proband is asked to sit on an examination bed without support of feet, eyes open and arms outstretched to the front.	◯ normal, no difficulties sitting >10 sec ◯ slight difficulties, intermittent sway ◯ constant sway, but able to sit > 10 s without support ◯ able to sit for > 10 s only with intermittent support ◯ unable to sit for >10 s without continuous support
Speech disturbance Speech is assessed during normal conversation.	◯ normal ◯ suggestion of speech disturbance ◯ impaired speech, but easy to understand ◯ occasional words difficult to understand ◯ many words difficult to understand ◯ only single words understandable ◯ speech unintelligible / anarthria

Finger chase (right hand side) Proband sits comfortably. If necessary, support of feet and trunk is allowed. Examiner sits in front of proband and performs 5 consecutive sudden and fast pointing movements in unpredictable directions in a frontal plane, at about 50 % of probands reach. Movements have an amplitude of 30 cm and a frequency of 1 movement every 2 s. Proband is asked to follow the movements with his index finger, as fast and precisely as possible. Average performance of last 3 movements is rated.	◯ no dysmetria ◯ dysmetria, under/ overshooting target < 5 cm ◯ dysmetria, under/ overshooting target < 15 cm ◯ dysmetria, under/ overshooting target > 15 cm ◯ unable to perform 5 pointing movements
Finger chase (left hand side) Proband sits comfortably. If necessary, support of feet and trunk is allowed. Examiner sits in front of proband and performs 5 consecutive sudden and fast pointing movements in unpredictable directions in a frontal plane, at about 50 % of probands reach. Movements have an amplitude of 30 cm and a frequency of 1 movement every 2 s. Proband is asked to follow the movements with his index finger, as fast and precisely as possible. Average performance of last 3 movements is rated.	◯ no dysmetria ◯ dysmetria, under/ overshooting target < 5 cm ◯ dysmetria, under/ overshooting target < 15 cm ◯ dysmetria, under/ overshooting target > 15 cm ◯ unable to perform 5 pointing movements
Finger chase mean	_____
Nose-finger test (right hand side) Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to point repeatedly with his index finger from his nose to examiner's finger which is in front of the proband at about 90 % of proband's reach. Movements are performed at moderate speed. Average performance of movements is rated according to the amplitude of the kinetic tremor.	◯ no tremor ◯ tremor with an amplitude < 2 cm ◯ tremor with an amplitude < 5 cm ◯ tremor with an amplitude > 5 cm ◯ unable to perform 5 pointing movements
Nose-finger test (left hand side) Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to point repeatedly with his index finger from his nose to examiner's finger which is in front of the proband at about 90 % of proband's reach. Movements are performed at moderate speed. Average performance of movements is rated according to the amplitude of the kinetic tremor.	◯ no tremor ◯ tremor with an amplitude < 2 cm ◯ tremor with an amplitude < 5 cm ◯ tremor with an amplitude > 5 cm ◯ unable to perform 5 pointing movements
Nose-finger test mean	_____

Fast alternating hand movements (right hand side) Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to perform 10 cycles of repetitive alternation of pro- and supinations of the hand on his/her thigh as fast and as precise as possible. Movement is demonstrated by examiner at a speed of approx. 10 cycles within 7 s. Exact times for movement execution have to be taken.	◯ normal, no irregularities (performs < 10s) ◯ slightly irregular (performs < 10s) ◯ clearly irregular, single movements difficult to distinguish or relevant interruptions, but performs < 10s ◯ very irregular, single movements difficult to distinguish or relevant interruptions, performs >10s ◯ unable to complete 10 cycles
Fast alternating hand movements (left hand side) Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to perform 10 cycles of repetitive alternation of pro- and supinations of the hand on his/her thigh as fast and as precise as possible. Movement is demonstrated by examiner at a speed of approx. 10 cycles within 7 s. Exact times for movement execution have to be taken.	◯ normal, no irregularities (performs < 10s) ◯ slightly irregular (performs < 10s) ◯ clearly irregular, single movements difficult to distinguish or relevant interruptions, but performs < 10s ◯ very irregular, single movements difficult to distinguish or relevant interruptions, performs >10s ◯ unable to complete 10 cycles
Fast alternating hand movements mean	_____
Heel-shin slide (right hand side) Proband lies on examination bed, without sight of his legs. Proband is asked to lift one leg, point with the heel to the opposite knee, slide down along the shin to the ankle, and lay the leg back on the examination bed. The task is performed 3 times. Slide-down movements should be performed within 1 s. If proband slides down without contact to shin in all three trials, rate 4.	◯ normal ◯ slightly abnormal, contact to shin maintained ◯ clearly abnormal, goes off shin up to 3 times during 3 cycles ◯ severely abnormal, goes off shin 4 or more times during 3 cycles ◯ unable to perform the task
Heel-shin slide (left hand side) Proband lies on examination bed, without sight of his legs. Proband is asked to lift one leg, point with the heel to the opposite knee, slide down along the shin to the ankle, and lay the leg back on the examination bed. The task is performed 3 times. Slide-down movements should be performed within 1 s. If proband slides down without contact to shin in all three trials, rate 4.	◯ normal ◯ slightly abnormal, contact to shin maintained ◯ clearly abnormal, goes off shin up to 3 times during 3 cycles ◯ severely abnormal, goes off shin 4 or more times during 3 cycles ◯ unable to perform the task
Heel-shin slide mean	_____
SARA total	_____

Myoclonus

Participant ID	_____
Does the patient have myoclonus?	◯ Yes ◯ No
Age of onset in years	☐ 0-5 ☐ 6-10 ☐ 11-15 ☐ 16-20 ☐ 21-25 ☐ 26-30 ☐ 31-35 ☐ 36-40 ☐ 41-45 ☐ 46-50 ☐ 51-55 ☐ 56-60
Frequency	☐ Rare ☐ Monthly ☐ Weekly ☐ Daily ☐ Hourly
Area Affected	☐ Generalised ☐ Focal
Stimulus provoked	◯ Yes ◯ No

Timed get up and go test

Participant ID	_____
Can the patient walk?	◯ Yes ◯ No
Instructions to patient When I say "Go," I want you to: 1. Stand up from the chair 2. Walk to the line on the floor at your normal pace 3. Turn 4. Walk back to the chair at your normal pace 5. Sit down again	_____ (Seconds)

Haematology

Participant ID	_____
Acanthocytes	_____ (%)
Haemaglobin	_____ (g/dl)
Red Cell Count	_____ (10^12/l)
MCV	_____ (fl)
MCH	_____ (pg)
MCHC	_____ (g/dl)
RDW	_____ (%)
White Cell Count	_____ (10^9/l)
Neutrophils	_____ (%)
Neutrophils Abs	_____ (10^9/l)
Lymphocytes	_____ (%)
Lymphocytes Abs	_____ (10^9/l)
Monocytes	_____ (%)

Monocytes Abs	_____ (10^9/l)
Eosinophils	_____ (%)
Eosinophils Abs	_____ (10^9/l)
Basophils	_____ (%)
Basophils Abs	_____ (10^9/l)
Platelets	_____ (10^9/l)

Radiology

Participant ID	_____
Total Brain Volume (TBV)	_____ (cm3)
Total CSF volume	_____ (cm3)
Area of midbrain	_____ (cm2)
Corpus Callosum Midline Area	_____ (cm2)
Intracranial Midline Area	_____ (cm2)
Corpus Callosum Midline Area to Intracranial Midline Area	_____ (cm2)
Right Caudate Nucleus Volume	_____ (cm3)
Left Caudate Nucleus Volume	_____ (cm3)
Total Caudate Nucleus Volume	_____ (cm3)
Combined caudate nucleus volume to TBV ratio	_____
Right caudate nucleus volume to TBV ratio	_____
Left caudate nucleus volume to TBV ratio	_____
Right Putamen Volume	_____ (cm3)

Left Putamen Volume	_____ (cm3)
Total Putamen Volume	_____ (cm3)
Putamen volume to TBV ratio	_____
Right Putamen Volume to TBV ratio	_____
Left Putamen Volume to TBV ratio	_____
Right Nucleus Accumbens	_____ (cm2)
Left Nucleus Accumbens	_____ (cm2)
Total Nucleus Accumbens Volume	_____
Total Nucleus Accumbens Volume to Total Brain Volume	_____
Maximal bifrontal ventricular distance	_____ (cm)
Transverse width of brain (Frontal)	_____ (cm)
Bifrontal cerebroventricular index	_____
Other Findings	_____

9.3 Appendix 3: Ethics, permissions, copyrights and “Turn it in” report.

1. University of the Witwatersrand Human Research Ethics Committee (Medical) ethics certificate
2. Co-author permission forms
3. Letter of permission to use the UHDRS
4. Turn it in report

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9.3.1 Ethics clearance certificate

R14/49 Dr David Graham Anderson et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140872

NAME: (Principal Investigator)	Dr David Graham Anderson et al
DEPARTMENT:	Neurology Wits Donald Gordon Medical Centre Charlotte Maxeke Johanesburg Academic Hospital Bloemfontein, Polokwane and Northen Gauteng
PROJECT TITLE:	The Phenotype of Huntington Disease Like 2 (HDL2)
DATE CONSIDERED:	29/08/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof A Krause
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	09/10/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date 31-10-2014

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

9.3.2 Co Author agreement Declarations for each publication

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG | FACULTY OF HEALTH SCIENCES

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, David Graham Anderson student number 9501360R, declare that this Thesis/Dissertation/Research Report is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Thesis/Dissertation/Research Report.

Signature of Student ..... **Date** 30 April 2018

Name of Primary Supervisor.....Prof Amanda Krause.....

Signature of Primary Supervisor ..... **Date** 22/04/2019

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article(s) by the student as part of his/her Thesis/Dissertation/Research Report. In cases where the student is not the 1st author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for his/her degree purposes.

Article: Title: A Systematic Review of the Huntington Disease-Like 2 Phenotype
Journal name, year, volume and page numbers: Journal of Huntington's Disease 6 (2017) 37–46

Authors	Name	Signature	Date
1st author	David G. Anderson		30 April 2018
2nd author	Ruth H. Walker		April 30 2018
3rd author	Myles Connor		06 May 2018
4th author	Jonathan Carr		1 March 2019
5th author	Russell L. Margolis		Mar 7 2019
6th author	Amanda Krause		22/04/2019

Comments by primary supervisor:

.....

.....

1

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FACULTY OF HEALTH SCIENCES

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, David Graham Anderson student number 9501360R, declare that this Thesis/Dissertation/Research Report is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Thesis/Dissertation/Research Report.

Signature of Student ..... **Date** 27.04.2018

Name of Primary Supervisor.....Prof Amanda Krause.....

Signature of Primary Supervisor ..... **Date** 22/04/2019

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Article: Title: Absence of Acanthocytosis in Huntington's Disease-like 2: A Prospective Comparison with Huntington's Disease

Journal name, year, volume and page numbers: Tremor and Other Hyperkinetic Movements December 5, 2017: 7; 512

Authors	Name	Signature	Date
1st author	David G. Anderson		27.04.2018
2nd author	Sergio Carmona		30 Apr.18
3rd author	Kubendran Naidoo		07/05/18
4th author	Theresa L. Coetzer		07/May/2018
5th author	Jonathan Carr		April 30 2018
6th author	Dobrila D. Rudnicki	Dobrila D. Rudnicki -S Digitally signed by Dobrila D. Rudnicki -S DN: c=US, o=U.S. Government, ou=HHS, ou=NIH, ou=People, 0.9.2342.19200300.100.1.1=2002144842, cn=Dobrila D. Rudnicki -S Date: 2018.05.07 07:49:59 -04'00'	
7th author	Ruth H. Walker		April 30 2018
8th author	Russell L. Margolis		7 Mar 2019
9th author	Amanda Krause		22/04/2019

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Signature of Student ..... **Date** 22 January 2019

Name of Primary Supervisor.....Prof Amanda Krause.....

Signature of Primary Supervisor .....**Date** 22/04/2019

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Article: Title: Emerging differences between Huntington's disease-like 2 and Huntington's disease: A comparison using MRI brain volumetry

Journal name, year, volume and page numbers: Tremor and Other Hyperkinetic Movements January, 2019: 21; 101666

Authors	Name	Signature	Date
1st author	David G. Anderson		22 January 2019
2nd author	Mark Haagensen		6 February 2019
3rd author	Aline Ferreira-Correia		18-Feb-2019
4th author	Ronald Pierson		1/30/19
5th author	Jonathan Carr		20 Feb 2019
6th author	Amanda Krause		22/04/2019
7th author	Russell L. Margolis		7 Mar 2017

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG | FACULTY OF HEALTH SCIENCES

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, David Graham Anderson student number 9501360R, declare that this Thesis/Dissertation/Research Report is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Thesis/Dissertation/Research Report.

Signature of Student ..... **Date**.....

Name of Primary Supervisor.....Prof Amanda Krause.....

Signature of Primary Supervisor .....**Date**.....22/04/2019.....

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Article: Comparison of the Huntington's disease-like 2 and Huntington's disease clinical phenotypes

Journal name, year, volume and page numbers: Accepted for publication Movement Disorders Clinical Practice, 13 February 2019,

Authors	Name	Signature	Date
1st author	David G. Anderson		14 Feb 2019
2nd author	Aline Ferreira-Correia		18-Feb-2019
3rd author	Filipe B. Rodrigues		14 Feb 2019
4th author	N. Ahmad Aziz		15 Feb 2019
5th author	Jonathan Carr		4 March 2019
6th author	Edward J. Wild		15 Feb 2019
7th author	Russell L. Margolis		7 March 2019
8th author	Amanda Krause		22/04/2019

Comments by primary supervisor:

.....

.....

9.3.3 Permission to use the Unified Huntington's Disease Rating Scale



August 22, 2014

David G. Anderson, MBBCh MMED FCNeurol
University of the Witwatersrand
Donald Gordon Medical Centre
19 Eton Rd.
Johannesburg 2193, South Africa

Re: Use of Unified Huntington Disease Rating Scale (UHDRS)

Dear Dr. Anderson,

The Huntington Study Group (HSG) has received your request to use the Unified Huntington Disease Rating Scale training video (UHDRS Video) for your personal education and familiarization with the UHDRS assessments (the "Purpose"). We are writing to confirm that the Huntington Study Group has granted approval for you to use the UHDRS Video.

Permission is required to use the UHDRS Video to be used exclusively for the Purpose specified above on satisfaction of the following conditions:

1) You will not reproduce, translate, modify, sell, or distribute any portion of the UHDRS Video;

2) You will not incorporate the UHDRS Video into clinical trials, training or certification programs or materials, software programs, or otherwise without written permission by the HSG;

3) You acknowledge that this authorization does not include permission to use the UHDRS Video for any commercially sponsored studies or other commercial purpose;

4) You agree that the fee to use the UHDRS Video is $100USD (includes shipping costs) and must be prepaid; and

5) You agree to ensure that the clinicians who use the UHDRS Video will understand the obligations contained herein including that the copyright designations on the UHDRS Video remain in its current format.

Please make checks payable to the "Huntington Study Group, Ltd." and send to: ATTN: Shari Kinel, Huntington Study Group, 2604 Elmwood Avenue, Suite 335, Rochester, NY 14618-2295. Alternatively, payment can be made online via our PayPal account here (please note that this is for video payment when submitting). Upon reception of payment and a signed letter agreement, we will send to you the UHDRS Video.

I trust this letter is responsive to your inquiry. Please contact Shari Kinel, JD, Executive Director of the Huntington Study Group (585.319.3591, shari.kinel@hsglimited.org) if you have any questions.

Sincerely yours,

Shari Kinel, JD
Executive Director
Huntington Study Group

By signing below, I agree to the terms stated herein.

Date

Signature

Printed Name

Title

Name of Institution

9.3.4 “Turn it in” report

NATIONAL HEALTH LABORATORY SERVICE
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DIVISION OF HUMAN GENETICS
Hospital Street, Johannesburg, 2001 | PO Box 1038, Johannesburg, 2000
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4 June 2019

Postgraduate Office
Faculty of Health Sciences

To whom it may concern

Re: Turnitin report Dr David Graham Anderson (student number 9501360R).

As the primary supervisor for the PhD of Dr Anderson, I hereby confirm that he has submitted his final Thesis entitled “The Phenotype of Huntington’s Disease Like 2” to the Turnitin review system and the report is satisfactory and included in his thesis.

Yours sincerely,

Professor Amanda Krause, MBBCh, PhD
Medical Geneticist/Head of Division
Amanda.krause@nhls.ac.za

This report is intended solely to record the observations and/or opinion of the writer. It does not constitute a medico-legal report.

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89	Eduardo Castro, Pablo Polosecki, Irina Rish, Dorian Pustina, John H. Warner, Andrew Wood, Cristina Sampaio, Guillermo A. Cecchi. "Baseline multimodal information predicts future motor impairment in premanifest Huntington's disease", NeuroImage: Clinical, 2018 Publication	<1%
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93	Arika Hasegawa, Takeshi Ikeuchi, Ryoko Koike, Nae Matsubara et al. "Long-term disability and prognosis in dentatorubral-pallidoluysian atrophy: A correlation with CAG repeat length", Movement Disorders, 2010 Publication	<1%
94	Mahner, M.. "What Exactly Are Genomes, Genotypes and Phenotypes? And What About Phenomes?", Journal of Theoretical Biology, 19970507	<1%

9.4 Appendix 4: Papers published from this study

1. **Anderson DG**, Walker RH, Connor M, Carr J, Margolis RL, Krause A. A systematic review of the Huntington disease-like 2 phenotype. Journal of Huntington's disease. 2017 Jan 1;6(1):37-46. http://doi.org/10.3233/JHD-160232

2. **Anderson DG,** Carmona S, Naidoo K, Coetzer TL, Carr J, Rudnicki DD, Walker RH, Margolis RL, Krause A. Absence of Acanthocytosis in Huntington's Disease-like 2: A Prospective Comparison with Huntington's Disease. Tremor and Other Hyperkinetic Movements. 2017;7. http://doi.org/10.7916/D81J9PDX

3. **Anderson DG**, Haagensen M, Ferreira-Correia A, Pierson R, Carr J, Krause A, Margolis RL. Emerging differences between Huntington's disease-like 2 and Huntington's disease: A comparison using MRI brain volumetry. NeuroImage: Clinical. 2019 Jan 1;21:101666. https://doi.org/10.1016/j.nicl.2019.101666

4. **Anderson, D.G.**, Ferreira-Correia, A., Rodrigues F.B., Aziz N.A., Carr, J., Wild E.J., Krause, A. and Margolis, R.L., 2019. Comparison of the Huntington's disease-like 2 and Huntington's disease clinical phenotypes. Movement Disorders Clinical Practice. 6(4): 302–311. doi: 10.1002/mdc3.12742

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A Systematic Review of the Huntington Disease-Like 2 Phenotype

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Abstract.
Background: Huntington Disease-like 2 (HDL2) is a neurodegenerative disorder similar to Huntington Disease (HD) in its clinical phenotype, genetic characteristics, neuropathology and longitudinal progression. Proposed specific differences include an exclusive African ancestry, lack of eye movement abnormalities, increased Parkinsonism, and acanthocytes in HDL2.
Objective: The objective was to determine the similarities and differences between HD and HDL2 by establishing the clinical phenotype of HDL2 with the published cases.
Methods: A literature review of all clinically described cases of HDL2 until the end of 2016 was performed and a descriptive analysis was carried out.
Results: Sixty-nine new cases were described between 2001 and 2016. All cases had likely African ancestry, and most were found in South Africa and the USA. Many features were found to be similar to HD, including a strong negative correlation between repeat length and age of onset. Chorea was noted in 48/57 cases (84%). Dementia was reported in 74% patients, and Parkinsonism in 37%. Psychiatric features were reported in 44 out of 47 cases. Patients with chorea had lower expanded repeat lengths compared to patients without chorea. Eye movements were described in 19 cases, 8 were abnormal. Acanthocytes were detected in 4 of the 13 patients tested. There were 19 out of 20 MRIs reported abnormal with findings similar to HD.
Conclusion: This review clarifies some aspects of the HDL2 phenotype and highlights others which require further investigation. Features that are unique to HDL2 have been documented in a minority of subjects and require prospective validation.

Keywords: Acanthocytosis, chorea, HDL2, Huntington disease-like 2, Huntington's disease, junctophilin-3

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INTRODUCTION

Huntington Disease like 2 (HDL2) is the Huntington Disease (HD) genocopy[1] that most strongly resembles HD clinically and radiologically [1, 2]. Both are trinucleotide repeat disorders. HD is caused by a CAG repeat expansion occurring in exon 1 of the *huntingtin* gene on chromosome 4p16.3, while HDL2 is due to a CTG/CAG expansion mutation in exon 2A of the *junctophilin-3* (*JPH*3) gene on chromosome 16q24.3 [3]. Since the discovery of the disease, there have been significant advances in the understanding of the genetics of HDL2, including data generated on the length of the repeat expansion and the haplotype associated with the mutation [4]. However, the clinical description so far derives from reports of small case series, scattered families, or isolated individuals. The phenotype has not been systematically or objectively reviewed.

Historical overview/background

The first eight cases of HDL2 were described in an African-American pedigree from the southeastern USA in 2001 [1]. The initial cases were found to have symptoms starting in the fourth decade of life with a movement disorder, psychiatric symptoms, weight loss, dementia, and a progressive and fatal course that was very similar to that of HD. The same authors identified the causative mutation in *JPH3* [3]. The gene encodes a junctional membrane protein (junctophilin-3) that binds the plasma membrane to endoplasmic reticulum in neurons. JPH3 is expressed predominantly in the brain and testes [5].

In 2002, a family with three cases was published and described as "autosomal dominant chorea–acanthocytosis" [6]. These cases showed dementia, chorea, and Parkinsonism but lacked the orolingual dystonia and other typical features commonly seen in chorea–acanthocytosis. The first patient in this family was initially clinically diagnosed with HD. The clinical phenotype, autosomal dominant inheritance pattern and presence of acanthocytes in all three cases, suggested that the disorder was a novel variant of chorea–acanthocytosis. Subsequently the affected individuals were found to have a mutation in the *JPH3* gene [7].

In Johannesburg, in 2002, seven additional cases were identified from a laboratory based cohort [8]. In black South African patients who tested positive for an HD-causing mutation, approximately 35% had a *JPH3* mutation and 65% an HD mutation. This added further weight to the hypothesis that HDL2 is an African disease. Larger studies have confirmed the high frequency of *JPH3* mutations in patients of African ancestry with a HD phenotype [4].

The HDL2 phenotype was established as an autosomal dominant inherited disorder that manifests in the third or fourth decade, characterized by chorea, dystonia, and/or Parkinsonism, progressive weight loss, and cognitive deficits. The disease progresses to death over the course of approximately 20 years [9].

Knowledge of the HDL2 phenotype remains fragmentary and incomplete, with the likely consequence that HDL2 is underdiagnosed. Improved understanding of the phenotype will be essential for the treatment and education of patients and their families. To achieve this, we need to more fully describe the phenotype, including its presentation, course, and complications.

We have systematically reviewed all the available published cases of HDL2, and analysed the available clinical data, to provide a more detailed description of the phenotype. This review is designed to assist the clinician in recognizing clinical features of HDL2, differentiating HDL2 from HD, and defining aspects of the HDL2 phenotype which are not yet clear and will therefore require future study.

METHODOLOGY

The details of the review process are summarized in Fig. 1. We searched MEDLINE, Google Scholar, EMBASE and Scopus in December 2016. The terms "Huntington's Disease Like 2" or "HLD2" or "junctophilin 3" or "JPH3" or "Huntington Disease" were always used in combination with the terms "phenotype" or "case report" or "case series" or "clinical" or "report" or "acanthocytosis" or "neuroacanthocytosis" or "Africa" or "African" or "phenocopy". Where possible, filters were set for studies pertaining to humans, and included articles written in all languages. No time limits were set to gather all articles published to the end of December 2016.

[1]The terms genocopy and phenocopy have been used interchangeably to define diseases of similar phenotype with different genetic causes. Strictly speaking this is the definition for the term "genocopy", where "phenocopy" refers to similar phenotypic traits caused by environmental factors. The term phenocopy will be used in this instance because it is most commonly used in the literature and in most cases, assumed to mean diseases with similar phenotypes of different genetic origins.

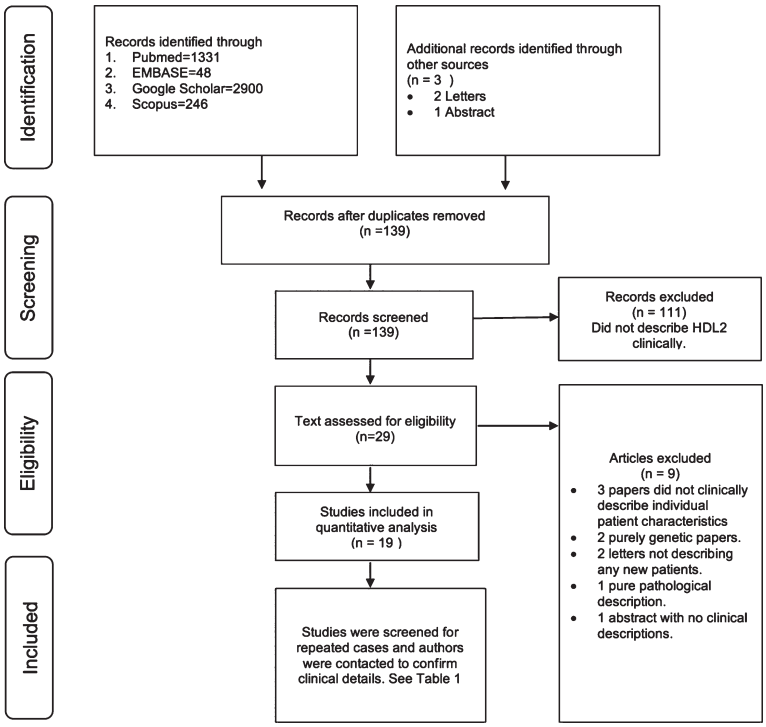

Fig. 1. Huntington Disease Like 2: Systematic review record selection. Strategy used to systematically review the HDL2 literature.

Articles were excluded if they did not have an association with HDL2, were purely laboratory based, did not describe HDL2 clinically, relied on previously described cases or did not add to the clinical description of previously described cases. Available clinical data were extracted and descriptive statistical analysis performed.

Descriptive analysis of the data was carried out using SAS version 9.4 for Windows. We analysed repeat size, age of onset, duration of disease, presenting symptoms (cognitive, psychiatric, or movement disorder), and the presence or absence of Parkinsonism, bradykinesia, chorea, dystonia, dementia, psychiatric symptoms, and acanthocytes. Age of onset was defined as the age of first onset of symptoms stated or implied in a publication.

RESULTS

We initially identified 30 articles in which new clinical features or new cases of HDL2 were described. Once publications with repeat descriptions of the same patients were excluded, 19 articles remained. These articles provided descriptions of clinical features in 69 novel cases of HDL2. All of these cases were confirmed to have the causative genetic mutation, although the size of the abnormal repeats were not always reported. Table 1 shows a breakdown of the origins of the cases. Authors were contacted for confirmation where doubt remained about duplicated cases.

While clinical descriptions were available for the 69 cases, many of these were incomplete. Positive clinical features were noted, but there were few records of negative or absent features. The majority of publications were case reports with minimal longitudinal follow up data available. Table 2 summarises the available clinical data on the 69 patients. Publications seldom provided family history or inheritance pattern information, and insufficient transmissions were documented to comment on anticipation in HDL2.

Table 1
Sources of HDL2 patients after the completed review process

Authors	Year	Number of patients	Number of new patients
Margolis R et al. [1]	2001	8	8
Holmes SE et al. [3]	2001	6	4
Stevanin G et al. [25]	2002	1	0
Walker RH et al. [6]	2002	3	3
Stevanin G et al. [54]	2003	2	1
Walker RH et al. [7]	2003	9	4
Walker RH et al. [9]	2003	5	0
Teive HAG et al. [55]	2007	1	1
Greenstein PE et al. [21]	2007	2	2
Bardien S et al. [18]	2007	3	3
Wild EJ et al. [2]	2008	1	1
Santos C et al. [22]	2008	1	1
Rodrigues GGR [23]	2008	4	4
Fischer CA et al. [19]	2012	1	1
Schneider SA et al. [13]	2012	6	6
Paradisi I et al. [10]	2012	4	4
Krause A et al. [4]	2015	22	22
Mariani LL et al. [20]	2016	3	3
Vasconcellos LFR et al. [16]	2016	1	1
Total			69

Table 2
Clinical results

	Characteristic	n[1]			
A	Gender Ratio (M:F)	35	23 : 12		
			Range	Mean	Median
	Repeat size	65	39–59	48	47
	Age of onset (years)	54	12–66	41.0	35
	Duration of disease (years)	27	1–22	9.1	17
B	First sign	24	Reported		
	Cognitive dysfunction		13		
	Psychiatric Symptoms		14		
	Movement disorder		11		
			Present	Absent	
C	Dementia	51	50	1	
	Psychiatric features	47	44	3	
	Chorea	57	48	9	
	Parkinsonism	26	26	–	
	Dystonia	16	16	–	
	Dysarthria	21	21	–	
	Dysphagia	4	4	–	
	Abnormal Eye movements	19	8	11	
	Myoclonus	4	4	–	
	Primitive Reflexes	16	10	6	
	Acanthocytes	13	4	8	

KEY. n[1]: Number of cases in whom information was available out of possible 69 cases. A: Demographic and Genetic Results. B: First sign findings. C: Clinical features.

Demographic and genetic findings

The majority of the patients were identified in South Africa and the USA, and were of African or African-American ancestry respectively (Table 3). The ancestry of patients with HDL2 is likely African in all cases, even if the ancestry is relatively distant. The shared common origin of these mutations is supported by studies that have shown a shared core haplotype in all patients tested, including those from South Africa, the USA, Mexico and the French West Indies [4] Although haplotyping has not been performed in the patients from Brazil and Venezuela, the historical links of these countries with Africa would suggest the origin of these patients is similar [9, 10].

Table 3
Countries of origin of described HDL2 cases

Country	Number of HDL2 reported cases	Percentage
South Africa	25	36%
United States of America	24	35%
Brazil	7	10%
Venezuela	4	6%
Mexico	3	4%
West Indies	2	3%
Congo	1	1%
Haiti	1	1%
Morocco	1	1%
United Kingdom	1	1%

The repeat length distribution is shown in Fig. 2. Although the median repeat expansion size (47 repeats) in HDL2 appears somewhat larger than in HD (43 repeats) [11], the mean age of onset for HDL2 is 41 years, very similar to that of HD of 40 years [12]. In HD the age of onset is defined as the age when movement abnormalities reportedly began; in the HDL2 publications initial symptoms included psychiatric and cognitive features. The age of onset showed a normal distribution with a range of 12 to 66 years. Gender was reported in 35 cases (12 females and 23 males). The ratio of 1.9 : 1 was not significantly different from 1 : 1 as seen in HD (chi-square test; $p = 0.10$).

There is a strong negative correlation between repeat length and age of onset in HDL2 (Pearson's $r = -0.76$; $p < 0.0001$; large effect size) (Fig. 3). This supports the findings of smaller HDL2 studies that showed similar correlations [1, 4, 13]. The coefficient of determination (r^2) value in this HDL2 group is = 0,585 as seen in Fig. 3. This is quite similar to the r^2 value of 0.642 in HD in a North American, European, and Australian sample (the HD MAPS cohort) [14].

The youngest age of onset, 12 years, was reported in a patient from Mexico with a repeat length of 49 repeats and a presentation of depression, mild chorea and aggressiveness [7]. The case is unusual for two

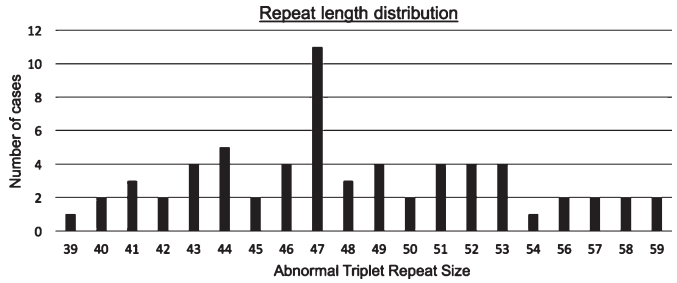

Fig. 2. Repeat length distribution. Graph showing the frequency of abnormal triplet repeats in *JPH3* from the described cases in the literature.

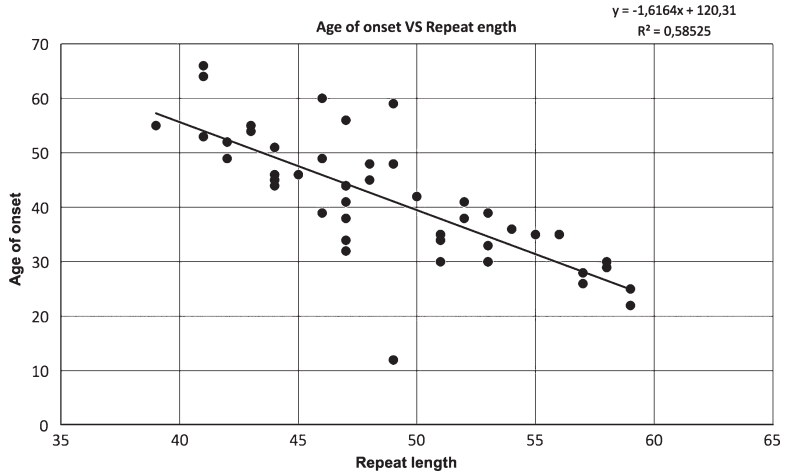

Fig. 3. Age of onset vs repeat length. Graph showing an inverse correlation of age of onset and triplet repeat length in HDL2 from all described cases in the literature.

reasons: the much younger than expected age at onset based on repeat length, and the absence of the Parkinsonian symptoms typically found in juvenile onset HD [15]. This case may demonstrate that patients with HDL2 may present at younger ages than would be predicted by their triplet repeat size and with unexpected clinical features. Alternatively, clinical signs unrelated to HDL2 may have led to the serendipitous finding of the HDL2 mutation.

Clinical features

The presence or absence of chorea was reported in 57 cases. Chorea was present in 48 cases (84%). Parkinsonism was reported in seven of the cases with chorea, and dystonia was reported in six of these seven. The median expanded repeat length was significantly lower for those with chorea (47; IQR 44–51) compared to those without chorea (52.5; IQR 50–54) ($n = 51$; Wilcoxon rank sum test; $p = 0.030$). The average repeat size for the group with chorea was 48 and age of onset was 41 years of age. The average disease duration for this group was 6.5 years.

Parkinsonism was reported present in 26 (38%) cases. Of the 26, 23 were reported to have bradykinesia, 17 rigidity and 10 dystonia. The mean repeat length for this group was 51 triplets, age of onset 39 years, and disease duration 8.7 years.

Dysarthria was reported in 21 of the 69 cases, and was noted as moderate or mild. Eye movements were reported to be abnormal in only 8/19 cases [1, 13, 16, 17]. Generalized epilepsy was not reported,

but four cases were reported to have myoclonus [13, 18].

The presence of dementia was noted in 51 of the 65 cases, with only one case from a retrospective record review from South African specifically recording its absence [4]. Prominent dementia is described in most cases, and in the single report which explored this further, there were prominent frontal lobe features, working memory deficits and psychiatric features, similar to HD [19]. The presence of primitive reflexes were reported in 10/16 cases, all in patients with an associated dementia. The Unified Huntington Disease Rating Scale (UHDRS) has only been reported in three more recently published papers [13, 16, 20]. In one paper the verbal fluency, Stroop and symbol digit modalities tests could not be completed due to mutism or severe dementia [13].

There were 44 out of 47 cases reported to have psychiatric findings, and only three cases in which there were no psychiatric features present. These three were again from the South African retrospective record review [4]. The psychiatric findings included depression (seven reports), hallucinations (two reports) and aggression (four reports). It has been suggested that the heterogeneous psychiatric phenotype is similar to that found in HD [19].

From the original family described, worsening of the chorea and an increase in bradykinesia was reported with disease progression [1]. With longitudinal descriptions in patients there have been reports of generalized spasticity, hyperactive deep tendon reflexes, and extensor plantar responses. Clinical progression has been described in only five of the papers reviewed [1, 19, 21–23].

Acanthocytes

Acanthocytes were detected in only four of the thirteen patients tested. [6, 9, 16, 18, 20, 23]. In those four patients, more than 30% of the erythrocyte population were acanthocytes, significantly greater than the normal population (less than 6.3% of erythrocytes are acanthocytes on a blood smear) but similar to the findings in chorea-acanthocytosis (typically higher than 28% [24]). To the best of our knowledge acanthocytosis has never been reported in HD.

Neuroimaging

There have been 20 reported MRIs in HDL2 cases; 19 were reported as abnormal. Generalized atrophy was described in all the cases with more severe atrophy of the caudate nucleus and putamen [1, 13, 18, 19, 22, 23, 25]. The single normal MRI is unexpected as this patient had a CT scan of the brain three years later which showed diffuse atrophy [6]. This is remarkable as this patient was found with two triplet expansion diseases and was also diagnosed with Fragile X syndrome. White matter lesions and atrophy are MRI features in Fragile X syndrome as well as in Fragile X-Associated Tremor/Ataxia Syndrome [26]. In two advanced cases, atrophy was generalized but affecting the parietal–occipital regions severely [13]. The brainstems and cerebellar regions are comparatively well-preserved when described.

DISCUSSION

Since HDL2 was first described in 2001 there have only been a small number of cases reported and the descriptions have often been incomplete. There are however a number of consistent observations that are useful.

Demographic and genetic findings

Mutations of *C9ORF72*, spinocerebellar ataxia (SCA) 17 and HDL2 are variably reported as the most common HD phenocopies globally [2, 27]. In South Africa, HDL2 accounts for one third of patients of black and mixed ancestry with an HD phenotype and a confirmed diagnosis [4]. In a recent study of HD and HDL2 in South Africa the estimated combined minimum disease frequency was found to be higher than previously thought. A figure of 0.25 : 100 000 in black and 2.10 : 100 000 in mixed ancestry populations was described as an underestimate due to the probable under-reporting of both diseases in an under-resourced country [28]. HDL2 is therefore an important consideration in the differential diagnosis of HD phenocopies.

HDL2 joins many of the other repeat expansion neurodegenerative diseases with an inverse correlation of repeat length and age of onset, including the SCAs, spinobulbar muscular atrophy (SMA), dentatorubropallidoluysian atrophy (DRPLA) and HD [15, 29, 30]. This has important implications in an attempt to understand the pathophysiology and potentially unravel the mechanism whereby these diseases have similar phenotypic features [31].

Huntington's disease and HDL2 have very similar repeat ranges associated with age of onset. Other CAG-repeat disorders have closely related, but

somewhat different repeat ranges associated with age of onset. Examples are the HD phenocopies due to expansions in SCA17 and DRPLA with ranges of 47–63 and 49–88 respectively [32, 33].

There is a similar strong negative correlation between repeat length and age of onset in polyglutamine repeat diseases [31]. We have shown that the co-efficient of determination values in HDL2 and HD are similar when compared to SCA17 (r^2–0.41) and DRPLA (r^2–0.865) [32, 33].

Movement disorder

Chorea was the most commonly reported movement abnormality in all published cases. Chorea was described as generalized or axial in most cases where the distribution was mentioned. Orofacial chorea was highlighted in two cases [19, 21]. In the original pedigree, chorea and dystonia were present in all of the patients [1], although this does not hold true in all subsequent descriptions.

In the HD phenotype, chorea is not always present throughout the progression of the disease. In most cases it is a dominating feature. However, as the disease progresses, dystonia and rigidity become more prominent and chorea less noticeable [15]. In early-onset of the disease, with a high number of repeats, chorea may be absent. Parkinsonism has been reported to be more common in HDL2 than in HD [1, 13]. Bradykinesia has long been recognised as a feature of HD when chorea is present and is aggravated by antipsychotic medication use [34]. A speculative picture emerges for HDL2 patients in which patients with shorter repeat lengths are more likely to have chorea, but the confounding factors of duration of disease, possibly coincident bradykinesia, and use of dopamine-blocking medications need to be taken into account.

Mild to moderate dystonia was reported in 14 cases, however the presence or absence of dystonia was not mentioned in the majority of reports. In the few cases with dystonia it was usually noted in conjunction with other features of Parkinsonism. Dystonia is reported in 95% of HD cases [35]. It may be that in the HDL2 cases it was not noted due to the prominence of other movement abnormalities, specifically chorea. Alternatively, dystonia may not be as common as it is in HD.

Dysarthria was reported in less than a third of the HDL2 cases in the literature, and was classified as moderate or mild. Dysarthria is common in HD occurring in up to 89% of cases [36]. Dysphagia was reported only in one study of six patients, where it was present in four cases, and absent in two [13]. The frequency and characteristics of dysphagia in HD are poorly described [37].

Preserved ocular motor function was suggested in the original family reported with HDL2 [1]. Abnormal eye movements are a cardinal and early feature of HD [38]. A third of cases from the described patients with HDL2 in the literature had abnormal eye signs. Whether this is a potential distinguishing feature between the two conditions, or if the eye signs change with the progression of HDL2 warrants further observation. When described the specific eye movement abnormalities were hypometric or jerky saccades and interrupted ocular pursuit [9, 13, 16]. It has also been suggested that there may be two phenotypes of HDL2, the first an early onset form which is more Parkinsonian and lacks ocular motor involvement and a second, late onset variant which is more typical of HD with the typical jerky horizontal saccades [39]. Eye movements are not often described in juvenile HD but have been described as abnormal in individual cases [40]. If eye movements are normal in HDL2 it would predict that the disease spares the brainstem and ocular motor control centres. This needs to be investigated on imaging and in neuropathological studies and dedicated systematic oculographic recordings.

Myoclonus has been reported in four cases. In one case, somatosensory evoked potentials (SEP) showed enlarged N20-P25 and P25-N33 components, indicating a possible cortical origin [18]. Epilepsy and myoclonus are unusual features in HD and usually occur in the juvenile variant of the disease. When myoclonus does present itself, large SEPs [41] are usually not found.

Cognition

Dementia is likely a universal feature in HDL2, but there are several unanswered questions. Is HDL2 dementia similar to the frontal and subcortical dementia seen in HD as has previously been suggested? Is HDL2 dementia always a heralding sign of disease onset, and does the dementia progress more rapidly than in HD? A significant challenge, where cognitive testing was performed, was that the cases were reported late when the cognitive impairment was already advanced. In order to describe the spectrum of cognitive dysfunction in HDL2, premanifest patients and early onset patients will need to be studied.

Psychiatric features

The psychiatric features of HDL2 are poorly documented but have been suggested to be similar to HD. In HD, depression is 4–6 times more common than in the general population but there is still no evidence to adequately guide antidepressant use [42]. Aggression is also a common feature, occurring in 66% of HD cases in some studies with no evidence-based treatment currently available [43].

First sign/s of disease (Table 2)

For the 24 cases in whom presenting signs and symptoms are described, similar frequencies of cognitive, psychiatric and movement features are documented. Some patients reported more than one presenting sign. It is important to define features of disease onset in HDL2. The TRACK-HD study defined clinical manifestations of HD and biomarkers longitudinally and has allowed for defined endpoints for therapeutic trials as well as a better understanding of the disease [44].

Disease progression

The papers that have described HDL2 patients longitudinally indicate a clinical picture of increasing Parkinsonism, corticospinal tract signs and worsening dementia similar to that in HD [1, 19, 21–23]. This might be expected as post mortem findings have shown that there is atrophy and disease pathology in the basal ganglia and both cortical and subcortical motor structures [45]. A similar progression has been noted in HD [46].

Acanthocytes

Neuroacanthocytosis syndromes are syndromes where acanthocytes are a cardinal feature in conjunction with neurological features. Mcleod Syndrome and Chorea Acanthocytosis are considered the "core" neuroacanthocytosis syndromes. Acanthocytes are not specific to these condtions and have been described in other conditions like pantothenate kinase–associated neurodegeneration [47].

HDL2 is currently classified as both an HD genoocopy and a neuroacanthocytosis syndrome, and often features in reviews of both disorders [2, 48, 49]. However, the association of HDL2 and acanthocytes remains unverified in the majority of patients [9]. No data exist on the relationship of acanthocytes to the trinucleotide repeat length or to duration of disease.

The association of HDL2 with acanthocytes may provide important clues about pathogenesis. Junctophilins are not thought to be present in red blood cell membranes [50]. In HDL2 and the core neuroacanthocytosis syndromes, chorea-acanthocytosis and Mcleod syndrome, abnormal phosphorylation of the proteins involved in connecting the membrane and cytoskeleton in the erythrocytes may be a common link to the production of acanthocytes [51].

Neuroimaging and neuropathology

Radiologically and pathologically, HDL2 is broadly similar to HD in its involvement of the neostriatum and basal ganglia and general atrophy with progression of disease [45]. HDL2 may differ, at least in some cases, in its degree of focal cortical involvement, notably of the occipital lobe. In one case with occipital lobe atrophy, visual failure was described. This patient had decreased visual acuity and was unable to follow visual commands [20]. HD was initially thought to preserve the nucleus accumbens but a strong correlation with poor functional and motor scores has now been found with atrophy of the nucleus accumbens [52]. In the HDL2 cases that have been studied, this nucleus was at least partially or significantly involved [21]. These differences between HD and HDL2 have been noted pathologically but only focal occipital atrophy has been noted radiologically in the cases of HDL2 and not nucleus accumbens involvement [13].

Research using a HDL2 mouse model has found overlapping polyQ-mediated mechanisms of pathogenesis in HD and HDL2 [53]. A more detailed understanding of HDL2 phenotype, an HD phenocopy caused by mutation in a gene with an entirely different function from *huntingtin,* may offer further evidence of a common pathogenesis and lead to the development of novel therapeutic approaches for both diseases.

CONCLUSION

The rarity of HDL2 makes describing the phenotype in a statistically meaningful way a challenge. Only 69 cases have been described over 15 years. This review has evaluated all published cases to the end of 2016 to consolidate the available data, and to provide the current view and understanding of the

clinical phenotype. The data are all retrospective, of varying quality, largely anecdotal and based on small patient groups. They also tend to be biased by the reporting of positive features, and the failure to report the absence of features. There is clearly a need for a further systematic and focused prospective study of cases. To address this need, the South African Huntington Disease Like 2 Study (SAHDLS), based in Johannesburg, where the HDL2 prevalence is the highest in the world, is currently collecting standardised clinical neuropsychological and MRI data and blood samples. We invite other clinicians to collect data from HDL2 patients using similar standardized methods to generate an international HDL2 database.

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REFERENCES

[1] Margolis RL, O'Hearn E, Rosenblatt A, Willour V, Holmes SE, Franz ML, et al. A disorder similar to Huntington's disease is associated with a novel CAG repeat expansion. Ann Neurol. 2001;50(3):373-80. doi:10.1002/ana.1124

[2] Wild EJ, Mudanohwo EE, Sweeney MG, Schneider SA, Beck J, Bhatia KP, et al. Huntington's disease phenocopies are clinically and genetically heterogeneous. Mov Disord. 2008;23(5):716-20. doi:10.1002/mds.21915

[3] Holmes SE, O'Hearn E, Rosenblatt A, Callahan C, Hwang HS, Ingersoll-Ashworth RG, et al. A repeat expansion in the gene encoding junctophilin-3 is associated with Huntington disease-like 2. Nat Genet. 2001;29(4):377-8. doi:10.1038/ng760

[4] Krause A, Mitchell C, Essop F, Tager S, Temlett J, Stevanin G, et al. Junctophilin 3 (JPH3) expansion mutations causing Huntington disease like 2 (HDL2) are common in South African patients with African ancestry and a Huntington disease phenotype. Am J Med Genet B Neuropsychiatr Genet. 2015;168(7):573-85. doi:10.1002/ajmg.b.32332

[5] Takeshima H, Komazaki S, Nishi M, Iino M, Kangawa K. Junctophilins: A novel family of junctional membrane complex proteins. Mol Cell. 2000;6(1):11-22. doi:10.1016/S1097-2765(05)00005-5

[6] Walker RH, Morgello S, Davidoff-Feldman B, Melnick A, Walsh MJ, Shashidharan P, et al. Autosomal dominant chorea-acanthocytosis with polyglutamine-containing neuronal inclusions. 2002;58(7):1031-7.

[7] Walker RH, Rasmussen A, Rudnicki D, Holmes SE, Alonso E, Matsuura T, et al. Huntington's disease–like 2 can present as chorea-acanthocytosis. Neurology. Lippincott Williams & Wilkins; 2003;61(7):1002-4. doi:10.1212/01.WNL.0000085866.68470.6D

[8] Krause A, Temlett J, Van der Meyden K, Ross CA, Callahan C, Margolis RL. CAG/CTG repeat expansions at the HDL2 locus are a common cause of Huntington disease in black South Africans. Am J Hum Genet. 2002;71,4:528-528.

[9] Walker RH, Jankovic J, O'Hearn E, Margolis RL. Phenotypic features of Huntington's disease-like 2. Mov Disord. 2003;18(12):1527-30. doi:10.1002/mds.10587

[10] Paradisi I, Ikonomu V, Arias S. Huntington disease-like 2 (HDL2) in Venezuela: Frequency and ethnic origin. J Hum Genet. 2012;58(1):3-6. doi:10.1038/jhg.2012.111

[11] Langbehn DR, Hayden MR, Paulsen JS, the PREDICT-HD Investigators of the Huntington Study Group. CAG-repeat length and the age of onset in Huntington disease (HD): A review and validation study of statistical approaches. Am J Med Genet B. 2010;153(2):397-408. doi:10.1002/ajmg.b.30992

[12] Ross CA, Tabrizi SJ. Huntington's disease: From molecular pathogenesis to clinical treatment. Lancet Neurol. 2011;10(1):83-98. doi:10.1016/S1474-4422(10)70245-3

[13] Schneider SA, Marshall KE, Xiao J, LeDoux MS. JPH3 repeat expansions cause a progressive akinetic-rigid syndrome with severe dementia and putaminal rim in a five-generation African-American family. Neurogenetics. 2012;13(2):133-40. doi:10.1007/s10048-012-0318-9

[14] Andresen JM, Gayán J, Djoussé L, Roberts S, Brocklebank D, Cherny SS, et al. The relationship between CAG repeat length and age of onset differs for Huntington's disease patients with juvenile onset or adult onset. Ann Hum Genet. 2006;71(3):295-301. doi:10.1111/j.1469-1809.2006.00335.x

[15] Walker FO. Huntington's disease. Lancet. 2007;369 (9557):218-28. doi:10.1016/S0140-6736(07)60111-1

[16] Vasconcellos LFR, Macêdo PJOM, Franck JB, Tumas V, Marques Júnior W, Spitz M. Huntington's Disease like 2 presenting with isolated Parkinsonism. J Neurol Sci. 2017;373:105-6. doi:10.1016/j.jns.2016.11.050

[17] Walker RH, Rasmussen A, Rudnicki D, Holmes SE, Alonso E, Matsuura T, et al. Huntington's disease–like 2 can present as chorea-acanthocytosis. Neurology. 2003;61(7):1002-4.

[18] Bardien S, Abrahams F, Soodyall H, van der Merwe L, Greenberg J, Brink T, et al. A South African mixed ancestry family with Huntington disease-like 2: Clinical and genetic features. Mov Disord. 2007;22(14):2083-9. doi:10.1002/mds.21672

[19] Fischer CA, Licht EA. The neuropsychiatric manifestations of Huntington's disease-like 2. J Neuropsychiatry ClinNeurosci. 2012;24(4):489-92. doi:10.1176/appi.neuropsych.11120358

[20] Mariani LL, Tesson C, Charles P, Cazeneuve C, Hahn V, Youssov K, et al. Expanding the spectrum of genes involved in Huntington disease using a combined clinical and genetic approach. JAMA Neurol. 2016;:1-10.

[21] Greenstein PE, Vonsattel J-PG, Margolis RL, Joseph JT. Huntington's disease like-2 neuropathology. Mov Disord. 2007;22(10):1416-23. doi:10.1001/jamaneurol.2016.2215

[22] Santos C, Wanderley H, Vedolin L, Pena S, Jardim L, Sequeiros J. Huntington disease-like 2: The first patient with apparent European ancestry. Clin Genet. 2008;73(5):480-5. doi:10.1111/j.1399-0004.2008.00981.x

[23] Rodrigues GGR, Walker RH, Brice A, Cazeneuve C, Russaouen O, Teive HAG, et al. Huntington's disease-like 2 in Brazil-Report of 4 patients. Mov Disord. 2008;23(15): 2244-7.

[24] Storch A, Kornhass M, Schwarz J. Testing for acanthocytosis. J Neurol. 2005;252(1):84-90. doi:10.1002/mds.22223

[25] Stevanin G, Camuzat A, Holmes SE, Julien C, Sahloul R, Dodé C, et al. CAG/CTG repeat expansions at the Huntington's disease-like 2 locus are rare in Huntington's disease patients. Neurology. 2002;58(6):965-7.
[26] Rodriguez-Revenga L, Gmez-Ansn B, Granell E, Pagonabarraga J, Mil M. Neuroimaging in Fragile X-associated. In: Bright P, edits. Neuroimaging - Clinical Applications. InTech; 2012. pp. 423-40. doi:10.5772/24016
[27] Moss DJH, Poulter M, Beck J, Hehir J, Polke JM, Campbell T, et al. C9orf72 expansions are the most common genetic cause of Huntington disease phenocopies. Neurology. 2014;82(4):292-9. doi:10.1212/WNL.0000000000000061
[28] Baine FK, Krause A, Greenberg LJ. The frequency of Huntington disease and Huntington disease-like 2 in the South African population. Neuroepidemiology. 2016;46(3):198-202. doi:10.1159/000444020
[29] Stevanin G, Durr A, Brice A. Clinical and molecular advances in autosomal dominant cerebellar ataxias: From genotype to phenotype and physiopathology. Eur J Hum Genet. 2000;8(1):4-18. doi:10.1038/sj.ejhg.5200403
[30] Piccioni F, Simeoni S, Andriola I, Armatura E, Bassanini S, Pozzi P, et al. Polyglutamine tract expansion of the androgen receptor in a motoneuronal model of spinal and bulbar muscular atrophy. Brain Res Bull. 2001;56(3-4):215-20. doi:10.1016/S0361-9230(01)00652-9
[31] Margolis RL, Ross CA. Expansion explosion: New clues to the pathogenesis of repeat expansion neurodegenerative diseases. Trends Mol Med. 2001;479-82.
[32] Stevanin G, Brice A. Spinocerebellar ataxia 17 (SCA17) and Huntington's disease-like 4 (HDL4). Cerebellum. 2008;7(2):170-8. doi:10.1007/s12311-008-0016-1
[33] Hasegawa A, Ikeuchi T, Koike R, Matsubara N, Tsuchiya M, Nozaki H, et al. Long-term disability and prognosis in dentatorubral-pallidoluysian atrophy: A correlation with CAG repeat length. Mov Disord. 2010;25(11):1694-700. doi:10.1002/mds.23167
[34] van Vugt JPP, van Hilten BJ, Roos RAC. Hypokinesia in Huntington disease. Mov Disord. 1996;11(4):384-8. doi:10.1002/mds.870110406
[35] Louis ED, Lee P, Quinn L, Marder K. Dystonia in Huntington's disease: Prevalence and clinical characteristics. Mov Disord. 1999;14(1):95-101. doi:10.1002/1531-8257(199901)14:1<95:AID-MDS1016>3.0.CO;2-8++++++
[36] Young AB, Shoulson I, Penney JB, Starosta-Rubinstein S, Gomez F, Travers H, et al. Huntington's disease in Venezuela: Neurologic features and functional decline. Neurology. 1986;36(2):244-9.
[37] Heemskerk A-W, Roos RAC. Dysphagia in Huntington's disease: A review. Dysphagia. 2010;26(1):62-6. doi:10.1007/s00455-010-9302-4
[38] Lasker AG, Zee DS. Ocular motor abnormalities in Huntington's disease. Vision Res. 1997;37(24):3639-45. doi:10.1016/S0042-6989(96)00169-1
[39] Margolis RL. Huntington Disease-Like 2. 2004 [Updated 2012 Apr 26]. In: Pagon RA, Adam MP, Ardinger HH, et al. GeneReviews®; 1993-2017. https://www.ncbi.nlm.nih.gov/books/NBK1529/
[40] Nance MA, Group UHDGT. Genetic testing of children at risk for Huntington's disease. Neurology. 1997;49(4):1048-53. doi:10.1212/WNL.49.4.1048
[41] Caviness JNJ, Kurth MM. Cortical Myoclonus in Huntington's disease associated with an enlarged somatosensory evoked potential. Mov Disord. 1997;12(6):1046-51. doi:10.1002/mds.870120633
[42] Moulton CD, Hopkins CWP, Bevan-Jones WR. Systematic review of pharmacological treatments for depressive symptoms in Huntington's disease. Mov Disord. 2014;29(12):1556-61. doi:10.1002/mds.25980
[43] Fisher CA, Sewell K, Brown A, Churchyard A. Aggression in Huntington's disease: A systematic review of rates of aggression and treatment methods. J Huntingtons Dis. 2014;3(4):319-32. doi:10.3233/JHD-140127
[44] Shannon KM, Fraint A. Therapeutic advances in Huntington's disease. Mov Disord. 2015;30(11):1539-46. doi:10.1002/mds.26331
[45] Rudnicki DD, Pletnikova O, Vonsattel J-PG, Ross CA, Margolis RL. A comparison of huntington disease and huntington disease-like 2 neuropathology. J Neuropathol Exp Neurol. 2008;67(4):366-74. doi:10.1097/NEN.0b013e31816b4aee
[46] Ross CA, Margolis RL. Huntington disease. Medicine (Baltimore). 1997;76:305-38.
[47] Hayflick SJ, Westaway SK, Levinson B, Zhou B, Johnson MA, Ching KHL, et al. Genetic, clinical, and radiographic delineation of Hallervorden-Spatz syndrome. N Engl J Med. 2003;348(1):33-40. doi:10.1056/NEJMoa020817
[48] Danek A, Walker RH. Neuroacanthocytosis. Curr Opin Neurol. 2005;18(4):386-92. doi:10.1097/01.wco.0000173464.01888.e9
[49] Velayos Baeza A, Dobson-Stone C, Rampoldi L, Bader B, Walker RH, Danek A, Monaco AP. Chorea-acanthocytosis. GeneReviews®, 1993-2017 http://www.ncbi.nlm.nih.gov/books/NBK1387/
[50] Nishi M, Mizushima A, Nakagawara K-I, Takeshima H. Characterization of human junctophilin subtype genes. Biochem Biophys Res Commun. 2000;273(3):920-7. doi:10.1006/bbrc.2000.3011
[51] Adjobo-Hermans MJ, Cluitmans JC, Bosman GJ. Neuroacanthocytosis: Observations, theories and perspectives on the origin and significance of acanthocytes. Tremor Other Hyperkinet Mov (N Y). 2015;5:328.
[52] van den Bogaard SJ, Dumas EM, Acharya TP, Johnson H, Langbehn DR, Scahill RI, et al. Early atrophy of pallidum and accumbens nucleus in Huntington's disease. J Neurol. 2011;258(3):412-20. doi 10.1007/s00415-010-5768-0
[53] Wilburn B, Rudnicki DD, Zhao J, Weitz TM, Cheng Y, Gu X, et al. An antisense CAG repeat transcript at JPH3 locus mediates expanded polyglutamine protein toxicity in Huntington's disease-like 2 mice. Neuron. 2011;70(3):427-40. doi:10.1016/j.neuron.2011.03.021
[54] Stevanin G, Fujigasaki H, Lebre A-S, Camuzat A, Jeannequin C, Dode C, et al. Huntington's disease-like phenotype due to trinucleotide repeat expansions in the TBP and JPH3 genes. Brain. 2003;126(Pt 7):1599-603. doi:10.1093/brain/awg155
[55] Teive HAG, Becker N, Munhoz RP, Brice A. Huntington's disease-like 2: The first case report in Latin America in a patient without African ethnic origin. Mov Disord. 2007;(Suppl. 16)(22):S27.

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Tremor and Other Hyperkinetic Movements

Brief Reports

Absence of Acanthocytosis in Huntington's Disease-like 2: A Prospective Comparison with Huntington's Disease

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Abstract

Background: Huntington's Disease-like 2 (HDL2) is classified as a neuroacanthocytosis; however, this remains unverified. We aim to determine if acanthocytes are present in HDL2 and whether acanthocytes can differentiate HDL2 from Huntington's disease (HD).

Methods: We prospectively compared 13 HD and 12 HDL2 cases against 21 unaffected controls in Johannesburg. Blood smears were prepared using international standards and reviewed by at least two blinded reviewers. An acanthocytosis rate of greater than 1.2% in the dry smear or greater than 3.7% in the wet smear was designated a priori as the threshold for clinical significance based on previously established standards. Flow cytometry was performed on all but four of the cases. Red cell membrane protein analysis was performed on all participants.

Results: There were 12 HDL2, 13 HD, and 21 controls enrolled. None of the HD or HDL2 participants had defined acanthocytosis or other morphological abnormalities. None of the HD or HDL2 cases had evidence of an abnormal band 3.

Discussion: Acanthocytosis was not identified in either HDL2 or HD in our patient population. Our results, based on the first prospective study of acanthocytes in HDL2 or HD, suggest that screening for acanthocytes will not help establish the diagnosis of HD or HDL2, nor differentiate between the two disorders and raises the question if HDL2 should be placed within the neuroacanthocytosis syndromes.

Keywords: Huntington's disease, Huntington's disease-like 2, acanthocytes, chorea

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Ethics Statement: This study was performed in accordance with the ethical standards detailed in the Declaration of Helsinki. The authors' institutional ethics committee has approved this study and all patients have provided written informed consent.

Introduction

Huntington's disease-like 2 (HDL2) is an autosomal dominant neurodegenerative disorder caused by a CTG/CAG expansion mutation in exon 2A of *junctophilin-3* (*JPH3*) on chromosome 16q24.3, which occurs in patients of African ancestry.[1] HDL2 is clinically and pathologically similar to Huntington's disease (HD), with progressive dementia and movement abnormalities comprising the predominant clinical phenotype.[2] Anecdotal case reports suggested that, compared to HD, eye movement abnormalities are less common and parkinsonism more common in HDL2.[3]

Acanthocytes were documented in some of the early reported cases of HDL2, resulting in diagnostic confusion with chorea–acanthocytosis.[4,5]

Acanthocytes are erythrocytes with spiky deformations of their membranes, and when found in high numbers are associated with many diseases. The source of acanthocytes in chorea–acanthocytosis, McLeod syndrome, and pantothenate kinase-associated neurodegeneration (and possibly also aceruloplasminemia) appears to be complex. Abnormal phosphorylation of the proteins connecting the membrane and cytoskeleton of erythrocytes, erythrocyte metabolism, the involvement of lipids in underlying signaling pathways, changes in lipid composition, and signal transduction have all been implicated.[6] The relationships of these potential mechanisms to neurodegeneration are unclear.[7]

A systematic review of all reported HDL2 patients showed acanthocytes were detected in four of 13 cases; however, the hematological findings in most HDL2 cases are unknown or unreported.[3] In three of these four cases, the affected individuals were members of the same pedigree, and one individual had a Fragile X mutation (660 triplet repeats), while the two affected relatives had repeats in the Fragile X pre-mutation range (57 and 56 repeats). Peripheral smears and electron microscopy revealed that 30–35% of the red blood cells were acanthocytes and red blood cell membrane extract showed an abnormal breakdown of band 3 proteins.[4] The fourth case was from a Mexican pedigree of likely African descent. Only one of the three family members with HDL2 had acanthocytes of 35% on peripheral smear, but cell membrane extracts did not show an abnormal band 3 protein.[5] These findings have led to the suggestion that HDL2 be classified as one the neuroacanthocytosis syndromes, and that the presence of acanthocytes might provide important insight into HDL2 pathogenesis, with implications for understanding HD and the neuroacanthocytoses. To our knowledge there have been no systematic efforts to document acanthocytes in HD.

We therefore designed a prospective systematic investigation to study acanthocytosis in HD and HDL2 patients of the same ethnicity, taking advantage of our finding that the area around Johannesburg, South Africa, contains the world's highest known concentration of HDL2 patients.[8]

Methods

HD and HDL2 patients with African or mixed ancestry were identified over 30 months at the Charlotte Maxeke Johannesburg Academic Hospital Huntington Disease Clinic as part of a larger HDL2 phenotype study.[3] Ethics clearance was obtained from the Human Research Ethics Committee of The University of the Witwatersrand (M140872) and informed consent was obtained from all study participants. Diagnoses were confirmed by mutation testing for the specific triplet repeat expansion in the genes causing HD and HDL2 at the Division of Human Genetics, National Health Laboratory Service. Control cases, also of African or mixed ancestry, were genetically unrelated family members or friends of the affected patients.

Blood samples were collected from both the control and either the HD or the HDL2 subject at the same study visit. Two 5-ml blood samples were collected, one in Ethylenediaminetetraacetic Acid (EDTA) and the other in Acid Citrate Dextrose (ACD) (BD Vacutainer Systems, UK). Each tube was labeled using a non-identifying study number and was processed within 20 minutes of the venipuncture. The laboratory was blinded to the diagnosis. Smears were prepared using the same techniques.

Methods for wet and fixed smears were performed according to previously established protocols.[9] In brief, an aliquot (~5 μL) of whole blood, collected in EDTA tubes, was used to prepare a fixed smear on a glass slide and stained using the Rapidiff Stain (Diagnostic Media Products, South Africa). In parallel, unfixed wet smears were prepared within an hour of the blood draw. A minimum of 10 fields of each preparation was examined with a Zeiss Axiostar (Carl Zeiss Jena GmbH, Germany) microscope under oil immersion at 1,000× magnification. Blood was obtained from an unaffected control at the same time and smears were prepared using the same techniques. An acanthocytosis rate of greater than 1.2% in the dry smear or greater than 3.7% in the wet smear was designated a priori as the threshold for clinical significance based on previously established standards.[9]

As a third approach to detecting abnormal hematological features in HDL2, red cell membranes were prepared from blood samples drawn in ACD tubes as described by Coetzer and Palek.[10] The protein concentration was determined using the Coomassie Plus Protein Assay Reagent (Pierce Biotechnology Incorporated, USA). Aliquots of solubilized erythrocyte membrane proteins were prepared by boiling for one minute in 10 mM Tris-HCl pH 8, 1 mM EDTA, 1% SDS, 5% sucrose, 2% β-mercaptoethanol, and 20–50 μg of protein was resolved on a 12% Laemmli SDS-polyacrylamide gel and a 4–17% Fairbanks gradient gel. Gels were stained with 0.05% Coomassie Blue (BDH Laboratory Supplies, UK) in 25% isopropanol, 10% acetic acid) and the amount of band 3 was quantitated using a Hoefer GS300 transmittance/reflectance scanning densitometer and GS365W software (Hoefer Scientific Instruments, USA).

The Fisher exact test was used to assess the relationship between categorized acanthocyte screening test results and groups. Data analysis was carried out using SAS version 9.4 for Windows. The 5% significance level was used.

Results

All HDL2 or HD cases enrolled had African or mixed ancestry. Control cases were genetically unrelated family members or acquaintances with African or mixed ancestry of the affected patients. The HD group included 11 separate pedigrees; two pedigrees each contributed two individuals to the study. The HDL2 group consisted of 12 unrelated individuals. Two HDL2 patients were also human immunodeficiency virus positive and receiving antiretroviral treatment. The results are presented in Table 1.

The acanthocyte counts remained below the defined normal thresholds in all of the HD, HDL2 and control cases in multiple biological replicates for both wet and dry smears. There were no other abnormal erythrocyte features noted on blood smear. The rest of the assessed flow cytometry for each group can be seen in Table 1 and all were within normal ranges. The flow cytometry evaluation was not available in four cases where there was a blood smear available. These were in three controls and one HD case. The amount of band 3 in the red cell

Table 1. **Comparative Results between HDL2, HD, and Control Groups**

	HDL2	HD	Control
Number of participants	12	13	21
Age (years)			
Mean age (SD)	49.2 (±9.5)	46.8 (±13.2)	49.5 (±14.9)
Median age	49	47	52
Range	32–66	32–68	21–79
Disease duration (years)			
Mean duration of disease (SD)	5.9 (±4.3)	8.2 (±4.7)	–
Median duration of disease	6	6	
Range	1–18	4–18	
Triplet repeat length			
Mean abnormal repeat length (SD)	46.3 (±3.3)	44.3 (±4.5)	–
Median abnormal repeat length	45	46	–
Range	43–53	40–49	
Hematology results			
Smear results			
Wet smear (defined as >3.7% erythrocytes being acanthocytes)			
Positive (number of patients)	0	0	0
Dry smear (defined as >1.2% erythrocytes being acanthocytes)			
Positive (number of patients)	0	0	0
Median % of acanthocytes (IQR)	0.21 (0.06–0.44)	0.06 (0.00–0.11)	0.06 (0.02–0.14)
Erythrocyte analysis (SD)			
Hemoglobin (g/dL)	14.62 (±1.40)	14.28 (±2.05)	15.56 (±3.99)
Red cell count (10^{12}/L)	5.01 (±0.63)	4.91 (±0.47)	5.08 (±0.56)
MCV (fL)	88.1 (±4.2)	87.1 (±5.6)	87.4 (±4.9)
MCH (pg)	29.5 (±2.0)	29.1 (±2.6)	29.1 (±2.0)
MCHC (g/dL)	33.3 (±1.5)	33.3 (±1.5)	33.3 (±1.2)
RDW (%)	13.3 (±0.9)	13.7 (±1.4)	13.6 (±0.8)

Abbreviations: IQR, Interquartile Range; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentrate; MCV, Mean Corpuscular Volume; RDW, Red cell Distribution Width; SD, Standard Deviation.

membranes of all the HD, HDL2, and control cases was normal, which correlates with the normal red cell morphology.

Discussion

There was no evidence of acanthocytosis in any of the HLD2 or HD patients. Assessments were performed using three different measures, and compared with a normal control group. We therefore conclude that acanthocytosis is not a significant component of HDL2 or HD in our patient population.

The presence of acanthocytosis in four previous cases of HDL2 remains intriguing. In the first three cases the technique for obtaining the smear described not stressing the erythrocytes with saline or aging them and that up to 5% acanthocytes/echinocytes was accepted as being within the normal range.[5] Echinocytes are difficult to differentiate from acanthocytes on light microscopy and are usually from smear processing errors whereas acanthocytes are of pathological relevance. The method we employed for the detection of acanthocytes by Storch et al.[9] was not published until 2005. This method is now considered to be the gold standard in testing for acanthocytes in neuroacanthocyte syndromes and reduces echinocytic stress.[9] The method used to detect acanthocytes in the original four cases where acanthocytes were seen was not described in sufficient detail to duplicate and therefore the Storch method was used by our group.

Acanthocytes may be an infrequent feature of HDL2, or may only develop in the presence of other genetic or environmental modifiers. Three of the four HDL2 cases with acanthocytes were related, and all carried either the Fragile X mutation or premutation. With hindsight it may have been useful to have had a Fragile X control group; however, acanthocytosis has not been reported in either Fragile X or FXTAS patients. The fourth case did not have abnormal band 3 proteins, and had HDL2-affected siblings without acanthocytes. All four cases have subsequently died and are therefore unfortunately not available for further study.

The pathogenesis of HDL2 appears to be complex, with potential contributions from a toxic gain-of-function of abnormal *JPH3* RNA,[11] loss of *JPH3* expression,[12] and, potentially, expression of an antisense gene encoding expanded polyglutamine repeats.[13] The present study, although a relatively small sample size, has nearly doubled the number of HDL2 patients tested for acanthocytes in the literature and for the first time examined acanthocytes in HD. Our results, based on the first prospective study of acanthocytes in HDL2 or HD, suggest that screening for acanthocytes will not help establish the diagnosis of HD or HDL2, nor differentiate between the two disorders. Further, the processes underlying acanthocyte formation are unlikely to substantially contribute to the pathogenesis of most cases of HDL2 and HD. The question is now raised as to whether HDL2 should be grouped with the neuroacanthocytosis syndromes. Finally, our results continue to demonstrate the remarkable phenotypic similarities between HD and HDL2.

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References

1. Holmes SE, O'Hearn E, Rosenblatt A, Callahan C, Hwang HS, Ingersoll-Ashworth RG, et al. A repeat expansion in the gene encoding junctophilin-3 is associated with Huntington disease-like 2. *Nat Genet* 2001;29:377–378. doi: 10.1038/ng760

2. Margolis RL, O'Hearn E, Rosenblatt A, Willour V, Holmes SE, Franz ML, et al. A disorder similar to Huntington's disease is associated with a novel CAG repeat expansion. *Ann Neurol* 2001;50:373–380. doi: 10.1002/ana.1312

3. Anderson DG, Walker RH, Connor M, Carr J, Margolis RL, Krause A, et al. A systematic review of the Huntington disease-like 2 phenotype. *J Huntington's Dis* 2017;6:37–46. doi: 10.3233/JHD-160232

4. Walker RH, Morgello S, Davidoff-Feldman B, Melnick A, Walsh MJ, Shashidharan P, et al. Autosomal dominant chorea-acanthocytosis with polyglutamine-containing neuronal inclusions. *Neurology* 2002;58:1031–1037. doi: 10.1212/WNL.58.7.1031

5. Walker RH, Rasmussen A, Rudnicki D, Holmes SE, Alonso E, Matsuura T, et al. Huntington's disease-like 2 can present as chorea-acanthocytosis. *Neurology* 2003;61:1002–1004. doi: 10.1212/01.WNL.0000085866.68470.6D

6. Adjobo-Hermans MJW, Cluitmans JCA, Bosman GJCGM. Neuroacanthocytosis: observations, theories and perspectives on the origin and significance of acanthocytes. *Tremor Other Hyperkinet Mov* 2015;5:328. doi: 10.7916/D8VH5N2M

7. Siegl C, Hamminger P, Jank H, Ahting U, Bader B, Danek A, et al. Alterations of red cell membrane properties in neuroacanthocytosis. *PLoS ONE* 2013;8:e76715. doi: 10.1371/journal.pone.0076715

8. Krause A, Mitchell C, Essop F, Tager S, Temlett J, Stevanin G, et al. Junctophilin 3 (JPH3) expansion mutations causing Huntington disease like 2 (HDL2) are common in South African patients with African ancestry and a Huntington disease phenotype. *Am J Med Genet B Neuropsychiatr Genet* 2015;168:573–585. doi: 10.1002/ajmg.b.32332

9. Storch A, Kornhass M, Schwarz J. Testing for acanthocytosis. *J Neurol* 2005;252:84–90. doi: 10.1007/s00415-005-0616-3

10. Coetzer TL, Palek J. Partial spectrin deficiency in hereditary pyropoikilocytosis. *Blood* 1986;67:919–924.

11. Rudnicki DD, Holmes SE, Lin MW, Thornton CA, Ross CA, Margolis RL, et al. Huntington's disease-like 2 is associated with CUG repeat-containing RNA foci. *Ann Neurol* 2007;61:272–282. doi: 10.1002/ana.21081

12. Seixas AI, Holmes SE, Takeshima H, Pavlovich A, Sachs N, Pruitt JL, et al. Loss of junctophilin-3 contributes to Huntington disease-like 2 pathogenesis. *Ann Neurol* 2012;71:245–257. doi: 10.1002/ana.22598

13. Wilburn B, Rudnicki DD, Zhao J, Weitz TM, Cheng Y, Gu X, et al. An antisense CAG repeat transcript at JPH3 locus mediates expanded polyglutamine protein toxicity in Huntington's disease-like 2 mice. *Neuron* 2011;70:427–440. doi: 10.1016/j.neuron.2011.03.021

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Emerging differences between Huntington's disease-like 2 and Huntington's disease: A comparison using MRI brain volumetry

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ABSTRACT

Huntington's Disease-Like 2 (HDL2), caused by a CTG/CAG expansion in *JPH3* on chromosome 16q24, is the most common Huntington's Disease (HD) phenocopy in populations with African ancestry. Qualitatively, brain MRIs of HDL2 patients have been indistinguishable from HD. To determine brain regions most affected in HDL2 a cross-sectional study using MRI brain volumetry was undertaken to compare the brains of nine HDL2, 11 HD and nine age matched control participants. Participants were ascertained from the region in South Africa with the world's highest HDL2 incidence. The HDL2 and HD patient groups showed no significant differences with respect to mean age at MRI, disease duration, abnormal triplet repeat length, or age at disease onset. Overall, intracerebral volumes were smaller in both affected groups compared to the control group. Comparing the HDL2 and HD groups across multiple covariates, cortical and subcortical volumes were similar with the exception that the HDL2 thalamic volumes were smaller. Consistent with other similarities between the two diseases, these results indicate a pattern of neurodegeneration in HDL2 that is remarkably similar to HD. However smaller thalamic volumes in HDL2 raises intriguing questions into the pathogenesis of both disorders, and how these volumetric differences relate to their respective phenotypes.

1. Introduction

Huntington's disease like 2 (HDL2) is the most common Huntington's disease (HD) phenocopy in populations with an African ancestry and is emerging as the most common HD phenocopy in South America, the USA and parts of Europe (Margolis et al., 2004; Mariani et al., 2016; Walker et al., 2018). South Africa has the highest number of HDL2 cases described despite the under ascertainment of patients due to lack of resources in rural communities (Anderson et al., 2017b; Baine et al., 2016).

Huntington's Disease is caused by a CAG/CTG repeat expansion in exon 1 of the *huntingtin* gene on chromosome 4p16.3, while HDL2 is caused by a similarly sized CTG/CAG expansion in exon 2A of *junctophilin-3* (*JPH3*) on chromosome 16q24.3 (Holmes et al., 2001). Both are autosomal dominant disorders characterized by psychiatric disease, movement disorder and cognitive dysfunction which progress and inevitably result in death approximately 15 to 20 years after clinical onset (Margolis et al., 2001). Observations of the HDL2 phenotype are from retrospective and anecdotal cases and reviews (Anderson et al., 2017b). Notwithstanding these genetic differences and the need for systematic study of the HDL2 clinical phenotype, HDL2 is the disease that has been described to most resemble HD (Walker et al., 2003).

Furthermore, qualitative MRI studies have not been able to differentiate between HDL2 and HD. To date these rare reports suggest similar patterns of atrophy between these two disorders (Anderson et al., 2017b). A systematic review of 69 published cases of HDL2 reported 20 MRI scans of HDL2 patients described as indistinguishable from HD (Anderson et al., 2017b). An exception was a patient with HDL2 who

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presented with parkinsonism and myoclonus. The MRI of this case showed typical cerebral atrophy but relative sparing of the caudate nucleus (Bardien et al., 2007). A report of a single case of HDL2 having two MRIs five years apart described progressive atrophy of the same areas as seen in HD (Rodrigues et al., 2008). Hyperintense putaminal rims on T2 imaging have been described in three cases with HDL2 from the same family (Schneider et al., 2012). The putaminal rim sign has also been detected in a number of other degenerative movement disorders including Multi-System Atrophy (MSA), Neuro-Acanthocytosis, Dentatorubral-Pallidoluysian Atrophy (DRPLA) and Spinocerebellar Ataxia Type 17 (SCA17).

Despite the fact that HD and HDL2 are caused by mutations in different genes, which are linked to different functions within the cell, the majority of the qualitative MRI reports, neuropathological analysis, and clinical phenotype, highlight the similarities between both disorders. We therefore hypothesized that quantitative MRI analysis of HDL2 patients would reveal similar findings to those detected in HD patients. To test this hypothesis, we performed a blinded systematic imaging study of matched HD and HDL2 patients with a normal control group, taking advantage of the relatively high number of HDL2 cases that have been reported in the region surrounding Johannesburg, South Africa (Anderson et al., 2017b; Krause et al., 2015).Comparing the brain MRIs of these clinically similar diseases has the potential to guide further investigation into the differences and similarities between HD and HDL2 and forms part of an ongoing systematic characterization of the phenotype of HDL2.

2. Methods

Patients with HDL2 and HD were identified and diagnosed through the Division of Human Genetics, National Health Laboratory Service and the School of Pathology, The University of the Witwatersrand, in Johannesburg, South Africa. Controls were unrelated healthy acquaintances matched for gender, age and race. Participants were enrolled between January 2015 and June 2017 and all subjects had African or mixed ancestry including a significant African component. The Human Research Ethics Committee of The University of the Witwatersrand approved this cross-sectional study (M140872).

MRIs of subjects were performed at the University of the Witwatersrand Donald Gordon Medical Centre Radiology Department on a 1.5 T Philips Intera MR scanner (Philips Medical Systems, Eindhoven). There were no relevant scanner upgrades during the study. The MRI sequences used in this study are available in the supplement.

We used the Brain Research: Analysis of Images, Networks, and Systems (BRAINS) protocol, which has been validated and extensively used to measure intracerebral volumes for HD in the large PREDICT-HD study (Paulsen et al., 2008). Scans were processed by Brain Image Analysis, LLC using the cross-sectional analysis stream of NeuroAnalytica version 1.3.09, a commercial image analysis platform based on AutoWorkup (Pierson et al., 2011). A discriminant tissue classification was then performed (Harris et al., 1999). Artificial neural networks for each subcortical structure were applied (Powell et al., 2008). Additional information about this technique is available in the supplement.

Comparison of age at MRI, disease duration, abnormal repeat length, and age at onset among the groups was performed using a one-way ANOVA. Comparison of variables among the three groups, controlling for age at MRI and separately, for disease duration, expanded repeat length, and age of disease onset was performed using a General Linear Model. Post-hoc comparisons were performed using the Tukey-Kramer adjustment. The false discovery rate was controlled by the Benjamini-Hochberg procedure and significance was calculated at $p < .0084$. Data analysis was carried out using SAS version 9.4 for Windows.

Table 1
Table showing subject demographic data and MRI volumes. a. Subject ages, age of disease onset, duration of disease and triplet repeat length. b. Unadjusted volumetric analysis of selected subcortical structures, hippocampi and total intracranial volumes in cm^3. HD = Huntington's Disease, HDL2 = Huntington's Disease like 2, n = number SD = Standard Deviation.

1a Demographics and Genetics	HDL2	HD	Controls
Number of participants	9	11	9
Male: Female	5:4	6:5	5:4
	Mean (SD)	Mean (SD)	Mean (SD)
Age at MRI (years)	47.6 (9.1)	46.0 (12.4)	42.6 (9.1)
Abnormal repeat length (n)	47.0 (3.5)	44.9 (3.2)	–
Duration of disease (years)	5.6 (3.0)	6.6 (4.8)	–
Age at onset(years)	42.0 (10.2)	39.4 (13.1)	–
1b Region Volume (cm^3)	Mean (SD)	Mean (SD)	Mean (SD)
Left thalamus	5.156 (0.671)	6.453 (0.868)	6.628 (0.698)
Right thalamus	4.885 (0.805)	6.232 (0.745)	6.269 (0.643)
Left putamen	2.147 (0.452)	2.615 (0.233)	4.771 (0.637)
Right putamen	2.249 (0.389)	2.698 (0.263)	4.714 (0.502)
Left caudate	1.010 (0.455)	1.528 (0.485)	3.359 (0.548)
Right caudate	1.080 (0.475)	1.667 (0.488)	3.392 (0.629)
Left globus pallidus	0.812 (0.190)	0.749 (0.103)	1.297 (0.311)
Right globus pallidus	0.772 (0.150)	0.766 (0.101)	1.371 (0.323)
Left amygdala	0.747 (0.230)	0.743 (0.143)	0.841 (0.171)
Right amygdala	0.665 (0.170)	0.755 (0.141)	0.834 (0.266)
Left nucleus accumbens	0.159 (0.050)	0.174 (0.043)	0.266 (0.075)
Right nucleus accumbens	0.179 (0.067)	0.232 (0.068)	0.289 (0.059)
Intracranial volume	1437.9 (112.7)	1424.1 (118.5)	1424.6 (156.5)

3. Results

Images of nine HDL2, 11 HD and nine normal control participants were included in the study. None of the HDL2 participants were related. Three of the HD pedigrees each contributed two participants to the study. There were no significant differences among the groups with respect to mean age at MRI ($F [2,26] = 0.54$; $p = .59$), disease duration ($F [1,18] = 0.57$; $p = .57$), abnormal repeat length ($F [1,18] = 0.18$; $p = .18$), or age at onset ($F [1,18] = 0.63$; $p = .63$) (Table 1).

The main aim of the study was to contrast HD and HDL2 MRIs. Initially a qualitative comparison of MRIs could not distinguish between both pathologies (see Fig. 1.) The quantitative comparison was conducted using BRAINS. We conducted an initial comparison between HD and healthy controls in order to ensure comparability with previous studies and validation of the present methodology. Subsequently, the HDL2 group was compared to controls with the objective of providing the first MRI brain volumetry characterization of HDL2. Lastly, HD and HDL2 MRIs were compared.

3.1. Comparison between patients with HD and normal controls

When compared to the normal control group and controlling for the age at the time of the MRI, the HD group showed significant less grey matter volume in both of the parietal and left temporal lobes with significantly smaller caudate nuclei, putamen and globus pallidum bilaterally (see Figs. 2 and 3 and Table 1b). These smaller grey matter volumes were reflected in corresponding increases in CSF volumes (Fig. 4). Notably the mean thalamic volumes were not statistically different between the HD group and the normal control group ($p < .01$) (see Table 2).

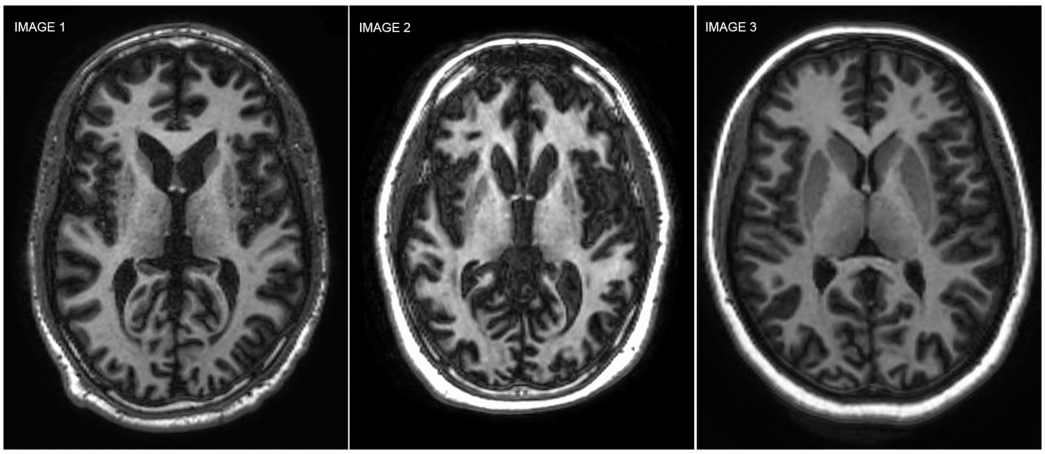

Fig. 1. Qualitative MRI Comparison. Three T1 axial 1.5 T MRIs comparing HDL2, HD and a normal control case. Image 1: 35-year-old HD subject with abnormal *HTT* allele = 49 triplet repeats. Image 2: 32-year-old HDL2 subject with abnormal *JPH3* = 53 triplet repeats. Image 3: 32-year-old normal control subject. The HDL2 and HD subjects show qualitatively similar grey and white matter atrophy with caudate nuclei and putaminal volume loss compared to the normal control.

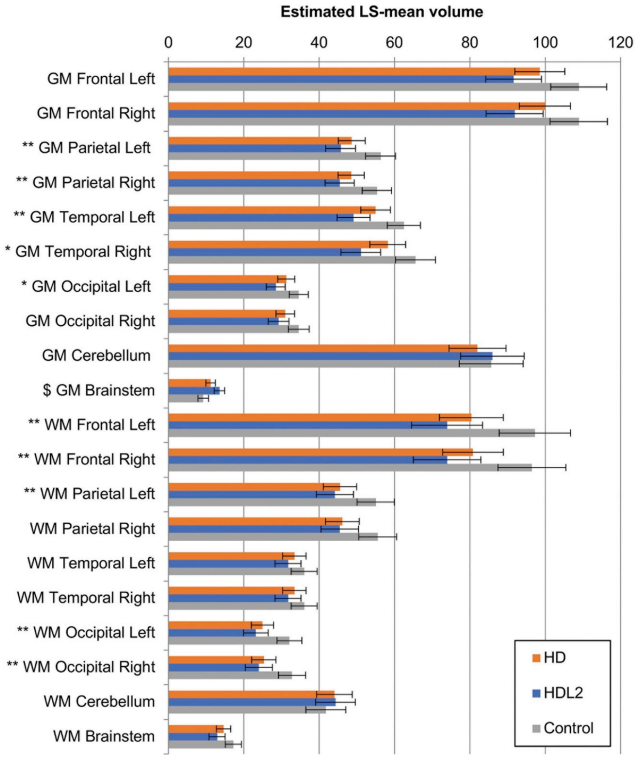

Fig. 2. HD, HDL2 and control groups grey matter and white matter volumes. The estimated least-squares (LS) means from the regression are depicted in a three-way comparison of grey and white matter volume. Error bars denote the 95% confidence intervals for the mean. Significance was set at $p < .008$. Symbols denote significant differences: **: HD, HDL2 < Control; *: HDL2 < Control; $: HD, Control < HDL2. GM = grey matter, WM = white matter.

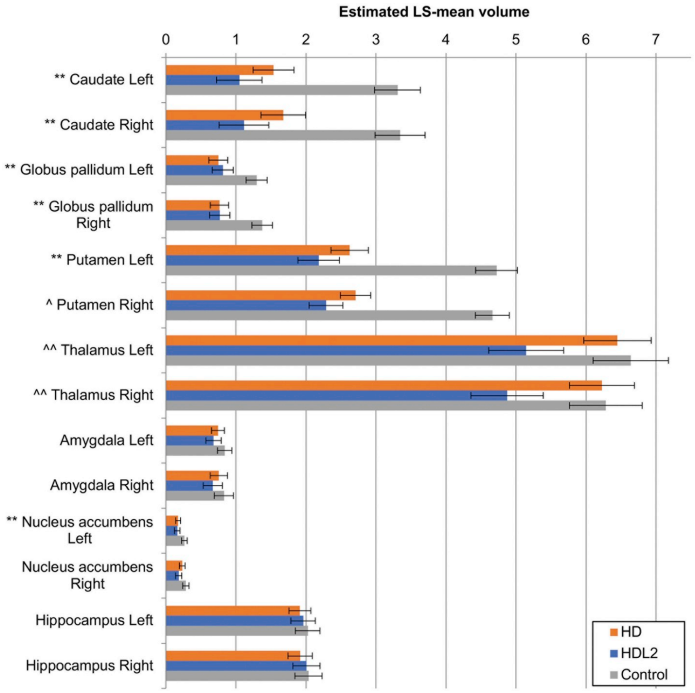

Fig. 3. HD, HDL2 and control groups basal ganglia, thalamic and hippocampal volumes. The estimated least-squares (LS) means from the regression are depicted in the figure in a three-way comparison among HD, HDL2 and control groups. Statistical significance is denoted by a symbol. Error bars denote the 95% confidence interval for the mean. Significance was set at $p < .008$. Symbols: **: HD, HDL2 < Control, ^: HDL2 < HD < Control, ^^: HDL2 < Control, HD where significance was reached. LS = Least-squares means, Nucleus acc = Nucleus accumbens.

3.2. Comparison between HDL2 and normal controls

Grey matter volume tended to be less in the HDL2 group compared to control groups when controlling for age at the time of the MRI (see Fig. 2). The HDL2 group showed significantly smaller grey matter volumes for both the parietal and temporal lobes and left occipital lobe and significantly smaller white matter volumes for both the frontal and occipital lobes and left parietal lobe compared to the normal control participants ($p < .008$). Almost all of the CSF spaces were significantly larger in the HDL2 group compared to the control group (see Fig. 4). Most of the basal ganglia including the thalamus were significantly smaller in the HDL2 group compared to the control group ($p < .008$) (see Table 1b and Fig. 3).

3.3. Comparison between HDL2 and HD

When comparing the HDL2 and HD groups, a similar pattern of grey and white matter volume was noted (see Fig. 2). The mean volumes of the caudate nucleus, globus pallidum, amygdala and the nucleus accumbens tended to be smaller in the HDL2 group compared to the HD group but this did not reach statistical significance when compared across all covariates (Table 1b and Fig. 3). When controlling for age at the time of the MRI, duration of disease, abnormal repeat length and age of onset, the HDL2 groups mean thalamic volumes were significantly smaller compared to the HD participants (Mean [STD] left and right thalamic volumes; HDL2 = left 5.156 cm^3 [0.671], right = 4.885 cm^3 [0.805], HD left = 6.453 cm^3 [0.86], right = 6.232 cm^3 [745]).

The HDL2 volumes were smaller when evaluating differences in the unadjusted putamen volumes in HDL2 and HD cases (Mean [STD] left and right putamen volumes; HDL2 = left 2.147 cm^3 [0.452], right = 2.249 cm^3 [0.389], HD left = 2.615 cm^3 [0.233], right = 2.698 cm^3 [0.263]). However, when applying the Benjamini-Hochberg procedure and applying a $p < .0084$, significance was not seen bilaterally for the putamen as was with the thalamus when controlling for age of onset. Furthermore, the thalamus (Left: $p < .0012$, Right: $p < .0006$) in comparison to the putamen (Left: $p < .011$, Right: $p < .0081$) had greater significant volume difference between the two diseases when controlling for age of disease onset (Table 2).

The unadjusted left and right caudate volumes suggest similar trends as seen in the thalamus and putamen when the unadjusted data are observed with the HDL2 volumes being smaller compared to the HD group caudate volumes (Mean [STD] left and right putamen volumes; HDL2 = left 1.010 cm^3 [0.455], right = 1.080 cm^3 [0.475], HD left = 1.528 cm^3 [0.485], right = 1.667 cm^3 [0.488]) (Table 1b). However, the LS mean caudate volumes are small: 3.2 for HD and 2.2 for the HDL2 groups and when applying the same procedure the difference between the HDL2 and HD caudate volumes did not reach significance when controlling for any of the covariates as noted in Table 2.

The relationship between duration of disease, abnormal repeat length, age at onset was separately determined for each volume measurement in both the HDL2 and HD groups. The trend was an inverse correlation for most measurements, as expected, but significance was not reached. In both diseases smaller volumes of the grey matter structures was seen with increasing duration of disease and larger repeat lengths.

Lastly, on blinded clinical review T2 images demonstrated bilateral putaminal rim hyperintensities in HD and HDL2 patients, but not in the control group.

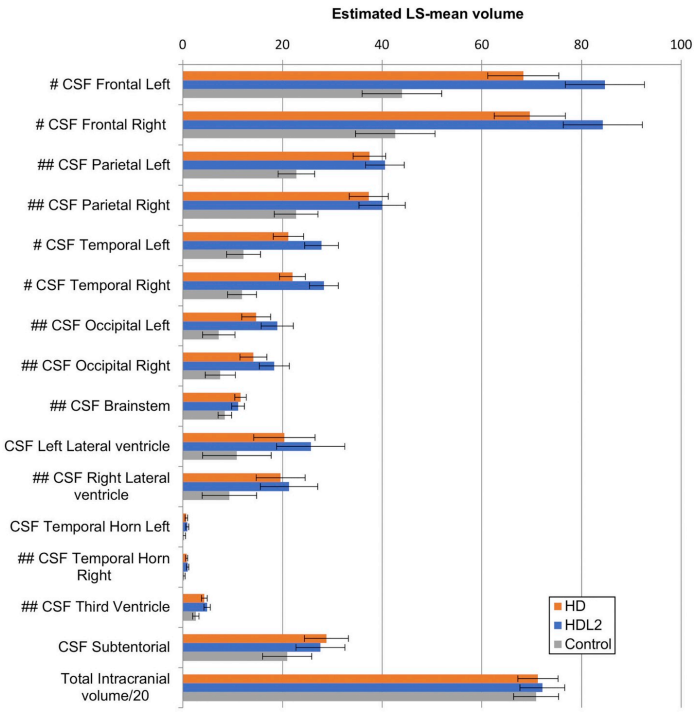

Fig. 4. HD, HDL2 and control cerebrospinal fluid and total intracranial volumes. The estimated least-squares (LS) means from the regression are depicted in the graph in a three-way comparison among the HD, HDL2 and control groups showing cerebrospinal fluid volumes and total intracranial volumes. Note that total intracranial volume was divided by 20 to fit to the graph scale. Statistical significance is shown with a symbol. Error bars denote the 95% confidence interval for the mean and significance was set at $p < .008$. Symbols denote #: Control < HD < HDL2. ##: HD, HDL2 < Control where significance was reached. LS = least squares mean, CSF = Cerebrospinal fluid.

4. Discussion

This is the first quantitative volumetric MRI study in patients with HDL2 and the first comparison to a HD group and an unaffected control group and done blind to the patients diagnosis. Consistent with the previously reported qualitative review of individual cases (Anderson et al., 2017b; Margolis et al., 2001). The analysis indicated similar atrophy of the grey and white matter structures including the basal ganglia and enlarged CSF spaces in the two disease groups compared to the normal control group. However, smaller thalamic volumes were noted in the HDL2 group compared to the HD group. There was no evidence of relative caudate sparing, as observed in one previous HDL2 case (Bardien et al., 2007).

The HD group showed white and grey matter loss, enlarged CSF spaces, and basal ganglia changes that replicated the findings of previous studies using similar analytic methods but much larger sample

Table 2
Table comparing the Huntington's Disease Like 2, Huntington's Disease and the Normal control groups basal ganglia mean volume significant differences across multiple covariates (controlling for age at MRI, controlling for duration of disease, controlling for abnormal repeat length and controlling for age at onset) Two-tailed significance: P < .008. *G. pallidus* = Globus Pallidus, HDL2 = Huntington's Disease Like 2, HD = Huntington's Disease, N. Accumbens = Nucleus accumbens.

Region	Comparison between HDL2, HD and control groups controlling for age at MRI		Comparison between HDL2 and HD groups controlling for duration of disease		Comparison between HDL2 and HD groups controlling for abnormal repeat length		Comparison between HDL2 and HD groups controlling for age at onset	
	Significant differences	*P* value	Significant differences	P value	Significant differences	P value	Significant differences	P value
Left Thalamus	HDL2 < HD, Control	0,0007	HDL2 < HD	0,0015	HDL2 < HD	0,0049	HDL2 < HD	0,0012
Right Thalamus	HDL2 < HD, Control	0,0005	HDL2 < HD	0,0008	HDL2 < HD	0,0032	HDL2 < HD	0,0006
Left Putamen	HDL2, HD < Control	< 0.0001	HDL2 < HD	0,0083	HDL2 < HD	0,0063	Not Significant	0,011
Right Putamen	HDL2 < HD < Control	< 0.0001	HDL2 < HD	0,0064	HDL2 < HD	0,0014	HDL2 < HD	0,0081
Left Caudate	HDL2, HD < Control	< 0.0001	Not Significant	0,023	Not Significant	0,027	Not Significant	0,034
Right Caudate	HDL2, HD < Control	< 0.0001	Not Significant	0,010	Not Significant	0,018	Not Significant	0,019
Left *G. pallidus*	HDL2, HD < Control	< 0.0001	Not Significant	0,45	Not Significant	0,41	Not Significant	0,42
Right G. Pallidus	HDL2, HD < Control	< 0.0001	Not Significant	0,81	Not Significant	0,80	Not Significant	0,97
Left Amygdala	Not Significant	0,11	Not Significant	0,35	Not Significant	0,27	Not Significant	0,35
Right Amygdala	Not Significant	0,27	Not Significant	0,22	Not Significant	0,16	Not Significant	0,25
Left N. Accumbens	HDL2, HD < Control	0,0016	Not Significant	0,38	Not Significant	0,16	Not Significant	0,54
Right N. Accumbens	Not Significant	0,011	Not Significant	0,018	Not Significant	0,10	Not Significant	0,10

sizes and provides support for the methods and patient population used in this study (Paulsen et al., 2008; Tabrizi et al., 2009; van den Bogaard et al., 2011). The key finding of this analysis is the similarity between the MRIs of HD and HDL2 patients, with the notable exception that the average thalamic volume was 21% smaller in the HDL2 group compared to the HD group. In contrast to this difference, the mean thalamic volume in the HD group was similar to those recorded in the normal control participants.

Initial neuroimaging studies found no difference in thalamic volumes between HD patients and controls (Rosas et al., 2003). Later studies have demonstrated thalamic volume loss in HD but have suggested that this reflects disease progression. Studies comparing MRI brain volumes in HD with normal controls often used different volumetric methods and showed HD thalamic volumes being between 2 and 8% less than the unaffected control groups (Coppen et al., 2018; Kassubek et al., 2004). Thalamic atrophy in HD appears to reflects whole brain atrophy and is proportional to overall degeneration (van den Bogaard et al., 2011). Therefore in keeping with previous reports of HD thalamic volumes, the mean thalamic volumes of the HD group in this study was 2% less than the control group. However, in the HD and HDL2 groups, regions like the putamen and caudate showed typically smaller volumes compared to the normal control group as has been described previously in HD (Tabrizi et al., 2009; van den Bogaard et al., 2011). The striking finding from this study was that HDL2 thalamic volume was significantly smaller than in the HD group when controlling for age at the time of the MRI, duration of disease, abnormal repeat length and age of onset of disease.

Attempts to find features differentiating HDL2 from HD are available, but findings have been anecdotal, inconsistent or disproved. For example, the hypothesis that HDL2 is a neuroacanthocytosis syndrome was recently refuted by a blinded controlled study (Anderson et al., 2017a). Descriptions of the HDL2's phenotype have introduced the possibility that HDL2 may disproportionately present with bradykinetic/parkinsonian features which likely reflects the unique phenotype and relatively long expanded repeat length, but this remains unverified (Margolis et al., 2001)(Schneider et al., 2012). The characterization of the clinical phenotype of HDL2 will remain incomplete while based only on retrospective case series (Anderson et al., 2017b). Thus, future research is needed, and our findings regarding the differences in thalamic volumes between HDL2 and HD may provide a new and exciting guide to explore into differentiating between these two mutations.

The possible clinical implications and pathogenic mechanisms causing greater thalamic atrophy in HDL2 would need to be accounted for. The pathogenesis of HDL2 is complex, with likely contributions from toxic properties of *JPH3* RNA with the repeat expansion, loss of expression of *JPH3*, and perhaps expanded tracts of polyglutamine expressed from a cryptic gene on the antisense strand at the HDL2 locus (Rudnicki et al., 2007; Seixas et al., 2012; Wilburn et al., 2011). The finding of smaller putamen and thalamic volumes in HDL2 compared to HD suggests differential neuronal and/or glial vulnerability in these areas to the HDL2 mutation compared to the HD mutation. This may have implications first in terms of phenotype. It has been suggested that there are clinical differences such as more myoclonus and parkinsonism although this needs further validation (Anderson et al., 2017b; Margolis et al., 2001). Secondly although little difference has been reported in the gross or histopathological findings between five patients with HDL2 compared to matched cases with HD (Rudnicki et al., 2008), it is possible that the thalamic differences may be the consequence of histopathological discrepancies between these mutations that require larger samples of HDL2 specimens (Rudnicki et al., 2008).

This study is based on the uniquely high prevalence of HDL2 in South Africa, and would be difficult to accomplish in any other location. Even in this region HDL2 is a rare disorder and therefore the sample size is small. The results do however establish the general neuroradiological similarity of HD and HDL2 and are consistent with their reportedly analogous clinical phenotypes. The selectively smaller thalamic volumes in HDL2 is an interesting finding, but the sample size imposes limits on data interpretation. In both HD and HDL2, the relationship between intracerebral volumes and duration of disease and abnormal repeat length do not reach significance, though most measurements trend to show smaller volumes with increasing duration of disease and larger abnormal repeat lengths. These are well established negative correlations for HD demonstrated in larger studies (Tabrizi et al., 2009). We would predict that in HDL2, like HD, these trends would be confirmed with larger cross-sectional cohorts of patients or with longitudinal analyses of smaller cohorts. In support of this, sequential MRIs five years apart have been reported for a single HDL2 case which qualitatively demonstrated progressive brain atrophy in a similar pattern to HD (Rodrigues et al., 2008).

Lastly, we found putaminal rim hyperintensities in all of the HD and HDL2 cases. This sign has been described before in HDL2 (Schneider et al., 2012). Other neurodegenerative disorders and normal controls have been shown to have putaminal rim hyperintensities and this is therefore unlikely to help distinguish HDL2 from other conditions (Lee et al., 2005; Tha et al., 2012). To our knowledge, the putaminal rim sign has not been commented on in HD previously.

5. Conclusion

Huntington's disease like 2 and HD are remarkably similar clinically and radiologically. When HDL2 is objectively compared to a HD group that is matched for age, abnormal repeat length and duration of disease there is a similar pattern emerging of smaller volumes in the grey and white matter as well as in the basal ganglia. The results presented here, now demonstrate the similarities between HDL2 and HD extend to spatial patterns of neurodegeneration, with the interesting exception of the thalamus, where selective thalamic volume loss tends to be greater in HDL2.

These findings raise intriguing possibilities about both the convergent and the divergent pathogenic pathways of the two diseases and opens future paths of research into how this relates to their phenotypes.

Disclosures and conflict of interest

Ronald Pierson is a shareholder in NeuroAnalytica and received fees from the University of the Witwatersrand for analysis of the MRIs.

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Data sharing and data accessibility

The authors encourage others to make use of this data and will make this large, anonymised data set available to any researchers or groups of good standing.

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Appendix A. Author contribution table

Name	Location	Role	Contribution
David G. Anderson	The University of the Witwatersrand Donald Gordon Medical Centre	Author	: Study concept and design, organization, acquisition of data, analysis and interpretation, writing of manuscript, critical revision of the manuscript for important intellectual content.
Mark Haagensen	The University of the Witwatersrand Donald Gordon Medical Centre	Author	: Acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content
Aline Ferreira-Correia	Department of Psychology, School of Human and Community Development, University of the Witwatersrand	Author	: Acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content.
Ronald Pierson	Brain Image Analysis, LLC, Simi Valley CA USA	Author	:Analysis and interpretation, critical revision of the manuscript for important intellectual content.
Jonathan Carr	Division of Neurology, Department of Medicine, University of Stellenbosch, Cape Town	Author	Study supervision, critical revision of the manuscript for important intellectual content.
Amanda Krause	Division of Human Genetics, National Health Laboratory Service and School of Pathology, Faculty of Health Sciences, The University of the Witwatersrand	Author	: Study supervision, Analysis and interpretation, critical revision of the manuscript for important intellectual content, senior author.
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Appendix B. Supplementary data

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References

Anderson, D.G., Carmona, S., Naidoo, K., Coetzer, T.L., Carr, J., Rudnicki, D.D., Walker, R.H., Margolis, R.L., Krause, A., 2017a. Absence of acanthocytosis in Huntington's disease-like 2: a prospective comparison with Huntington's disease. Tremor Other Hyperkinet Mov (N Y) 7, 512. https://doi.org/10.7916/D81J9PDX.

Anderson, D.G., Walker, R.H., Connor, M., Carr, J., Margolis, R.L., Krause, A., 2017b. a systematic review of the Huntington disease-like 2 phenotype. J. Huntingtons Dis. 6, 37–46. https://doi.org/10.3233/JHD-160232.

Baine, F.K., Krause, A., Greenberg, L.J., 2016. The frequency of Huntington disease and Huntington disease-like 2 in the South African population. Neuroepidemiology 46, 198–202. https://doi.org/10.1159/000444020.

Bardien, S., Abrahams, F., Soodyall, H., van der Merwe, L., Greenberg, J., Brink, T., Carr, J., 2007. A South African mixed ancestry family with Huntington disease-like 2: clinical and genetic features. Mov. Disord. 22, 2083–2089. https://doi.org/10.1002/mds.21672.

Coppen, E.M., Jacobs, M., van den Berg-Huysmans, A.A., van der Grond, J., Roos, R.A.C., 2018. Grey matter volume loss is associated with specific clinical motor signs in Huntington's disease. Parkinsonism Relat. Disord. 46, 56–61. https://doi.org/10.1016/j.parkreldis.2017.11.001.

van den Bogaard, S.J.A., Dumas, E.M., Acharya, T.P., Johnson, H., Langbehn, D.R., Scahill, R.I., Tabrizi, S.J., van Buchem, M.A., van der Grond, J., Roos, R.A.C., TRACK-HD Investigator Group, 2011. Early atrophy of pallidum and accumbens nucleus in Huntington's disease. J. Neurol. 258, 412–420. https://doi.org/10.1007/s00415-010-5768-0.

Harris, G., Andreasen, N.C., Cizadlo, T., Bailey, J.M., Bockholt, H.J., Magnotta, V.A., Arndt, S., 1999. Improving tissue classification in MRI: a three-dimensional multispectral discriminant analysis method with automated training class selection. J. Comput. Assist. Tomogr. 23, 144–154.

Holmes, S.E., O'Hearn, E., Rosenblatt, A., Callahan, C., Hwang, H.S., Ingersoll-Ashworth, R.G., Fleisher, A., Stevanin, G., Brice, A., Potter, N.T., Ross, C.A., Margolis, R.L., 2001. A repeat expansion in the gene encoding junctophilin-3 is associated with Huntington disease-like 2. Nat. Genet. 29, 377–378. https://doi.org/10.1038/ng760.

Kassubek, J., Juengling, F.D., Ecker, D., Landwehrmeyer, G.B., 2004. Thalamic atrophy in Huntington's disease co-varies with cognitive performance: a morphometric MRI analysis. Cereb. Cortex 15, 846–853. https://doi.org/10.1093/cercor/bhh185.

Krause, A., Mitchell, C., Essop, F., Tager, S., Temlett, J., Stevanin, G., Ross, C., Rudnicki, D., Margolis, R., 2015. Junctophilin 3 (JPH3) expansion mutations causing Huntington disease like 2 (HDL2) are common in South African patients with African ancestry and a Huntington disease phenotype. Am. J. Med. Genet. B Neuropsychiatr. Genet. 168, 573–585. https://doi.org/10.1002/ajmg.b.32332.

Lee, W.-H., Lee, C.-C., Shyu, W.-C., Chong, P.-N., Lin, S.-Z., 2005. Hyperintense putaminal rim sign is not a hallmark of multiple system atrophy at 3T. AJNR Am. J. Neuroradiol. 26, 2238–2242.

Margolis, R.L., O'Hearn, E., Rosenblatt, A., Willour, V., Holmes, S.E., Franz, M.L., Callahan, C., Hwang, H.S., Troncoso, J.C., Ross, C.A., 2001. A disorder similar to Huntington's disease is associated with a novel CAG repeat expansion. Ann. Neurol. 50, 373–380. https://doi.org/10.1002/ana.1124.

Margolis, R.L., Holmes, S.E., Rosenblatt, A., Gourley, L., O'Hearn, E., Ross, C.A., Seltzer, W.K., Walker, R.H., Ashizawa, T., Rasmussen, A., Hayden, M., Almqvist, E.W., Harris, J., Fahn, S., MacDonald, M.E., Mysore, J., Shimohata, T., Tsuji, S., Potter, N., Nakaso, K., Adachi, Y., Nakashima, K., Bird, T., Krause, A., Greenstein, P., 2004. Huntington's disease-like 2 (HDL2) in North America and Japan. Ann. Neurol. 56, 670–674. https://doi.org/10.1002/ana.20248.

Mariani, L.-L., Tesson, C., Charles, P., Cazeneuve, C., Hahn, V., Youssov, K., Freeman, L., Grabli, D., Roze, E., Noël, S., Peuvion, J.-N., Bachoud-Levi, A.-C., Brice, A., Stevanin, G., Durr, A., 2016. Expanding the spectrum of genes involved in Huntington disease using a combined clinical and genetic approach. JAMA Neurol. 1–10. https://doi.org/10.1001/jamaneurol.2016.2215.

Paulsen, J.S., Langbehn, D.R., Stout, J.C., Aylward, E., Ross, C.A., Nance, M., Guttman, M., Johnson, S., MacDonald, M., Beglinger, L.J., Duff, K., Kayson, E., Biglan, K., Shoulson, I., Oakes, D., Hayden, M., Predict-HD Investigators and Coordinators of the Huntington Study Group, 2008. Detection of Huntington's disease decades before diagnosis: the Predict-HD study. J. Neurol. Psychiatry 79, 874–880. https://doi.org/10.1136/jnnp.2007.128728.

Pierson, R., Johnson, H., Harris, G., Keefe, H., Paulsen, J.S., Andreasen, N.C., Magnotta, V.A., 2011. Fully automated analysis using BRAINS: autoworkup. NeuroImage 54, 328–336. https://doi.org/10.1016/j.neuroimage.2010.06.047.

Powell, S., Magnotta, V., Johnson, H.J., Jammalamadaka, V.K., Pierson, R., Andreasen, N., 2008. Registration and machine learning-based automated segmentation of subcortical and cerebellar brain structures. NeuroImage 39, 238–247. https://doi.org/10.1016/j.neuroimage.2007.05.063.

Rodrigues, G.G.R., Walker, R.H., Brice, A., Cazeneuve, C., Russaouen, O., Teive, H.A.G., Munhoz, R.P., Becker, N., Raskin, S., Werneck, L.C., Junior, W.M., Tumas, V., 2008. Huntington's disease-like 2 in Brazil-report of 4 patients. Mov. Disord. 23, 2244–2247. https://doi.org/10.1002/mds.22223.

Rosas, H.D., Koroshetz, W.J., Chen, Y.I., Skeuse, C., Vangel, M., Cudkowicz, M.E., Caplan, K., Marek, K., Seidman, L.J., Makris, N., Jenkins, B.G., Goldstein, J.M., 2003. Evidence for more widespread cerebral pathology in early HD: an MRI-based morphometric analysis. Neurology 60, 1615–1620.

Rudnicki, D.D., Holmes, S.E., Lin, M.W., Thornton, C.A., Ross, C.A., 2007. Huntington's disease–like 2 is associated with CUG repeat-containing RNA foci. Ann. Neurol. 61, 272–282. https://doi.org/10.1002/ana.21081.

Rudnicki, D.D., Pletnikova, O., Vonsattel, J.-P.G., Ross, C.A., Margolis, R.L., 2008. A comparison of Huntington disease and Huntington disease-like 2 neuropathology. J. Neuropath Neuro. 67, 366–374. https://doi.org/10.1097/nen.0b013e31816b4aee.

Schneider, S.A., Marshall, K.E., Xiao, J., LeDoux, M.S., 2012. JPH3 repeat expansions cause a progressive akinetic-rigid syndrome with severe dementia and putaminal rim in a five-generation African-American family. Neurogenetics 13, 133–140. https://

doi.org/10.1007/s10048-012-0318-9.
Seixas, A.I., Holmes, S.E., Takeshima, H., Pavlovich, A., Sachs, N., Pruitt, J.L., Silveira, I., Ross, C.A., Margolis, R.L., Rudnicki, D.D., 2012. Loss of junctophilin-3 contributes to Huntington disease-like 2 pathogenesis. Ann. Neurol. 71, 245–257. https://doi.org/10.1002/ana.22598.
Tabrizi, S.J., Langbehn, D.R., Leavitt, B.R., Roos, R.A., Durr, A., Craufurd, D., Kennard, C., Hicks, S.L., Fox, N.C., Scahill, R.I., Borowsky, B., Tobin, A.J., Rosas, H.D., Johnson, H., Reilmann, R., Landwehrmeyer, B., Stout, J.C., 2009. Biological and clinical manifestations of Huntington's disease in the longitudinal TRACK-HD study: cross-sectional analysis of baseline data. Lancet Neurol. 8 (9), 791–801. https://doi.org/10.1016/S1474-4422(09)70170-X.
Tha, K.K., Terae, S., Tsukahara, A., Soma, H., Morita, R., Yabe, I., Ito, Y.M., Sasaki, H., Shirato, H., 2012. Hyperintense putaminal rim at 1.5 T: prevalence in normal subjects and distinguishing features from multiple system atrophy. BMC Neurol. 12, 39. https://doi.org/10.1186/1471-2377-12-39.
Walker, R.H., Jankovic, J., O'Hearn, E., Margolis, R.L., 2003. Phenotypic features of Huntington's disease-like 2. Mov. Disord. 18, 1527–1530. https://doi.org/10.1002/mds.10587.
Walker, R.H., Gatto, E.M., Bustamante, M.L., Bernal-Pacheco, O., Cardoso, F., Castilhos, R.M., Chana-Cuevas, P., Cornejo-Olivas, M., Estrada-Bellmann, I., Jardim, L.B., López-Castellanos, R., López-Contreras, R., Maia, D.P., Mazzetti, P., Miranda, M., Rodríguez-Violante, M., Teive, H., Tumas, V., 2018. Huntington's disease-like disorders in Latin America and the Caribbean. Parkinsonism Relat. Disord. 1–12. https://doi.org/10.1016/j.parkreldis.2018.05.021.
Wilburn, B., Rudnicki, D.D., Zhao, J., Weitz, T.M., Cheng, Y., Gu, X., Greiner, E., Park, C.S., Wang, N., Sopher, B.L., La Spada, A.R., Osmand, A., Margolis, R.L., Sun, Y.E., Yang, X.W., 2011. An antisense CAG repeat transcript at JPH3 locus mediates expanded polyglutamine protein toxicity in Huntington's disease-like 2 mice. Neuron 70, 427–440. https://doi.org/10.1016/j.neuron.2011.03.021.

Movement Disorders
CLINICAL PRACTICE

RESEARCH ARTICLE

Comparison of the Huntington's Disease like 2 and Huntington's Disease Clinical Phenotypes

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ABSTRACT: Background: Huntington's disease like 2 (HDL2) is the most common Huntington's disease (HD) phenocopy in many countries and described as the phenocopy with the greatest resemblance to HD. The current clinical description of HDL2 is based on retrospective data. It is unknown whether HDL2 has clinical features that distinguish it from HD.

Objective: To describe the HDL2 phenotype and compare it to HD systematically.

Methods: A blinded cross-sectional design was used to compare the HDL2 (n = 15) and HD (n = 13) phenotypes. African ancestry participants underwent assessments, including the Unified Huntington's Disease Rating Scale (UHDRS). The UHDRS motor component was video recorded and evaluated by blinded experts and the inter-rater reliability calculated.

Results: Both groups were homogeneous in terms of demographics and disease characteristics. However, HDL2 patients presented three years earlier with more prominent dysarthria and dystonia. Raters could not distinguish between the two diseases with a high level of agreement. No significant differences in the TMS between HDL2 and HD were found. In both disorders, disease duration correlated with motor scores, with the exception of chorea. Psychiatric and cognitive scores were not significantly different between the groups.

Conclusions: The HDL2 phenotype is similar to HD and is initially characterized by dementia, chorea, and oculomotor abnormalities, progressing to a rigid and bradykinetic state, suggesting the UHDRS is useful to monitor disease progression in HDL2. Although HDL2 patients scored higher on some UHDRS domains, this did not differentiate between the two diseases; it may however be emerging evidence of HDL2 having a more severe clinical phenotype.

Introduction

Since its discovery,[1] Huntington disease like 2 (HDL2) has emerged as the most common Huntington disease (HD) phenocopy in patients with African ancestry.[2] In the South African black population, for every two cases diagnosed with HD there is one case diagnosed with HDL2.[3] Importantly HDL2 is the most frequent HD phenocopy in South America, the United States, and parts of Europe.[4–6]

The index HDL2 pedigree was followed in the Baltimore Huntington's Disease clinic for many years as a family believed to have HD. The HD mutation, caused by a CAG repeat expansion

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occurring in exon 1 of the *huntingtin* (HTT) gene on chromosome 4p16.3, was reported in 1993.[7] When genetic testing then became available, this African American family was tested and found not to have the HD mutation.[1] A subsequent study led to the discovery that the disease in this family was caused by a CTG expansion mutation in exon 2A of *junctophilin-3* (*JPH3*) on chromosome 16q24.3, and to the designation of the disease as HDL2.[8]

Both disorders present with movement abnormalities, progressive subcortical dementia, and psychiatric symptoms that progress to death 15 to 20 years after the onset of the disease.[1,9] However, previous reports suggested that HDL2 presents with two different phenotypes:[10] a parkinsonian, earlier-onset form without oculomotor involvement, or second, a later-onset variant that is akin to the classical HD phenotype with typical oculomotor involvement. Overall, the HDL2 phenotype has been considered to have greater parkinsonism, less dysarthria, and relatively preserved oculomotor function compared to HD.[9,11] A systematic review of 69 cases of HDL2 corroborated some of these reported findings.[12] Parkinsonism was reported in 37% of HDL2 cases. However, this was without the specific parkinsonian features of rigidity and bradykinesia being described. Previous definitions of parkinsonism have implied the presence of a tremor with other classic hypokinetic features.[14] Therefore, defining specific features of hypokinesia would allow for a more accurate description of the HDL2 phenotype. Dystonia was described in conjunction with parkinsonism and/or chorea in only 16 cases of HDL2. Dysarthria was identified in one-third of the HDL2 cases and was reported as mild to moderate. Only eight of the 19 cases in which an oculomotor exam was recorded had an abnormal oculomotor function, potentially differentiating HDL2 from HD, as early loss of oculomotor function is considered a cardinal sign in HD.[12,13] Myoclonus has been reported in four cases of HDL2.[11,15] Like HD, dementia was seen in almost all cases, chorea was the most common motor finding, and a strong negative correlation was observed between repeat length and age of onset of the disease.[12]

Descriptions of the HDL2 phenotype have consisted of small case series with limited detailed documentation of particular clinical signs being either present or absent. To date, there have been only three reports where the Unified Huntington's Disease Rating Scale (UHDRS) was used to assess HDL2 patients.[5,11,16] Therefore, the current understanding of the HDL2 phenotype remains incomplete.[12] Systematic examination of specific phenotypic features has yielded findings that were not consistent with initial reports. For instance, reports of acanthocytosis in HDL2 were not confirmed in a blinded, controlled study.[17] Furthermore, volumetric comparisons between matched HD and HDL2 subject's MRIs have demonstrated remarkable similarities, as expected from previous case series of HDL2 neuroimages. However, an exception of greater thalamic atrophy in HDL2 has been reported in a blinded study for the first time, and could potentially distinguish HDL2 from HD when using quantitative MRI image analysis.[18]

To compare the HDL2 and HD clinical phenotype systematically, we took advantage of the relatively high prevalence of HDL2 in South Africa.[2] Using standardized rating scales and video examinations by experienced clinicians that were blinded to the patients' genetic diagnosis, we developed a protocol for rating clinical phenotypes. Our results demonstrate remarkable similarities between the two disorders, and go some way to clarify the HDL2 phenotype.

Materials and Methods

Study Design

A cross-sectional design with blinded assessment was used to compare the phenotypes of patients with HDL2 (n = 15) and HD (n = 13). Patients with a genetically confirmed diagnosis of HDL2 or HD, tested at The Division of Human Genetics, National Health Laboratory Service (NHLS) in Johannesburg South Africa, were invited to enroll into an ongoing prospective study of the HDL2 phenotype as previously described.[12] HDL2 has been exclusively identified in patients with African and patients with mixed ancestry to date.[2] (Mixed ancestry individuals in South Africa are those whose gene pool is derived from one or more of the indigenous African populations [San, Khoikhoi or Bantu speaking]; European immigrants from western Europe; and/or slaves and indentured laborers from Madagascar, the Malaysian archipelago, and India.[2]) Therefore, both cohorts were restricted to these ethnic groups to avoid potential unblinding. The Human Research Ethics Committee of The University of the Witwatersrand approved this study (M140872).

Data Ascertainment

A movement disorder neurologist (DGA) interviewed all the participants and a friend/family member. The age of disease onset was defined as the age at which the subject initially experienced abnormal movements. These data were obtained from interviewing the patients and their accompanying friends/family members. They were then asked whether the initial symptom of the disease was: cognitive, psychiatric, or movement abnormality. Participants were then examined using the UHDRS Total Motor Score (TMS); the examination was recorded on video. Three raters (AA, DGA, FBR), experienced in the clinical examination of HD, independently viewed the videos, and scored the TMS for each subject. Raters were blinded to the genetic diagnosis of the patients. All TMS raters are Enroll-HD TMS certified and have at least three years' experience using the scale. After reviewing the videos, evaluators also assigned each participant a disease diagnosis of HDL2 or HD. The TMS scores were averaged among the three examiners. Patients underwent a cognitive assessment using the cognitive components of the UHDRS by an experienced neuropsychologist (AFC). A subgroup of patients could not complete cognitive components of the UHDRS due to the presence of important confounding variables on neuropsychological performance or inability to take the cognitive tests. Specifically, 11 cases were excluded; four of them presented with advanced dementia, three were human immunodeficiency virus

positive (HIV+), three were not proficient in English, and one had motor aphasia.

Data Analysis

Comparison of abnormal repeat length, age at onset, age at diagnosis, and disease duration between diseases was performed using the Wilcoxon rank sum test. Comparison of the difference between age at onset (defined as the age at which the subject initially experienced abnormal movements) and age at diagnosis between phenotypes was done using the independent samples t-test. The relationship between age at onset and abnormal repeat length, overall and by group, was determined by a General Linear Model (GLM) with age at onset as the dependent variable and abnormal repeat length and disease group, and their interaction, as the independent variables. Comparison of manifesting symptom, ethnicity, and gender between phenotypes was performed using Fisher's exact test. Inter-rater reliability (IR) analysis is discussed in the Supporting Data.

Composite motor assessment scores for each dimension were calculated by averaging the scores of the three raters. Comparison of these motor assessment dimensions and total scores, as well as the UDHRS functional assessment scores between phenotypes, were done with a GLM with the score as the dependent variable, and phenotype and disease duration as the independent variables. The phenotype-disease duration interaction was non-significant in all cases and was removed from the model. We controlled for disease duration (rather than age of onset, or repeat length) since this had the largest difference between the two phenotypes. The z-test for proportions was used to determine the accuracy of the clinician assigning the subject diagnosis as either HDL2 or HD. A 5% significance level was used.

Results

Demographic and Genetic Data

Thirteen patients with HD and 15 with HDL2 were studied from predominantly the north of South Africa (Fig. 1). In the HD group, three of the ten pedigrees each contributed two participants to the data. The HDL2 group consisted of 15 unrelated individuals. There was no significant difference between the abnormal triplet repeat length, age of disease onset, age at diagnosis, gender, ethnicity, or initial manifesting symptom between the two groups (Table 1). The median duration of disease was longer in the HD group (7y; IQR 6–8) compared to the HDL2 group (4y; IQR 3–7y; $P = 0.029$).

Age at onset (defined as the age at which the subject initially experienced abnormal movements) was strongly associated with the abnormal repeat length in both diseases ($P < 0.0001$), and the slope of the relationship varied with the disease group ($P = 0.022$). For every triplet increase in abnormal repeat length, the age at onset decreased by 3.2 years for the HD group, but by only 1.8 years for the HDL2 group (Fig. 2). The estimated delay between onset and molecular diagnosis was 6.0 ± 1.6y (95% CI) for the HD group, compared to 3.3 ± 1.5y for the HDL2 group ($P = 0.021$). This suggests that the time to present for medical assistance and diagnosis from the time of disease onset in the HDL2 patients was approximately three years earlier compared to HD patients. However, the age of disease onset is later for a given abnormal triplet repeat length in HDL2 compared to HD patients. The CAG repeat size on the normal allele has been shown to influence the age of disease onset in HD.[19] However, the age at onset was not significantly related to the normal triplet repeat length when controlling for either disease.

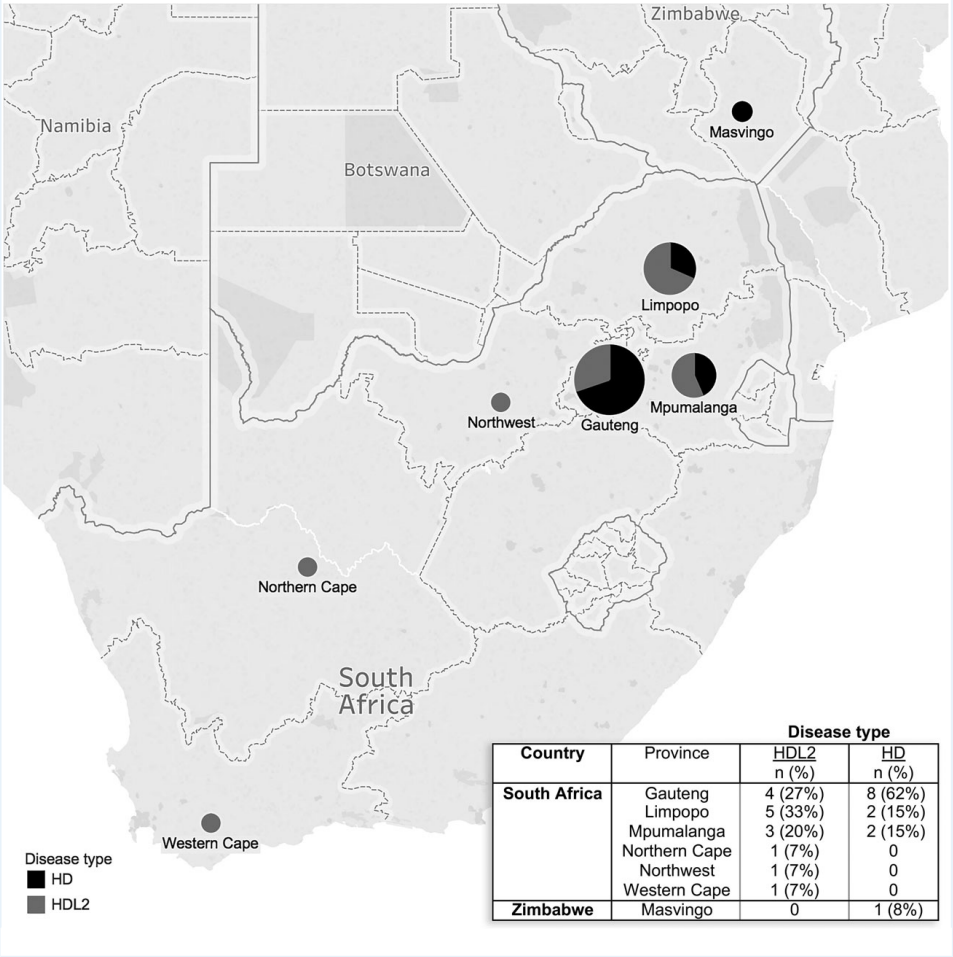
Comorbidities and Concomitant Medications

Two of the HDL2 and one of the HD patients were HIV+, and none of them had other infections. The HIV+ cases had MRI scans at the time of the study showing atrophic changes in keeping with HD. The MRIs did not show any focal lesions or HIV-associated infections. One of the HDL2 HIV+ cases had a CD4 cell count below 200 cells/μL. This subject was awaiting HIV treatment. The other two HIV+ cases had CD4 cell counts of over 500 cells/μL and were receiving antiretroviral treatment. Eight HDL2 and eight HD patients were taking dopamine antagonists. Three HD patients and none of the HDL2 group had myoclonus. Two were brothers who also had generalized seizures. Both brothers had an abnormal, expanded HD allele with a relatively long 47 triplet repeats. The disease onset was also relatively young at 23 and 28 years of age for each subject, with chorea as the manifesting symptom. However, apart from the myoclonus and seizures they did not differ significantly from the rest of the HD cases. EEG reports for both brothers were reported as generalized spike and wave discharges, and both were treated with sodium valproate. Their MRIs at the time of the study showed typical HD changes with striatal atrophy without focal brain lesions or other radiological findings that could account for the seizures. All three reported on history that the myoclonus and seizures began after the onset of HD motor symptoms.

Motor Phenotype

Across all patients, the Brennan-Prediger coefficient IR for the TMS was 0.80 (95% CI = 0.70–0.90), in the "substantial-to-almost-perfect" range, with an intraclass correlation (ICC) = 0.95, nearly identical to the standard set in the original development of the UHDRS.[20] IR ranged from moderate to near perfect for other subscores, similar to previously established standards[21] (Supporting Data).

When controlling for duration of disease, there was a significant increase in motor scores, with increased disease duration in both disease groups. The rate of increase was similar between diseases (Fig. 3). The total motor score increased by 4.4 ± 1.3 points and this did not differ significantly between disease groups ($P = 0.37$). However, for a given disease duration, the HDL2 patients had TMS scores that were higher on average, but this difference did not reach statistical significance ($P = 0.06$). In both disease groups, most individual component scores increased by an estimated



Country	Province	Disease type: HDL2 n (%)	Disease type: HD n (%)
South Africa	Gauteng	4 (27%)	8 (62%)
	Limpopo	5 (33%)	2 (15%)
	Mpumalanga	3 (20%)	2 (15%)
	Northern Cape	1 (7%)	0
	Northwest	1 (7%)	0
	Western Cape	1 (7%)	0
Zimbabwe	Masvingo	0	1 (8%)

FIG. 1. Distribution of Huntington's disease like 2 and Huntington's disease patients.
Map of Southern Africa showing the distribution of Huntington's disease like 2 and Huntington's disease patients.
Abbreviations: HD, Huntington's disease; HDL2, Huntington's disease like 2; n = number of participants.

0.2 ± 0.1 units for every year of disease ($P < 0.0001$). The chorea scores were the exception to this trend and were not higher in individuals with longer disease duration in either disease. This finding is consistent with previous reports in HD where the severity of chorea tends to plateau from the early to moderate stages of the disease.[22]

On average, the HDL2 group had worse results for the combined dystonia score (t = -2.6; $P = 0.015$), dysarthria score (t = -3.33; $P = 0.003$), and the Luria test scores (t = -3.38; $P = 0.002$) when compared to the HD group and controlling for duration of disease (Table 2A). We found no other significant differences, either overall or on any sub-score, in the TMS between the two groups.

Blinded raters' success at assigning a diagnosis of HD or HDL2 to each case was no better than chance ($P = 0.28$).

Behavior and Functional Components

All three UHDRS functional assessments demonstrated increased impairment in each disease with longer disease duration, but no

TABLE 1 *Comparison of demographic, genetic, and manifesting symptom data between Huntington's disease like 2 and Huntington's disease groups*

Demographic/genetic variable		HDL2 (n = 15) Median	HDL2 (n = 15) IQR	HD (n = 13) Median	HD (n = 13) IQR	*P* value between groups
Abnormal triplet repeat length		46	44-50	46	42-47	0.19
Disease duration (y)		4	3-7	7	6-8	**0.029**
Age at onset (y)		41	35-50	40	28-43	0.41
Age at diagnosis (y)		47	38-54	45	34-50	0.68
Years of education		12	9-15	12	12-14	0.80
		n (%)		**n (%)**		
Gender	Female	7 (47%)		7 (54%)		0.71
	Male	8 (53%)		6 (46%)		
Race	Black	12 (80%)		6 (46%)		0.061
	Mixed	3 (20%)		7 (54%)		
Manifesting symptom	Cognitive	3 (20%)		2 (15%)		0.57
	Movement	10 (67%)		7 (54%)		
	Psychiatric	2 (13%)		4 (31%)		

Table comparing the demographic, genetic and manifesting symptom data between HDL2 and HD groups. Note Two-tailed significance at $P < 0.05$ for *P*-value between groups with statistically significant findings noted in bold font.
Abbreviations: HD, Huntington's Disease; HDL2, Huntington's disease like 2; IQR, interquartile range; y, years.

difference between the two diseases controlling for duration of disease (Table 2B).

The behavioral component score of the UHDRS, designed to measure psychiatric symptoms in HD, did not significantly differ between the two groups and did not change with the disease duration (Table 2C).

Cognition

Cognitive scores were not significantly different between the two groups except for a higher score on the Stroop Colour-Word test for the HD patients when controlling for covariates as described ($P = 0.04$; Table 2D).

Discussion

This is the first comparison of the HDL2 and HD clinical phenotypes by blinded raters using the UHDRS. In summary, the demographic features of HD and HDL2 patients were comparable, the results support the previously described phenotypic similarity between HDL2 and HD despite their genetic differences,[1,2,12] and the diseases remain clinically indistinguishable at various points of disease duration.

When comparing the HDL2 and HD patients using the UHDRS, all four components of the scale showed similar scores. After scoring the UHDRS TMS, blinded specialists were unable to differentiate HDL2 from HD. This has important implications for clinical practice. Clinicians should consider testing for the *JPH3* mutation in patients with African ancestry with a HD phenotype but no *HTT* expansion mutation.

Dementia is a prominent feature in HDL2. Nevertheless, identifying a more refined neurocognitive profile in HDL2 has been challenging because many patients presenting with advanced disease are unable to complete comprehensive testing.[23] Although this remains to be further investigated, the present study detected no quantitative difference between HDL2 and HD except that the Stroop Color and Word test median score for the HD patients was significantly higher than that of the HDL2 patients. In addition, HDL2 patients performed worse than HD patients on the Luria test, although this test has not been useful in differentiating different dementias.[24] This suggests that the cognitive dysfunction in HDL2 may be similar to that of HD, although it introduces the possibility that HDL2 dementia may be different from HD in terms of severity and progression. Dedicated research to the neuropsychology of HDL2 is needed, as the present study did not control for relevant neuropsychological cofounders.[25]

The behavior and independence components of the UHDRS did not differentiate HDL2 from HD. The independence results, for both diseases, worsened with disease duration as was seen in the UHDRS cognitive and motor scores. The psychiatric measures showed no correlation with duration for either disease, a finding that has been previously described in HD[20] and likely reflects the unpredictable and nonlinear nature of the psychiatric syndromes in these disorders.

Due to the lack of systematic descriptions from the literature and reports of motor features that have been described to differentiate HDL2 from HD, the motor phenotype was of particular interest to the authors. Eye movements, initially reported as relatively preserved in HDL2, were abnormal in all HDL2 cases. Abnormal eye movements were detected in patients in the early stages of HDL2, and appeared worse with longer disease duration, mirroring the findings in HD.

Chorea was not significantly different between the HDL2 and HD groups and was present in all but one HDL2 case. In both diseases, the chorea score was the only clinical sign that did not increase with disease duration. This has been described previously in HD.[22]

Previous accounts of HDL2 have highlighted a higher rate of parkinsonism.[12] This was not shown in this study, as bradykinesia and rigidity were similar in the two diseases. Previous descriptions of HDL2 patients with a more parkinsonian phenotype may be attributable to evaluations performed later in the course of the disease when parkinsonism is more prominent.[1,11]

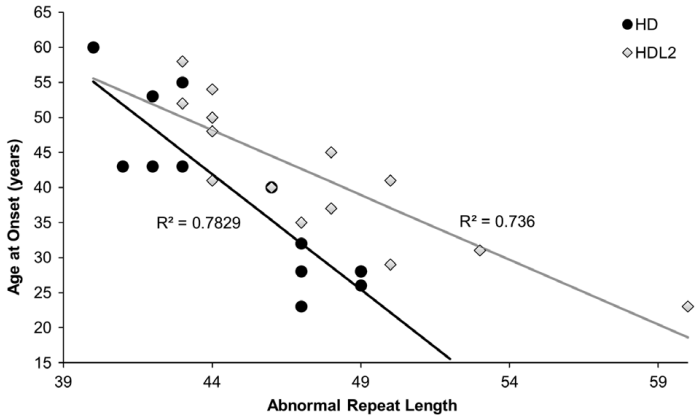


FIG. 2. Age of disease onset vs. abnormal repeat length.
Scatterplot showing inverse relationship between age of onset and abnormal triplet repeat length for both HDL2 and HD.
Abbreviations: HD, Huntington's disease; HDL2, Huntington's disease like 2.

Myoclonus has been previously described in HDL2.[12] All cases of myoclonus from this current study were in the HD group. The distribution of patients with myoclonus being only in the HD group and none in the HDL2 group is possibly due to the small sample size. This may result in a larger margin of error, and therefore this finding may not be a true reflection of the myoclonus in HD and HDL2. The existence of generalized epilepsy in two of our adult-onset patients is of interest as epilepsy associated with HD has only been described in the juvenile onset form of the disease.[26] Both patients with epilepsy from this study were brothers, and both had 47 CAG repeat expansions in the HD locus. The disease manifested relatively in their twenties in both cases. However, phenotypically the HD did not differ from the other HD study subject with predominant chorea, and their MRI scans showed typical HD changes with no focal cause for seizures.

While most movement abnormalities were similar in the HD and HDL2 groups, the dysarthria and dystonia scores were worse in the HDL2 patients. Dystonia is recognized as the TMS item with the poorest interrater reliability due to the subjectivity and difficulty with interpretation.[21] Our interrater agreement for dystonia showed substantial agreement (0.67), which is much higher than recently reported (0.34).[21]

Compared to HD patients, the age of disease onset in the HDL2 group was later for a given abnormal triplet repeat length. This has been was previously reported in a retrospective analysis of 41 HDL2 cases.[2] However, HDL2 patients in this study presented for diagnosis on average three years sooner once the disease had manifested when compared to the HD patients. This finding suggests that once the disease manifests in HDL2, there may be more prominent features resulting in these patients seeking medical care sooner after onset. The Luria test, dysarthria, and dystonia are more severe in HDL2 compared to HD as the UHDRS findings have shown in this study which may account for the HDL2 cases seeking aid earlier.

Given the similarity of HD and HDL2 phenotypically, identifying other features that HDL2 and HD share may help elucidate pathogenic pathways implicated in both diseases. Common features of triplet repeat disorders include a strong negative correlation between age of onset and the number of triplet repeats. The two groups had very similar inverse correlations (HDL2 [$R^2 = 0.74$], HD [$R^2 = 0.78$]), which has been previously noted in a systematic review of HDL2 cases.[12] Another feature that many CAG repeat disorders share is that they manifest clinically at a threshold of 35 to 40 triplet repeats.[27] This has been shown to be the threshold that polyglutamine proteins begin to aggregate in vitro studies.[28] Although there is some debate to the degree that polyglutamine proteins play in the pathogenesis of HDL2,[29] the identical number of 40 triplet repeats shared between HDL2 and HD at which the diseases manifest[8] may suggest some molecular overlap.

Current evidence suggests that the HD expansion mutation results in disease primarily through the expression of a protein with an excessively long stretch of polyglutamine residues, with contributions from toxic properties of the mutant RNA and loss of normal protein function.[30] In HDL2, in which pathogenic pathways are less established, evidence suggests that loss of *JPH3* expression and toxic properties of the mutant *JPH3* RNA contribute to neurodegeneration, with a role for cryptic antisense expression of a transcript encoding a protein with an expanded stretch of polyglutamine residues.[29,31,32] The similarities between the two diseases genetically, clinically, pathologically, and radiologically,

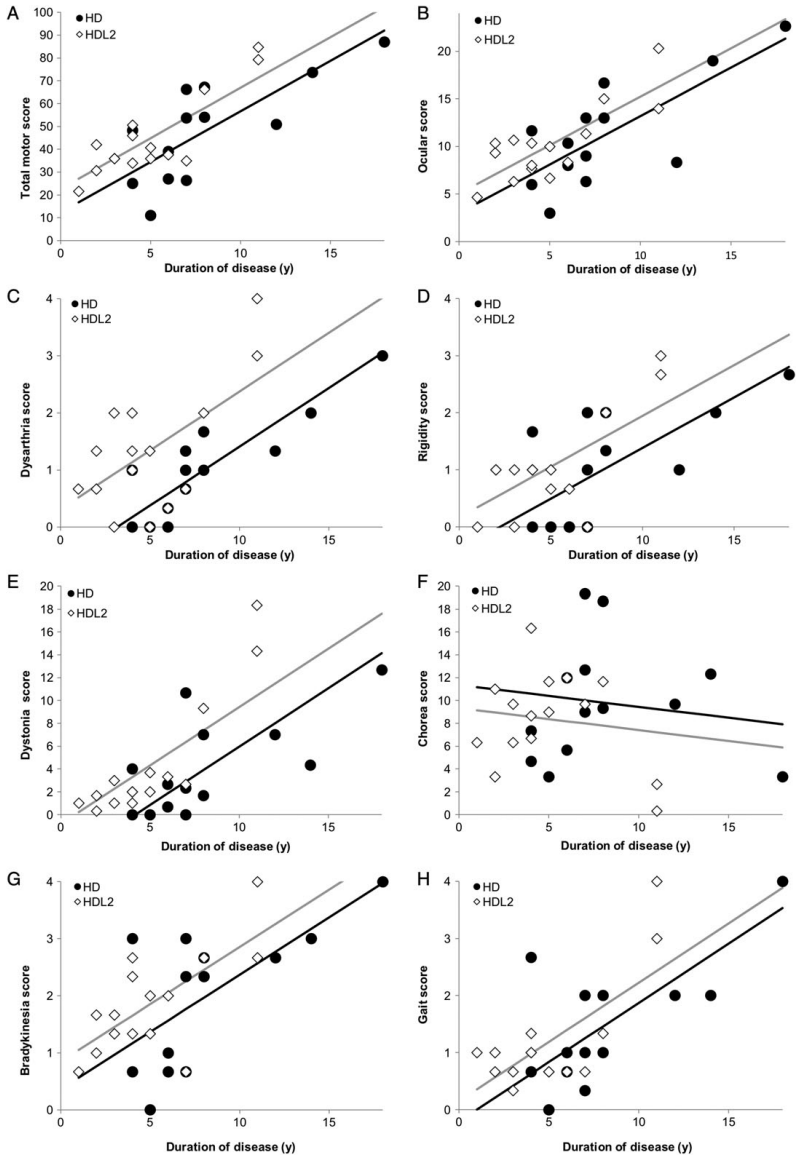


FIG. 3. Scatterplots for Unified Huntington's Disease Rating Scale Total Motor Score and sub-scores vs. duration of disease. Scatterplots demonstrating the TMS and some sub-scores from the UHDRS. (A) Total Motor Score (B) Ocular Score (C) Dysarthria Score (D) Rigidity Score (E) Dystonia (F) Chorea (G) Bradykinesia (H) Gait.
Abbreviations: HD, Huntington's Disease; HDL2, Huntington's Disease like 2; UHDRS TMS, Unified Huntington's Disease Rating Scale Total Motor Score; y, year.

TABLE 2 *Comparisons of the Unified Huntington's Disease Rating Scale between Huntington's disease like 2 and Huntington's disease groups*

UHDRS	HDL2			HD			*P* value between groups
A. UHDRS-TMS	**N**	**Mean**	**STD**	**N**	**Mean**	**STD**	
Total motor score	15	45.1	18.0	13	48.4	22.1	0.06
	N	**Median**	**IQR**	**N**	**Median**	**IQR**	
Combined ocular score	15	10.0	7.7-11.3	13	10.3	8.0-13.0	0.12
Dysarthria	15	1.3	0.7-2.0	13	1.0	0.3-1.3	**0.003**
Tongue protrusion	15	1.0	0.0-2.7	13	1.3	1.0 -2.0	0.13
Combined rigidity	15	1.0	0.7-1.0	13	1.0	0.0-2.0	0.06
Luria	15	4.0	2.3-4.0	13	2.3	1.0-3.7	**0.002**
Combined dystonia	15	2.0	1.0-3.7	13	2.7	0.7-7.0	**0.015**
Combined chorea	15	9.0	6.3-11.7	13	9.3	5.7-12.3	0.31
Bradykinesia	15	2.3	0.7-3.0	13	2.3	0.7-0.3	0.16
Retropulsion	15	1.7	1.0-2.0	13	2.0	2.0-2.0	0.71
Gait	15	1.0	0.7-1.3	13	1.0	0.7-2.0	0.26
Tandem gait	15	1.0	0.3-3.3	13	2.3	1.0-3.0	0.70
B. Functional scores	**N**	**Median**	**IQR**	**N**	**Median**	**IQR**	
Functional capacity assessment	15	5.0	3.0-9.0	13	5.0	2.0-7.0	0.23
Functional checklist	15	15.0	7.0-21.0	13	14.0	9.0-18.0	0.11
Independence score	15	70	60- 90	13	70	60-90	0.05
C. Behavioral assessment	**N**	**Median**	**IQR**	**N**	**Median**	**IQR**	
Behavior score	15	20.0	9.0-23.0	13	19.0	13.0-33.0	0.34
D. Cognitive assessment	**N**	**Median**	**IQR**	**N**	**Median**	**IQR**	
MoCA	7	13	11-16	10	16.5	13-20	0.15
SDMT written	5	14	10-20	10	15	11-21	0.82
SDMT oral	5	20	11-24	10	17.5	10-21	0.57
% Speed/accuracy	4	88.5	80.5-89.5	10	87.5	76-92	0.68
Stroop word	7	44	39-81	10	52	30-61	0.60
Stroop color	7	19	16-50	10	26	23-35	0.60
Stroop interference	7	7	2-12	10	18.5	15-21	**0.04**

(A) UHDRS Total Motor Scores - Noting some of the scores are combined. (B) Functional Scores (C) Total Behavioural Score (D) Cognitive Assessments. Note: Two-tailed significance at $P < 0.05$ for *P*-value between groups with statistically significant findings noted in bold font.
Abbreviations: HD, Huntington's Disease; HDL2, Huntington's disease like 2; IQR, interquartile range; MoCA, Montréal Cognitive Assessment; N, number; SDMT, Symbol Digit Modality Test; STD, Standard deviation; UHDRS TMS, Unified Huntington's Disease Rating Scale Total Motor Score.

are striking and suggest common mechanisms of pathogenesis. However, it is likely that divergent aspects of their pathogenesis result in subtle phenotypic differences like dysarthria and dystonia, or more striking symptoms at onset, leading to an earlier diagnosis. We have shown in this study that differences between HDL2 and HD cannot be differentiated by examining individual patients.

The key limitation of this study is the small sample size, reflecting the low prevalence of HDL2 even in a population in which the disease is more common, and the difficulty in ascertaining cases in areas with poor healthcare services.[3] Nonetheless, our analysis is the first systematic comparison of HD and HDL2. While subtle differences between the diseases cannot be excluded, our results strongly support that the clinical phenotype of the two diseases is indistinguishable in the clinical setting.

A possible limitation could be the inclusion of mixed ancestry patients into the analysis as a way of introducing other genetic modifying variables into the study. However, HDL2 has been exclusively reported in black and mixed ancestry patients around the world.[2,6,12] In Johannesburg, a significant proportion of the cases with HDL2 have mixed ancestry.[2] Therefore, the mixed ancestry group represents an important subsection of the HDL2 phenotype. Excluding this group would not be a true reflection of the HDL2 phenotype and furthermore, this would have reduced the number of cases available to study.

Another potential limitation of the study was the inclusion of HIV+ participants; however, there were important reasons for their inclusion. First, the HIV+ cases are representative of this population as South Africa has the highest prevalence of HIV in the world with seven million people infected.[33] Second, the HD phenotype has not been described as different in HIV from previous studies, although HD may manifest earlier in the presence of HIV.[34] HIV-associated neurocognitive disorders are common in HIV; therefore, the three HIV+ cases from this study were excluded from the cognitive analysis.[35] Comparatively, HIV associated movement disorders are rare, affecting 2 to 3% of cases with chorea and parkinsonism related to HIV often have secondary causes like toxoplasmosis or cerebral infarcts,[36] which were not present in these cases as evidenced by the MRI findings of the HIV+ cases.

Conclusions

Our results indicate that, like HD, HDL2 is heterogenous in its clinical phenotype and is characterized by dementia, chorea and oculomotor abnormalities as the manifesting symptoms, with progression to a rigid and bradykinetic state with worsening dystonia. However, compared to HD, HDL2 patients presented three years earlier for diagnosis from the time of the disease manifesting, had poorer scores on dysarthria and dystonia testing, and had worse results on the Luria and Stroop interference tests. These differences may suggest a more severe course in HDL2 compared to HD. However, this did not help in distinguishing

the two diseases when blinded examiners assessed the patients. Therefore, we suggest testing for HDL2 if the HD mutation is negative in a subject with African ancestry.

Previous studies have shown the UHDRS to be a sensitive tool to measure motor changes in HD. We have now demonstrated that HDL2 and HD share the same clinical features and that their motor phenotypes are indistinguishable. Thus, with the current evidence, developing a separate rating scale for HDL2 appears unnecessary. Therefore, we suggest that the UHDRS, once further validated, may be a useful method of monitoring HDL2 longitudinally and for determining treatment outcomes as it has been for HD.

Finally, the findings of this study support common pathogenic pathways between HD and HDL2 that remain unknown. Detecting these pathogenic mechanisms could assist in identifying therapeutic targets for both diseases.

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Author Roles

1. Research Project: A. Conception, B. Organization, C. Execution; 2. Statistical Analysis: A. Design, B. Execution, C. Review and Critique; 3. Manuscript Preparation: A. Writing the First Draft, B. Review and Critique.

D.G.A.: 1A, 1B, 1C, 2B, 2C, 3A, 3B
A.F.C.: 1A, 1B, 1C, 2B, 2C, 3B
F.B.R.: 1C, 2C, 3B
N.A.A.: 1C, 3B
E.J.W: 2B, 2C, 3B
J.C.: 1A, 2B, 2C, 3B
A.K.: 1A, 1B, 2B, 2C, 3B
R.L.M.: 1A, 2B, 2C, 3B

Aline Ferreira-Correia: Acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content.

Filipe B. Rodrigues: Review of videos, analysis and interpretation, critical revision of the manuscript for important intellectual content.

Ahmad Aziz: Review of videos, analysis and interpretation, critical revision of the manuscript for important intellectual content.

Edward J Wild: Analysis and interpretation, critical revision of the manuscript for important intellectual content.

Jonathan Carr: Study supervision, critical revision of the manuscript for important intellectual content.

Amanda Krause: Study supervision, Analysis and interpretation, critical revision of the manuscript for important intellectual content, senior author.

Russell L. Margolis: Study supervision, Analysis and interpretation, critical revision of the manuscript for important intellectual content, senior author.

Disclosures

Ethical Compliance Statement: The authors confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines. The authors confirm that The Human Research Ethics Committee of The University of the Witwatersrand approved this study (M140872) and subject consent was obtained for this work.

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References

1. Margolis RL, O'Hearn E, Rosenblatt A, et al. A disorder similar to Huntington's disease is associated with a novel CAG repeat expansion *Ann Neurol* 2001;50(3):373–380.
2. Krause A, Mitchell C, Essop F, et al. Junctophilin 3 (JPH3) expansion mutations causing Huntington disease like 2 (HDL2) are common in South African patients with African ancestry and a Huntington disease phenotype. *Am J Med Genet B Neuropsychiatr Genet* 2015 Oct;168(7):573–585.
3. Baine FK, Krause A, Greenberg LJ. The frequency of Huntington disease and Huntington disease-like 2 in the South African population. *Neuroepidemiology* 2016;46(3):198–202.
4. Margolis RL, Holmes SE, Rosenblatt A, et al. Huntington's disease-like 2 (HDL2) in North America and Japan. *Ann Neurol* 2004;56(5):670–674.
5. Mariani LL, Tesson C, Charles P, et al. Expanding the spectrum of genes involved in Huntington disease using a combined clinical and genetic approach. *JAMA Neurol* 2016;73(9):1–10.
6. Walker RH, Gatto EM, Bustamante ML, et al. Huntington's disease-like disorders in Latin America and the Caribbean. *Parkinsonism Relat Disord* 2018;53:1–12.
7. MacDonald ME, Ambrose CM, Duyao MP, et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1993;72(6):971–983.
8. Holmes SE, O'Hearn E, Rosenblatt A, et al. A repeat expansion in the gene encoding junctophilin-3 is associated with Huntington disease-like 2. *Nat Genet* 2001;30:29(4):377–378.
9. Walker RH, Jankovic J, O'Hearn E, Margolis RL. Phenotypic features of Huntington's disease-like 2. *Mov Disord* 2003;9:18(12):1527–1530.
10. Margolis RL. Huntington Disease-Like 2 - GeneReviews® - NCBI Bookshelf. httpwww.ncbi.nlm.nih.govbooksNBK 2004:1–14. Accessed 25 July, 2018.
11. Schneider SA, Marshall KE, Xiao J, LeDoux MS. JPH3 repeat expansions cause a progressive akinetic-rigid syndrome with severe dementia and putaminal rim in a five-generation African-American family. *Neurogenetics* 2012;13(2):133–140.

12. Anderson DG, Walker RH, Connor M, Carr J, Margolis RL, Krause A. A systematic review of the Huntington disease-Like 2 phenotype. *J Huntingtons Dis* 2017;6(1):37–46.
13. Jankovic J. Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosur Ps* 2008;79(4):368–376.
14. Lasker AG, Zee DS. Ocular motor abnormalities in Huntington's disease. *Vision Res* 1997;37(24):3639–3645.
15. Bardien S, Abrahams F, Soodyall H, et al. A South African mixed ancestry family with Huntington disease-like 2: clinical and genetic features. *Mov Disord* 2007;22(14):2083–2089.
16. Vasconcellos LFR, Macêdo PJOM, Franck JB, Tumas V, Marques Júnior W, Spitz M. Huntington's Disease like 2 presenting with isolated Parkinsonism. *J Neurol Sci* 2017;373:105–106.
17. Anderson DG, Carmona S, Naidoo K, et al. Absence of acanthocytosis in Huntington's disease-like 2: a prospective comparison with Huntington's disease. *Tremor Other Hyperkinet Mov (NY)* 2017;7:512.
18. Anderson DG, Haagensen M, Ferreira-Correia A, et al. Emerging differences between Huntington's disease-like 2 and Huntington's disease: a comparison using MRI brain volumetry. *Neuroimage Clin* 2019;21:101666.
19. Djoussé L, Knowlton B, Hayden M, et al. Interaction of normal and expanded CAG repeat sizes influences age at onset of Huntington disease. *Am J Med Genet* 2003 23;119A(3):279–282.
20. Huntington Study Group. Unified Huntington's Disease Rating Scale: reliability and consistency. *Mov Disord* 1996;11(2):136–142.
21. Winder JY, Roos RAC, Burgunder J-M, Marinus J, Reilmann R. Interrater Reliability of the Unified Huntington's Disease Rating Scale-total motor score certification. *Mov Disord Clin Pract* 2018;15;5(3):290–295.
22. Rosenblatt A, Kumar BV, Mo A, Welsh CS, Margolis RL, Ross CA. Age, CAG repeat length, and clinical progression in Huntington's disease. *Mov Disord* 2011;27(2):272–276.
23. Fischer CA, Licht EA. The neuropsychiatric manifestations of Huntington's disease-like 2. *J Neuropsych Clin N* 2012;24(4):489–492.
24. Weiner MF, Hynan LS, Rossetti H, Falkowski J. Luria's three-step test: what is it and what does it tell us? *Int Psychogeriatr* 2011;23(10):1602–1606.
25. Watts AD, Shuttleworth-Edwards AB. Neuropsychology in South Africa: confronting the challenges of specialist practice in a culturally diverse developing country. *Clin Neuropsychol* 2016;30(8):1305–1324.
26. Cloud LJ, Rosenblatt A, Margolis RL, et al. Seizures in juvenile Huntington's disease: frequency and characterization in a multicenter cohort. *Mov Disord* 2012;27(14):1797–1800.
27. Paulson H. Repeat expansion diseases. *Handb Clin Neurol*. 2018;147:105–123.
28. Brignull HR, Moore FE, Tang SJ, Morimoto RI. Polyglutamine proteins at the pathogenic threshold display neuron-specific aggregation in a pan-neuronal *Caenorhabditis elegans* model. *J Neurosci* 2006;26(29):7597–7606.
29. Seixas AI, Holmes SE, Takeshima H, et al. Loss of junctophilin-3 contributes to Huntington disease-like 2 pathogenesis. *Ann Neurol* 2012;71(2):245–257.
30. Romo L, Mohn ES, Aronin N. A fresh look at Huntingtin mRNA processing in Huntington's disease. *J Huntingtons Dis* 2018;7(2):101–108.
31. Rudnicki DD, Holmes SE, Lin MW, Thornton CA, Ross CA. Huntington's disease--like 2 is associated with CUG repeat-containing RNA foci. *Ann Neurol* 2007;61(3):272–282.
32. Wilburn B, Rudnicki DD, Zhao J, et al. An antisense cag repeat transcript at JPH3 locus mediates expanded polyglutamine protein toxicity in Huntington's disease-like 2 Mice *Neuron* 2011;70(3):427–440.
33. Statistics South Africa. Mid-year population estimates: 2017. https://www.statssa.gov.za/publications. 2017. Accessed 7 December, 2018.
34. Schultz JL, Nopoulos PC, Gonzalez-Alegre P. Human immunodeficiency virus infection in Huntington's disease is associated with an earlier age of symptom onset. *J Huntingtons Dis* 2018;7(2):163–166.
35. Sacktor N, Skolasky RL, Seaberg E, et al. Prevalence of HIV-associated neurocognitive disorders in the Multicenter AIDS Cohort Study. *Neurology* 2016;86(4):334–340.
36. Tse W, Cersosimo MG, Gracies J-M, Morgello S, Olanow CW, Koller W. Movement disorders and AIDS: a review. *Parkinsonism Relat Disord* 2004;10(6):323–334.

Supporting Information

Supporting information may be found in the online version of this article.

Supporting Data. Assessment of the inter-rater reliability for Unified Huntington's Disease Rating Scale comparing the motor scores of the Huntington's disease like 2 and Huntington's disease groups.

9.5 Appendix 5: Supplementary data

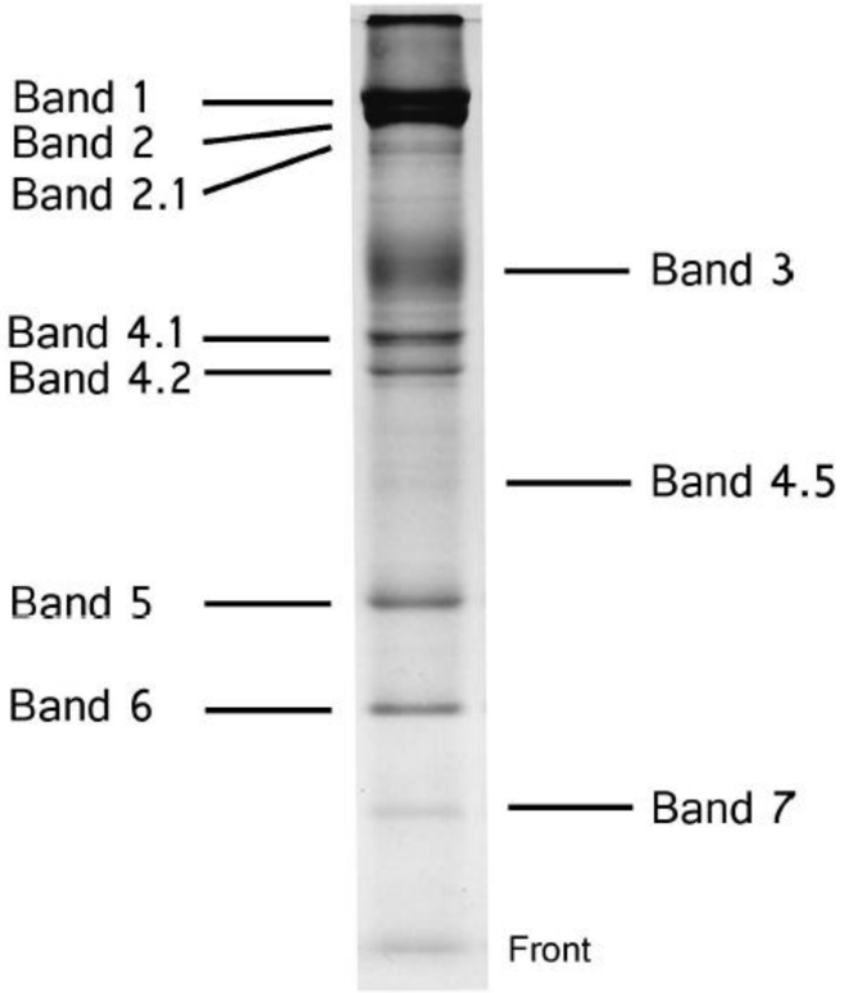

Figure 9.1 Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of human red blood cell (Aoki, 2017).

For the preparation of red blood cell membrane proteins, it is necessary to solubilize the phospholipid bilayer by sodium dodecyl sulfate, and then the extracted membrane proteins are separated by acrylamide gel electrophoresis (SDS-PAGE). Band 3 is a glycoprotein and is detected as a diffuse band on SDS gel due to the microheterogeneity of the attached oligosaccharides. The band 3 protein [is the most abundant of the red cell membrane proteins and composes approximately 25% of these proteins. For Band 3, approximately 1.0×10^6 copies are present in each red blood cell. The molecular mass of band 3 is estimated at approximately 100 kDa by SDS-PAGE. By mild proteolysis, the band 3 protein, which consists of 911 amino acid residues, can be divided into a hydrophilic cytoplasmic fragment of 41 kDa and a hydrophobic membrane fragment of 52 kDa. (Aoki, 2017)

Table 9.1:The differences in white and grey matter volumes between the groups (with p values) that are graphically represented in Figures 5.2 - 5.4

Volume	p-value for group effect			
	3 groups, control for age at MRI	2 groups, control for duration of disease	2 groups, control for abnormal repeat length	2 groups, control for age at onset
L Frontal Gm	0,0044	0,18	0,12	0,26
L Frontal Csf	<0.0001	0,01	0,03	0,01
R Frontal Wm	0,0041	0,16	0,01	0,22
R Frontal Csf	<0.0001	0,01	0,07	0,02
L Parietal Gm	0,0022	0,27	0,22	0,29
L Parietal Wm	0,0071	0,43	0,46	0,62
L Parietal Csf	<0.0001	0,18	0,41	0,29
R Parietal Gm	0,0036	0,26	0,19	0,27
R Parietal Csf	<0.0001	0,26	0,62	0,44
L Temporal Gm	0,0008	0,10	0,03	0,08
L Temporal Csf	<0.0001	0,02	0,01	0,02
R Temporal Gm	0,0023	0,07	0,04	0,07
R Temporal Csf	<0.0001	0,02	0,01	0,01
L Occipital Gm	0,0070	0,12	0,05	0,13
L Occipital Wm	0,0014	0,23	0,18	0,35
L Occipital Csf	<0.0001	0,07	0,10	0,09
R Occipital Wm	0,0030	0,44	0,33	0,58
R Occipital Csf	<0.0001	0,07	0,10	0,09
Brainstem Gm	0,0007	0,04	0,03	0,04
Brainstem Csf	0,0037	0,69	0,80	0,59
L Caudate	<0.0001	0,02	0,03	0,03
R Caudate	<0.0001	0,01	0,02	0,02
L Globus	<0.0001	0,45	0,41	0,42
R Globus	<0.0001	0,81	0,80	0,97
L Putamen	<0.0001	0,01	0,01	0,01
R Putamen	<0.0001	0,01	0,00	0,01
L Thalamus	0,0007	0,01	0,05	0,00
R Thalamus	0,0005	0,01	0,00	0,00
L Accumbens	0,0016	0,38	0,16	0,54
R Lateral Ventricle	0,0082	0,65	0,52	0,73
R Temporal Horn	0,0003	0,31	0,30	0,33
Third Ventricle	<0.0001	0,27	0,13	0,27

CSF= cerebrospinal fluid Gm = Grey Matter, R= right, L= left Wm = White Matter

Table 9.2 All data for the UHDRS with p values

Variable	Group	n	Mean	Std Dev	Median	Interquartile range		Min	Max	p-value for effect of disease group	Effect of disease group on score: HDL2 vs HD (estimate ± 95% CI)	p-value for effect of disease duration	Effect of each year of disease on score (estimate ± 95% CI)
Ocular (6 items)	Overall	28	10,7	4,7	10,2	7,8	13,0	3,0	22,7				
	HD	13	11,3	5,6	10,3	8,0	13,0	3,0	22,7	0,61	1.0 ± 3.7	0,81	-0.1 ± 0.5
	HDL2	15	10,2	3,9	10,0	7,7	11,3	4,7	20,3				
Dysarthria	Overall	28	1,2	1,0	1,0	0,5	1,8	0,0	4,0				
	HD	13	1,0	0,9	1,0	0,3	1,3	0,0	3,0	0,38	-0.4 ± 0.8	0,77	0.0 ± 0.1
	HDL2	15	1,4	1,1	1,3	0,7	2,0	0,0	4,0				
Tongue protrusion	Overall	28	1,4	1,3	1,2	0,0	2,3	0,0	4,0				
	HD	13	1,4	1,1	1,3	1,0	2,0	0,0	4,0	0,99	0.0 ± 1.0	0,85	0.0 ± 0.1
	HDL2	15	1,4	1,4	1,0	0,0	2,7	0,0	4,0				
Rigidity	Overall	28	1,1	0,9	1,0	0,0	1,8	0,0	3,0				
	HD	13	1,1	1,0	1,0	0,0	2,0	0,0	2,7	0,99	0.0 ± 0.7	0,83	0.0 ± 0.1
	HDL2	15	1,1	0,9	1,0	0,7	1,0	0,0	3,0				
Finger tap (2 items)	Overall	28	2,1	1,0	2,0	1,1	2,5	1,0	4,0				
	HD	13	2,3	1,1	2,3	1,5	3,0	1,0	4,0	0,53	0.3 ± 0.8	0,48	0.0 ± 0.1
	HDL2	15	1,9	1,0	1,8	1,0	2,3	1,0	4,0				
Pronate/supinate (2 items)	Overall	28	2,0	1,0	2,0	1,3	2,3	0,3	4,0				
	HD	13	2,1	1,0	2,0	1,5	2,3	0,3	4,0	0,70	0.1 ± 0.7	0,24	-0.1 ± 0.1
	HDL2	15	1,8	1,0	1,8	1,0	2,2	0,5	4,0				
Luria	Overall	28	2,7	1,4	3,0	1,3	4,0	0,0	4,0				
	HD	13	2,1	1,6	2,3	1,0	3,7	0,0	4,0	0,039	-1.2 ± 1.0	0,93	0.0 ± 0.1
	HDL2	15	3,2	1,1	4,0	2,3	4,0	0,7	4,0				
Dystonia trunk	Overall	28	1,1	1,0	0,7	0,3	1,3	0,0	3,7				
	HD	13	1,1	1,1	0,7	0,0	1,3	0,0	3,7	0,98	0.0 ± 0.8	0,70	0.0 ± 0.1
	HDL2	15	1,0	0,9	0,7	0,3	1,3	0,0	3,0				
Dystonia UL (2 items)	Overall	28	1,0	1,1	0,7	0,2	1,3	0,0	4,0				
	HD	13	0,9	0,9	0,7	0,0	1,7	0,0	2,3	0,85	-0.1 ± 0.8	0,72	0.0 ± 0.1
	HDL2	15	1,0	1,2	0,7	0,2	1,0	0,0	4,0				
Dystonia LL (2 items)	Overall	28	0,6	1,0	0,3	0,0	0,7	0,0	4,0				
	HD	13	0,6	0,8	0,2	0,0	0,7	0,0	2,5	0,98	0.0 ± 0.5	0,66	0.0 ± 0.1
	HDL2	15	0,6	1,1	0,3	0,0	0,5	0,0	4,0				
Dystonia ALL (5 items)	Overall	28	4,2	4,7	2,5	1,0	5,7	0,0	18,3				
	HD	13	4,1	4,2	2,7	0,7	7,0	0,0	12,7	0,95	-0.1 ± 3.8	0,75	0.1 ± 0.5
	HDL2	15	4,4	5,3	2,0	1,0	3,7	0,3	18,3				
Chorea face	Overall	28	1,2	0,6	1,2	0,7	1,7	0,3	2,7				
	HD	13	1,3	0,7	1,3	0,7	1,7	0,3	2,7	0,66	0.1 ± 0.5	0,16	0.0 ± 0.1
	HDL2	15	1,1	0,5	1,0	0,7	1,3	0,3	2,3				
Chorea bol	Overall	28	1,3	0,7	1,3	0,7	1,7	0,0	3,0				
	HD	13	1,5	0,8	1,7	0,7	1,7	0,3	3,0	0,38	0.3 ± 0.6	0,64	0.0 ± 0.1

	HDL2	**15**	1,2	0,7	1,3	0,7	1,7	0,0	2,7				
Chorea trunk	**Overall**	**28**	1,4	0,8	1,3	0,8	2,3	0,0	3,0				
	HD	**13**	1,3	0,8	1,3	0,7	1,7	0,3	2,7	0,32	-0.3 ± 0.7	0,33	0.0 ± 0.1
	HDL2	**15**	1,6	0,9	1,7	1,0	2,3	0,0	3,0				
Chorea UL (2 items)	**Overall**	**28**	1,4	0,7	1,4	1,0	1,9	0,0	3,0				
	HD	**13**	1,6	0,8	1,3	1,0	2,0	0,5	3,0	0,67	0.1 ± 0.6	0,24	0.0 ± 0.1
	HDL2	**15**	1,3	0,7	1,5	1,0	1,8	0,0	2,5				
Chorea LL (2 items)	**Overall**	**28**	1,1	0,7	1,3	0,3	1,6	0,0	2,7				
	HD	**13**	1,3	0,8	1,3	1,0	1,7	0,0	2,7	0,31	0.3 ± 0.6	0,33	0.0 ± 0.1
	HDL2	**15**	0,9	0,7	1,3	0,3	1,5	0,0	1,7				
Chorea ALL (7 items)	**Overall**	**28**	9,0	4,6	9,2	6,0	11,8	0,3	19,3				
	HD	**13**	9,8	5,2	9,3	5,7	12,3	3,3	19,3	0,63	0.9 ± 3.6	0,27	-0.3 ± 0.4
	HDL2	**15**	8,4	4,2	9,0	6,3	11,7	0,3	16,3				
Bradykinesia	**Overall**	**28**	1,9	1,1	2,0	1,0	2,7	0,0	4,0				
	HD	**13**	2,0	1,2	2,3	0,7	3,0	0,0	4,0	0,84	0.1 ± 0.8	0,70	0.0 ± 0.1
	HDL2	**15**	1,9	0,9	1,7	1,3	2,7	0,7	4,0				
Retropulsion	**Overall**	**28**	1,9	0,8	2,0	1,0	2,0	0,7	4,0				
	HD	**13**	2,1	0,7	2,0	2,0	2,0	1,0	4,0	0,13	0.5 ± 0.6	0,96	0.0 ± 0.1
	HDL2	**15**	1,6	0,9	1,7	1,0	2,0	0,7	4,0				
Gait	**Overall**	**28**	1,3	1,0	1,0	0,7	2,0	0,0	4,0				
	HD	**13**	1,5	1,1	1,0	0,7	2,0	0,0	4,0	0,42	0.3 ± 0.8	0,62	0.0 ± 0.1
	HDL2	**15**	1,2	1,0	1,0	0,7	1,3	0,3	4,0				
Tandem walking	**Overall**	**28**	1,8	1,3	1,7	0,8	3,0	0,0	4,0				
	HD	**13**	2,1	1,1	2,3	1,0	3,0	0,0	4,0	0,26	0.3 ± 0.8	0,62	0.0 ± 0.1
	HDL2	**15**	1,5	1,4	1,0	0,3	3,3	0,0	4,0				
Motor testing total (31 items)	**Overall**	**28**	46,7	19,7	41,3	34,5	60,2	11,0	87,0				
	HD	**13**	48,4	22,1	51,0	27,0	66,3	11,0	87,0	0,76	2.5 ± 15.7	0,70	-0.4 ± 1.9
	HDL2	**15**	45,1	18,0	37,7	35,0	50,7	21,7	84,7				
Total behaviour score	**Overall**	**28**	20,5	13,1	19,5	11,0	25,5	2,0	67,0				
	HD	**13**	24,2	16,0	19,0	13,0	33,0	6,0	67,0	0,34	3.9 ± 7.8	0,73	0.2 ± 0.9
	HDL2	**15**	17,2	9,3	20,0	9,0	23,0	2,0	36,0				
Functional capacity total	**Overall**	**28**	5,7	3,7	5,5	2,5	8,5	1,0	13,0				
	HD	**13**	5,5	3,9	5,0	2,0	7,0	1,0	13,0	0,63	-0.7 ± 2.9	0,54	-0.1 ± 0.4
	HDL2	**15**	5,9	3,7	6,0	3,0	9,0	1,0	13,0				
Functional checklist score	**Overall**	**28**	13,6	7,6	14,5	8,5	20,0	0,0	25,0				
	HD	**13**	13,6	7,9	14,0	9,0	18,0	0,0	25,0	0,75	-1.0 ± 5.9	0,23	-0.5 ± 0.7
	HDL2	**15**	13,6	7,6	15,0	7,0	21,0	0,0	23,0				
Independence score	**Overall**	**28**	70,7	20,2	70,0	60,0	90,0	20,0	100,0				
	HD	**13**	70,0	23,1	70,0	60,0	90,0	20,0	100,0	0,65	-3.7 ± 15.8	0,28	-1.1 ± 1.9
	HDL2	**15**	71,3	18,1	70,0	60,0	90,0	30,0	100,0				