

**THE UTILIZATION AND OUTCOME OF DIAGNOSTIC, PREDICTIVE  
AND PRENATAL GENETIC TESTING FOR HUNTINGTON DISEASE IN  
JOHANNESBURG FROM 1998 TO 2006.**

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fulfilment of the requirements for the degree of  
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## DECLARATION

**I, Elaine Bernadene Sizer, declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine in Genetic Counselling at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.**

.....

.....day of..... 2008

This thesis is dedicated to my family  
– my father, Genard, my mother, Heather and my sister, Catherine –  
for providing me with love, support and motivation  
always.

## ABSTRACT

Huntington Disease (HD) is a neurodegenerative disorder that is inherited in an autosomal dominant manner, and for which testing is available. The aim of this retrospective file-based study was to analyse the numbers and demographics of individuals who had diagnostic, predictive or prenatal genetic counselling and/or testing for HD between January 1998 and December 2006 through the Division of Human Genetics, National Health Laboratory Service and University of the Witwatersrand, Johannesburg.

Files for 287 individuals who had genetic counselling and/or testing for HD were included in this study, with 77% being diagnostic cases, 20% predictive and 3% prenatal. When the results obtained in this study were compared to a study by Kromberg et al. (1999) done previously in the same Division, it was found that there has been an increase in the number of diagnostic and predictive tests done per year during this study, with diagnostic tests making up a greater percentage of the total number of tests performed.

One of the objectives of this study was to characterise the individuals who requested HD testing and to compare the characteristics of those in the diagnostic testing group to those in the predictive testing group. The median age of the individuals in the predictive testing group was 30 years, which was significantly different from the median age of 49 years for individuals in the diagnostic testing group ( $p < 0.001$ ). It was found that there were significantly more women than men requesting predictive testing ( $p = 0.02$ ), while the number of males and females in the diagnostic testing group was similar ( $p = 1.00$ ). There was also a greater percentage of employed (76.4%) versus unemployed (23.6%) individuals in the predictive testing group, while the percentages of employed and unemployed individuals in the diagnostic testing group were similar (45.5% and 54.5% respectively). Significantly more individuals in the diagnostic testing group had children (74.5%) compared to those in the predictive testing group, where 44.6% of individuals had one or more children. There was a greater percentage of white individuals in the predictive testing group (91% white; 3.5% black) compared to the diagnostic testing group (48% white, 42% black).

The completion rate of the predictive testing process was 66.7%. In the predictive testing group, 39.5% of individuals tested positive for HD, and in the diagnostic testing group 53% of individuals

tested positive for HD. Nine prenatal tests were requested by five different couples, and 7 tests were performed. Three of these fetuses tested positive for HD (including a set of twins) and these two pregnancies were terminated.

Overall, there seems to be a lack of awareness of and/or access to the genetic services offered for HD through the Division of Human Genetics, National Health Laboratory Service and University of the Witwatersrand, Johannesburg, particularly among black individuals and the professionals treating them. Information generated from this study can be used to understand the individuals seeking genetic counselling and/or testing for HD better, and can direct efforts to improve awareness and access amongst groups noted to be under-represented. It also serves as a starting point for further research.

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## NOMENCLATURE

%	Percentage
Amniocentesis	A prenatal test usually performed between 16 and 20 weeks gestation, where amniotic fluid is cultured and tested for chromosome abnormalities, biochemical abnormalities or other genetic conditions.
BDNF	Brain-Derived Neurotrophic Factor
CAG	Three base pair repeat sequence - Cytosine, Adenine, Guanine
CTG	Three base pair repeat sequence - Cytosine, Thymine, Guanine
CUG	Three base pair repeat sequence - Cytosine, Uracil, Guanine
CVS	Chorionic Villus Sampling – A prenatal test usually performed between 10 and 12 weeks gestation, where chorionic villus cells are removed from the placenta and tested for chromosome abnormalities or genetic conditions.
DNA	Deoxyribonucleic acid
Division	Division of Human Genetics, National Health Laboratory Service and University of the Witwatersrand Johannesburg
HC	Huntington Chorea
HD	Huntington Disease
HD1	Huntington Disease 1
HDL2	Huntington Disease-Like 2
IT15	Important transcript 15
IVF	<i>In Vitro</i> Fertilization
JPH3	Junctophilin 3
kb	Kilobase
kDa	Kilodalton
Microsatellite	A DNA sequence consisting of a known number of nucleotides which are tandemly repeated and are used as markers to track the inheritance of genes
MNBL1	Muscleblind-like protein 1
N-terminal	The end of a protein corresponding to the 5' end of the coding sequence
NHLS	National Health Laboratory Service
PCR	Polymerase Chain Reaction
PolyQ	Polyglutamine
p-value	A measure of probability
RNA	Ribonucleic acid
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
TOP	Termination of pregnancy

# **1 INTRODUCTION**

Huntington disease (HD) is a neurodegenerative disorder that is inherited in an autosomal dominant manner (Purdon, Mohr, Ilivitsky et al. 1994). Symptoms of HD include progressive dementia, cognitive dysfunction, psychiatric problems and choreiform movements. It is estimated that the worldwide prevalence of HD is about 4 to 8 per 100,000 but varies considerable (from 0.5 to 10 per 100,000) in different population groups (Harper 1996, Rimoin, Connor, Pyeritz et al. 2002; Landles and Bates 2004). The disease seems to be less common in the Japanese, Chinese, and Finnish populations and in African black populations, but much more common in individuals of European descent (Harper, 1996; Rimoin et al. 2002). HD is caused by the presence of an expanded number of CAG repeats in the important transcript 15 (*IT15*) gene on chromosome four (Huntington's Disease Collaborative Research Group 1993), but a second disease-causing allele has been found in black African patients (Holmes, O'Hearn, Rosenblatt et al. 2001). This is also an expansion mutation which leads to HD-like symptoms, but it occurs in the Junctophilin 3 (*JPH3*) gene and involves a CAG/CTG repeat expansion (Holmes et al. 2001).

Testing is currently offered in South Africa to detect repeat expansions in these two genes. It can be used for diagnostic testing when individuals are symptomatic, predictive testing for at-risk individuals, or prenatal testing. Genetic counselling is also offered to both at-risk and affected individuals and their families for HD predictive, diagnostic or prenatal testing.

The purpose of this study was to assess the number of patients seen for genetic counselling and/or genetic testing for HD in the Division of Human Genetics, National Health Laboratory Services and University of the Witwatersrand, Johannesburg (here forth referred to as the Division) between January 1998 and December 2006. This study also characterised these patients by obtaining demographic information about them, their motivations for testing and test results. This may give genetic counsellors and clinicians within the Division further insight into their patients and their needs. It is hoped that such information will be useful in the provision of both clinical and laboratory services and will allow appropriate services to be provided.

## **1.1 CLINICAL FEATURES OF HUNTINGTON DISEASE**

The age of onset of this disorder is usually in the third or fourth decade of life, but can present in early childhood or as late as in the eighties (Young 2003; Landles and Bates 2004). The symptoms of HD are progressive and once an individual starts showing symptoms, their survival time is generally not more than 20 years (Purdon et al. 1994).

### **1.1.1 Neurological symptoms of HD**

Neurological symptoms are often the first presentation of HD (Harper 1996). Early motor/neurological signs of HD include restlessness, hyper-reflexia, abnormal eye movements and rapid and inappropriate movement of the fingers, hands or toes (Rimoin et al. 2002). Abnormal eye movements include a decreased ability to initiate small, rapid, jerky movements of the eyes (saccades) when trying to focus on objects, such as while turning the head or moving, as well as increased blinking (Harper 1996). Dysarthria is also common in early HD. Dysarthria is the disturbance of speech, such as the rhythm of speaking as well as the speed, and may later cause speech to be unintelligible (Harper 1996).

Later neurological symptoms include choreiform movements which are involuntary movements such as jerking or writhing that may affect the limbs and the face, and lead to difficulties talking and swallowing (Purdon et al. 1994). This chorea is continuously present while the individual is awake and tends to worsen during times of stress (Rimoin et al. 2002). The chorea associated with HD is variable, but always includes some degree of dystonia which causes sustained contractions of muscles leading to twisting movements, abnormal posture and slow abnormal movements (Harper 1996; Rimoin et al. 2002). As the disease advances, other symptoms such as rigidity and loss of voluntary movements as well as the dystonia, become more apparent (Harper 1996; Rimoin et al. 2002). Difficulties in swallowing may also become more obvious and this increases the risk of asphyxiation (Harper 1996). Weight loss is common in the later stages of HD, as is incontinence (Harper 1996).

Other neurological abnormalities may include epilepsy and cerebellar dysfunction, but these are less common in the adult onset form of HD, compared to the juvenile form of HD. HD can also

lead to changes in gait. The gait of an individual with HD, especially one who has had chorea for many years, tends to be wide-based and staggering (Harper 1996). Some individuals may have a slow, unsteady gait due to bradykinesia and are more likely to fall (Harper 1996).

### **1.1.2 Cognitive symptoms of HD**

Cognitive symptoms associated with HD include progressive dementia and cognitive dysfunction such as memory loss, a decreased ability to learn new material, and attention and concentration problems (Purdon et al. 1994; Harper 1996; Young 2003). Language difficulties are common in HD and can affect both the ability to speak effectively and to understand others. Writing is also usually affected in individuals with HD, due to both neurological and cognitive difficulties (Harper 1996). Individuals with HD may also experience a decline in visual perception (Purdon et al. 1994; Harper 1996; Young 2003). They may also have decreased problem-solving abilities and difficulty with concept formation (Harper 1996). The cognitive symptoms associated with HD may lead to problems with social interaction and inappropriate emotional responses (Harper 1996).

The symptoms of HD result from the loss of striatal neurons (nerve cells located within the dorsal striatum) and atrophy of the caudate nucleus and the putamen in the brain (Rimoin et al. 2002). The caudate nucleus and putamen are located near the centre of the brain, forming the dorsal striatum, and are involved in learning and memory (Rimoin et al. 2002).

### **1.1.3 Psychiatric symptoms of HD**

Psychiatric symptoms associated with HD are variable and can include irritability, depression and suicidal ideation (Purdon et al. 1994; Harper 1996; Young 2003). Less common psychiatric manifestations in HD can include paranoia and delusions, mania, personality disorder and schizophrenia (Harper 1996).

#### **1.1.4 Features of HDL2**

Features of HDL2 are similar to those seen in patients with HD1. They include neurological symptoms such as dysarthria, hyper-reflexia, chorea, dystonia, rigidity and bradykinesia, as well as tremor (Margolis, Holmes, Rosenblatt et al. 2004). HDL2 also presents with the cognitive and psychiatric symptoms seen in HD1 (Margolis et al. 2004; Greenstein, Vonsattel, Margolis et al. 2007). Individuals with HDL2, like individuals with HD1, experience progressive worsening of symptoms over a period of 10-20 years, but they tend to present later than individuals with HD1, usually only in the 4<sup>th</sup> decade (Margolis et al. 2004).

#### **1.1.5 Features of Juvenile HD**

HD that presents before the age of 20 years is termed “Juvenile HD” (Nance and Myers 2001). Symptoms in children and adolescents include severe mental deterioration, cerebellar abnormalities, abnormalities of eye movement, epilepsy, rigidity, ataxia, bradykinesia and general motor abnormalities (Teisberg 1995; Harper 1996; Nance and Myers 2001). They rarely present with chorea (Harper 1996). In South Africa, the prevalence of juvenile HD is about 4% in the white HD population, and as high as 15.7% in the mixed ancestry HD population (Hayden, MacGregor, Saffer et al. 1982), compared to 7-10% worldwide (Harper 1996).

### **1.2 INHERITANCE OF HUNTINGTON DISEASE**

HD has an autosomal dominant inheritance pattern and therefore the child of an affected individual has a 50% chance of inheriting the expanded allele. Because this disease is usually completely penetrant, an individual who has inherited the gene will at some stage present with symptoms of this disorder (Purdon et al. 1994; Landles and Bates 2004).

## **1.3 THE GENETICS OF HUNTINGTON DISEASE**

### **1.3.1 Huntington Disease 1**

The HD1 gene, *IT15*, is located on chromosome 4p16.3. This gene is comprised of 67 exons, and is 180kb in length. The resulting protein, called Huntingtin (htt), consists of 3144 amino acids and has a molecular mass of 348kDa. Htt has two forms – a 13.7kb transcript and a 10.3kb transcript (Warby, Graham and Hayden 2007).

In unaffected individuals, the first exon of this gene contains between 9 and 35 CAG repeats, each of which codes for the amino acid glutamine (Huntington Disease Collaborative Research Group 1993). This polyglutamine tract forms part of the htt protein that is located in the cytoplasm of many cells in the body, particularly in the neurons of the brain (Cattaneo, Rigamonti, Goffredo et al. 2001; Young 2003). HD is caused by an expansion in the number of CAG repeats in *IT15* to more than 40 repeats (Huntington Disease Collaborative Research Group 1993). As the number of CAG repeats increases, so does the number of glutamine residues in the htt protein and this causes the protein to undergo a conformational change, forming mutant htt (Cattaneo et al. 2001; Landles and Bates 2004). Incomplete penetrance can occur when the number of CAG repeats is 36 to 39 (Lopez 2007). The incomplete penetrance associated with this allele range means that it is uncertain whether or not an individual will present with symptoms. Some individuals with repeats in this range may present with many of the symptoms of HD, while others may have no symptoms at all or may have only mild symptoms, such as restlessness (Rubinsztein, Leggo, Coles et al. 1996). There may also be genetic or environmental factors that could influence the development of HD in individuals with 36 to 39 repeats (Rubinsztein et al. 1996), making it difficult to provide these individuals with definitive answers regarding their HD status. It has been found that individuals with intermediate alleles (27 to 35 CAG repeats) are not at risk of developing HD themselves, but the repeat is unstable and can expand when passed from a parent to their child (Semake, Creighton, Warby et al. 2006; Lopez 2007). Various factors can influence the expansion of intermediate CAG repeats to the “incomplete penetrance” range. These include the length of the CAG repeat, with longer repeats being more likely to expand, as well as the gender and age of the parent carrying the intermediate allele, with the risk being greater when the allele is passed on from a father to his children, particularly if the father is of advanced paternal age (older than 35)

(Lopez 2007). It is therefore important to counsel the patient appropriately if repeat numbers in these ranges are found.

### **1.3.2 Huntington Disease-like 2**

In a few individuals who present with clinical features of HD, an *IT15* CAG repeat expansion is not detected (Stevanin, Fujigasaki, Lebre et al. 2003). In some of these patients, molecular studies have shown the presence of a CAG/CTG repeat expansion in another gene, *JPH3*, located on chromosome 16q24.3 (Holmes et al. 2001). This gene is 95.4 kb in length and consists of 5 exons (GenAtlas 2003). The *JPH3* gene encodes a 748 amino-acid protein – junctophilin-3 (JHP3) – which has a molecular weight of 81kDa and is a component of junctional membrane complexes (Margolis 2006).

Individuals with HDL2 have an expanded number of CAG/CTG repeats to more than 40 (Margolis 2006). Normal allele ranges are 6 to 28 CAG/CTG repeats; reduced penetrance alleles, which may expand when passed on, range from 29-39, and more than 40 repeats results in fully penetrant HDL2 (Margolis 2006; Greenstein et al. 2007). This expanded CTG repeat is alternatively spliced, forming either poly-leucine or poly-alanine amino acid tracts in the junctophilin-3 protein or may be spliced out (Holmes et al. 2001). It is not understood how this causes HD-like symptoms (Margolis, Rudnicki and Holmes 2005).

An expansion of the number of CAG/CTG repeats in *JPH3* has not yet been detected in Caucasian populations, but occurs in individuals of African ancestry (Stevanin et al. 2003; Margolis et al. 2005; Margolis 2006). Worldwide prevalence rates are not known, but it is thought to occur in only 1% of patients with HD-like symptoms who test negative for HD1 (Margolis 2006). Although HDL2 is considered to be rare in many parts of the world, it is thought to occur in about one third of black South African HD patients (A. Krause, personal communication, November 2007).

### **1.3.3 Juvenile HD**

There is a phenomenon that can occur in HD families, where the number of repeats expands when passed on to successive generations. This is termed “anticipation”, and in HD, this tends to occur

when a father passes on the disease gene to his children. The increased number of repeats in children of affected individuals often leads to the earlier-onset juvenile form of HD in these children (Nance and Myers 2001). Juvenile HD usually occurs when the number of repeats is greater than 60 (Teisberg 1995; Harper, Lim and Craufurd 2000; Nance and Myers 2001)

## **1.4 THE PATHOGENESIS OF HUNTINGTON DISEASE**

### **1.4.1 Huntington Disease 1**

Mutant htt impacts on many cell processes, and the disruption of these processes is thought to induce the neurodegeneration seen in patients with HD1 (Cattaneo et al. 2001; Young 2003). Mutant htt disrupts the transportation of vesicles containing brain-derived neurotrophic factor (BDNF) in neurons and the transportation of organelles within cells (Gutekunst, Levey, Heilman et al. 1995; Gauthier, Charrin, Borrell-Pagès et al. 2004). Because the striatal neurons do not receive sufficient BDNF, a pro-survival factor that contributes to survival signals the cells receive, the cells become susceptible to cell death, which leads to neurodegeneration (Zuccato, Ciammola, Rigamonti et al. 2001; Gauthier et al. 2004; Borrell-Pages, Zala, Humbert et al. 2006)

Mutant htt has been found, in mouse models, to disrupt the functioning of mitochondria, leading to calcium ( $\text{Ca}^{2+}$ ) dysregulation (Bezprozvanny and Hayden 2004). Excess calcium in the cytoplasm of cells, along with decreased amounts of BDNF, is thought to lead to the abnormal activation of proteases which cleave the mutant htt, resulting in the formation of N-terminal fragments containing the glutamine residues (termed polyQ stretches) (Hermel, Gafni, Propp et al. 2001; Gafni, Hermel, Young et al. 2004; Hermel, Hart, Gunduz et al. 2004; Borrell-Pages et al. 2006). These fragments are then transported into the nucleus, where they induce death of the spiny striatal neurons by interfering with transcription of various genes as well as interfering with intracellular transport (Luthi-Carter, Strand, Peters et al. 2000; Borrell-Pagès, Canals, Cordelières et al. 2006). These polyQ fragments also form aggregates which can be deposited in the cytoplasm (where they are termed cytoplasmic aggregates or inclusions), in the nucleus (intranuclear inclusions) or in striatal neurons (neuritic inclusions), and are commonly found in the cortex of the brain (Li H, Li SH, Johnston H et al. 2000; Cattaneo et al. 2001; Young 2003; Borrell-Pagès et al. 2006). These

aggregates have been shown to impair mitochondrial transport, which may be an important factor in the pathogenesis of HD1 (Chang, Rintoul, Pandipati et al. 2006).

#### **1.4.2 Huntington Disease-like 2**

Not much information is known about how the expanded CAG/CTG repeat in *JPH3* causes HDL2. *JPH3* is expressed primarily in the brain and is involved in forming a junctional complex between the cytoplasmic membrane and the endoplasmic reticulum and ion channels within the cells (Margolis 2006; Rudnicki, Holmes, Lin et al. 2007). In HDL2, RNA transcripts with expanded CUG repeats form foci in the frontal cortex, with portions of the transcript being incompletely processed (Rudnicki et al. 2007). These transcripts have been shown to be cytotoxic and may have the same pathogenesis as in HD1, which is one way in which expanded CAG/CTG repeats in HDL2 are thought to cause the HDL2 phenotype (Rudnicki et al. 2007). Another protein, MBNL1 (muscleblind-like protein 1), which is involved in the regulation of splicing of transcripts, has been shown to co-localize with these RNA foci, resulting in there being less available MBNL1 within the cell (Rudnicki et al. 2007). It is thought that this may lead to missplicing of other genes, but more research is needed to confirm this (Margolis 2006; Rudnicki et al. 2007).

Another possible mode of pathogenesis in HDL2 relates to the function of *JPH3* within the cell. Alteration in the structure of *JPH3* due to the expanded CAG/CTG repeats may interfere with these junctional complexes and lead to calcium dysregulation (Margolis 2006). The presence of the incompletely processed *JPH3* transcripts leads to reduced expression of *JPH3*, which may also lead to disruption of its function and contribute to the disease pathogenesis (Rudnicki et al. 2007).

### **1.5 MOLECULAR GENETIC TESTING FOR HUNTINGTON DISEASE**

Direct mutation testing has been available for HD1 testing since 1994. Samples collected in the Division, were initially sent to laboratories overseas (mainly in England and the Netherlands) and later to the Department of Human Genetics at the University of Cape Town (Kromberg, Krause, Spurdle et al. 1999). Since 2001 samples have been analyzed at the Division, using direct mutation

analysis. This involves using PCR primers to amplify the region where the CAG repeats are found to determine the exact number of repeats that are present in the gene. A result indicating more than 40 repeats is considered positive for HD1.

As the symptoms associated with HD1 are difficult to distinguish from HDL2 (Margolis et al. 2005) the Division currently also offers all individuals of black or mixed ancestry diagnostic testing for HDL2. Samples have also been tested retrospectively. This testing has been available at the Division since 2004 and is done using direct mutation testing. The presence of an expanded number of CAG/CTG repeats (more than 40) is considered a positive result for HDL2.

Before direct mutation testing became available, linkage analysis was done and is still available for rare cases when exclusion testing is requested. Linkage analysis involves the detection of markers that are known to flank the disease gene. These markers are usually single nucleotide polymorphisms (SNPs) or microsatellites that are near enough to the disease gene not to be subject to homologous recombination (Ball, Tyler and Harper 1994). If an individual is known to have HD it is possible to trace the markers that are linked to the disease gene through each generation in the family, and determine who is at risk of developing HD (Ball et al. 1994). Linkage analysis can only be used to modify an individual's risk of developing HD and cannot determine with certainty whether or not the mutated gene is actually present in an individual (Ball et al. 1994). This is because there is a small possibility of recombination occurring between the gene and marker.

### **1.5.1 Diagnostic Testing for HD**

Diagnostic testing is used for the purpose of confirming the diagnosis of HD in individuals who are symptomatic (Kromberg et al. 1999). Diagnostic testing for HD is currently done at the Division using direct mutation analysis.

### **1.5.2 Predictive Testing for HD**

Asymptomatic, at-risk individuals may wish to know whether or not they have inherited HD from an affected parent. Since 1989, these individuals have been offered predictive (also termed pre-

symptomatic) HD testing, which was done by linkage analysis up until 1994, and is now done by direct mutation testing.

There is an internationally accepted protocol which is followed before an asymptomatic individual is tested for the presence of the expanded CAG repeat causing HD1 or for the CTG repeat causing HDL2. The protocol followed in the Division is based on the one which was developed by the UK Huntington Disease Association in 1991 (Crauford, Tyler and U.K. Huntington's Prediction Consortium 1992). For an individual to be eligible to enter the predictive testing program, they should meet the following criteria:

1. They must have a confirmed family history of HD or one which is highly suggestive of the disease. It is important to have a confirmed (positive) HD result in an affected family member, if possible, to ensure that the disease being inherited and tested for in the family is actually HD (Kromberg et al. 1999).
2. They should be at 25-50% risk of having inherited the mutated gene but an individual with a lower risk will not necessarily be excluded.
3. They must be at least 18 years of age at the time of testing. Rarely, exceptions can be made in the case of mature minors.
4. They must give informed consent.
5. Individuals who request predictive testing, but are found to have a severe mental illness or who are at high risk of suicide are counselled against participating in the predictive testing program, but cannot be excluded.
6. It is advisable that the individual undergoing testing has a support person who accompanies them to all the appointments, especially the result-giving session. This support person can be a spouse or friend, but should preferably not be someone who is themselves at risk of inheriting HD and who may wish to undergo predictive HD testing later.

Individuals eligible for inclusion in the predictive testing program are required to undergo various assessments before they will be tested for the HD gene mutation. The first step in the predictive testing program involves one or more appointments with a genetic counsellor where a family history is obtained and where the individual's understanding of HD, their motivation for undergoing testing and their thoughts regarding receiving a positive HD result and receiving a negative HD result are discussed (Kromberg et al. 1999). The predictive testing protocol is also

explained during one of the genetic counselling sessions. The individual is then referred to a neurologist, a psychologist and a psychiatrist who each interview and examine the individual using a variety of tests. This is to determine whether or not the individual has symptoms of HD, and if there is any reason for them to be discouraged from undergoing predictive HD testing (Crauford et al. 1992; Kromberg et al. 1999). If they are found to show symptoms of HD, then they are not required to complete the predictive testing protocol and diagnostic testing is offered instead.

After the assessments, the individual returns to the genetic counsellor who will have been informed of the findings of the neurologist, psychiatrist and psychologist and can discuss with the individual whether they want to proceed with testing (Kromberg et al. 1999). If they do wish to proceed, blood will be taken for direct mutation analysis. When the results are available, the genetic counsellor will discuss the results with them at another appointment. After a result is given, the individual will be offered follow-up appointments, should they need them, with the genetic counsellor, psychologist, psychiatrist and/or neurologist (Kromberg et al. 1999).

### **1.5.3 Prenatal Testing for HD**

Prenatal testing for HD is also available in South Africa. Prior to 1994 this was done using linkage analysis, but is now done using direct mutation testing or exclusion testing in rare cases. Prenatal testing can be requested on the fetus of an individual who is affected or at 50% risk of having inherited HD and termination of pregnancy can be offered when a fetus is found to have an expanded repeat or is found to be high risk by linkage analysis.

## **1.6 GENETIC COUNSELLING FOR HD**

### **1.6.1 Genetic Counselling**

HD is an inherited disorder and individuals who have the disease or are at risk of developing it, should be offered genetic counselling. The most up-to-date definition of genetic counselling is that given by the National Society of Genetic Counselors (National Society of Genetic Counselors' Definition Task Force, Resta, Biesecker et al. 2006). They define genetic counselling as “the

process of helping people understand and adapt to the medical, psychological, and familial implications of the genetic contributions to disease” and includes “interpretation of family and medical histories”, education of patients about the disorder or condition (inheritance, risks, medical information, cause, treatments, prevention), as well as counselling patients to enable them to make informed choices regarding the condition (National Society of Genetic Counselors' Definition Task Force et al. 2006).

### **1.6.2 Diagnostic HD testing issues**

A diagnostic test is done to confirm that the patient’s clinical features are caused by HD. Receiving a positive or negative result may have psychological consequences for both the individual and his/her family. A positive result helps to identify the cause of the symptoms but the individual testing positive for HD, and their family, has to come to terms with the progression of the disorder and understand that there is no cure. The family may also have to care for the affected individual and make decisions regarding both their future and that of the affected individual (Tibben 2007). Treatment and care of an individual with HD may also place a financial burden on the family, especially as symptoms progress and the affected individual can no longer work (Harper 1996). Another aspect of a positive test result which would impact on the family is that the individual’s children are at 50% risk of inheriting HD (Harper 1996; Tibben 2007). Many affected individuals may already have children when they start showing symptoms of HD, so reproductive decision-making is no longer an option (Harper 1996).

A negative test result for HD means that the patient’s symptoms are not caused by HD. The individual and their family may find it reassuring that the individual’s symptoms are not due to HD, but they may then have to deal with a different diagnosis, or with not finding a diagnosis. The risk to their children may also then be unknown.

### **1.6.3 Predictive HD testing issues**

Individuals who are at risk of inheriting HD but are not currently showing symptoms suggestive of HD are termed “pre-symptomatic” and should receive genetic counselling. If they wish to know

their status, they need to go through a predictive testing protocol (discussed in section 1.5.2) (Chapman 1992). The reason for this is that there are many psychosocial issues associated with HD including emotional consequences of testing and decision-making (Chapman 1992). These need to be discussed to help make the result easier to deal with, regardless of whether it is positive or negative (Bloch, Adam, Wiggins et al. 1992).

Part of the genetic counselling session will involve exploring the individual's feelings around HD as well as their experiences of the disease, discussing their motivation for entering the HD predictive testing program, and helping them to think about how they may feel and what their future plans may be if the result is negative or positive (van 't Spijker and ten Kroode 1997). During these sessions the counsellor may also be alerted to difficulties in the individual's life, such as depression, suicidal ideation, relationship difficulties and denial, which need to be carefully addressed before the individual continues with predictive HD testing (Chapman 1992).

Many emotions can be present in individuals wanting to undergo predictive HD testing. These can include fear and anxiety regarding the test result, and its impact on their future and their children's future (van 't Spijker and ten Kroode 1997). Individuals may also experience depression, uncertainty, confusion and/or anger towards an affected parent or unaffected sibling (Forrest Keenan, Miedzybrodzka, van Teijlingen et al. 2007). Many emotions may arise as a result of witnessing a parent's struggle with HD, or from a family member's predictive/diagnostic test result (Forrest Keenan et al. 2007). There may also be issues regarding their own identity and their expected status and what finding out their status will do to this identity (Bloch et al. 1992; Chapman 1992; Huggins, Bloch, Wiggins et al. 1992). There is also the possibility of discrimination against a person who tests positive for HD with regard to employment opportunities and insurance coverage (Quaid and Morris 1993; Almqvist, Bloch, Brinkman et al. 1999)

For many patients, undergoing predictive testing for HD can be very stressful, regardless of the outcome of the test (Almqvist, Brinkman, Wiggins et al. 2003). Problems following predictive testing can include sadness, low self-esteem, anxiety, suicidal ideation, aggression, irritation, obsessions and compulsions (Witjes-Ane', Vegter-van der Vlis, van Vugt et al. 2003). A study by Codori, Slavney, Rosenblatt (2004) showed that approximately 20% of patients undergoing predictive HD testing, who test positive, experience depression severe enough to require treatment, and a small percentage of individuals (about 6.9%) who test positive for HD cannot cope with the

result, and this may lead to “catastrophic events” (Almqvist et al. 2003). This could include severe depression, hospitalization or suicide (Almqvist et al. 2003). The reason for this is thought to be an identity crisis, with most of these catastrophic events or adverse reactions occurring within a year of receiving the positive result (Almqvist et al. 2003). However, long term studies have shown that most individuals are eventually able to deal with the results and integrate the new information into their lives (Almqvist et al. 2003).

A positive HD result may also put a strain on a relationship, as the result may drastically change the dynamics of a relationship (van 't Spijker and ten Kroode 1997). The individual who tested positive for HD may become concerned about being a burden to their partner as their disease progresses, or may fear being abandoned by their partner as their symptoms start to show (Tassicker 2005). Individuals with HD may also start to suffer from various psychiatric problems including irritability, depression and suicidal ideation which may place a strain on their partner (Purdon et al. 1994; Young 2003). There is also a 50% chance that an affected individual's children may inherit the gene. This may result in many emotional reactions, such as guilt and worry (van 't Spijker and ten Kroode 1997).

There are also psychosocial effects when the outcome of the predictive HD test is negative. Individuals may experience relief and happiness that they tested negative for HD and that their children are no longer at risk of inheriting HD (van 't Spijker and ten Kroode 1997). It may also have positive effects with regard to medical aid rates and coverage as well as employment opportunities (Quaid and Morris 1993). Individuals who test negative for HD may feel that they can now make different decisions regarding studying, travelling and having a family, knowing that they are not at risk of developing HD (Huggins et al. 1992). They may also feel relieved that they will not be a burden to others due to HD progression (Huggins et al. 1992).

A negative result may not always bring feelings of relief and happiness (Reviewed in van 't Spijker and ten Kroode, 1997). A small percentage of individuals who test negative for HD cannot cope with the result (Huggins et al. 1992). These individuals may have expected to test positive for HD, and may have made life decisions based on the anticipated result, making the negative result difficult to deal with (Meiser and Dunn 2001). If an individual's siblings or other family members have tested positive for HD, an unaffected individual may also experience “survivor” guilt (Huggins et al. 1992; van 't Spijker and ten Kroode 1997; Decruyenaere, Evers-Kiebooms,

Boogaerts et al. 2005). Survivor guilt occurs when an individual tests negative for HD and experiences guilt due to other family members having tested positive (van 't Spijker and ten Kroode 1997).

#### **1.6.4 Prenatal HD testing issues**

Individuals who know they are affected or at risk of having inherited HD, may choose to have prenatal testing in a pregnancy. There are many issues related to this such as testing options, consequences of testing and decision-making around TOP when a pregnancy is affected.

Testing options include chorionic villus sampling (CVS) and amniocentesis, both of which are invasive procedures and are associated with a miscarriage risk. This needs to be discussed with the couple. There is also a 50% chance that the fetus will test positive for HD if a parent has the gene mutation. The genetic counsellor needs to discuss with the couple what they may choose to do should the prenatal HD test be positive. The options available include terminating the pregnancy because the fetus will develop HD later in life or continuing the pregnancy with this knowledge. This may raise issues related to termination of pregnancy, such as ethical dilemmas, religious and cultural beliefs and feelings of guilt, loss or regret. If a couple chooses not to terminate, issues arise regarding the parents' knowledge of their child's HD status and the removal of the child's autonomy to decide whether or not to be tested (Richards and Rea 2005). As it is against the Division's code of ethics to test minors (individuals under 18 years of age) for adult-onset disorders such as HD, couples who indicate that they will still continue a pregnancy if they receive a positive prenatal HD test, may be refused prenatal diagnosis in that pregnancy.

Alternate reproductive methods such as donor eggs/sperm, adoption and pre-implantation genetic diagnosis (not available in South Africa) may also need to be discussed. The first step in pre-implantation diagnosis (PGD) involves the fertilization of ova by sperm using *in-vitro* fertilization (Mateizel, De Temmerman, Ullmann et al. 2006). The zygotes are allowed to develop and at about day 3 when the embryos consist of approximately 6 cells, one cell is removed from each zygote (Mateizel et al. 2006). These cells are then tested for HD using direct mutation testing (Moutou, Gardes and Viville 2004; Mateizel et al. 2006). Only unaffected embryos are implanted into the uterus (Moutou et al. 2004)

The uptake of prenatal testing for HD is estimated to be between 8% and 18% (reviewed in Richards and Rea 2005). The reason for the low uptake rate is thought to be because many parents are against termination of pregnancy, especially when the condition has an onset in adulthood. Other psychological issues such as denial of the condition or hope of a cure for their child, if they are found to be positive for HD in the future, also comes into play (Richards and Rea 2005).

## **1.7 TREATMENT FOR HUNTINGTON DISEASE**

There is currently no cure for HD. However, supportive treatments are available which may help to temporarily improve the quality of life of individuals with HD. These may include occupational therapy, speech therapy and physiotherapy and it is useful for the patient to see a psychologist and psychiatrist to help deal with depression and other mental changes. Ideally, individuals with HD should be cared for by a team including neurologists, psychiatrists, psychologists, occupational therapists, physiotherapists, speech therapists, social workers and genetic counsellors. Medication is also available to help reduce symptoms, such as antidepressants for psychological changes, and neuroleptics to suppress involuntary movements (Borrell-Pagès et al. 2006).

Various drugs are now being researched because of their effect on the pathways involved in causing HD (Borrell-Pagès et al. 2006). These include antioxidants (e.g. Coenzyme Q10) which may improve mitochondrial function and increase BDNF levels, transcription regulators (e.g. mithramycin) which can improve motor and neurological function and anti-apoptotics and protein aggregation inhibitors, which may also be used as potential treatments for HD (Ferrante, Andreassen, Dedeoglu et al. 2002; Ferrante, Ryu, Kubilus et al. 2004; Gauthier et al. 2004; Borrell-Pagès et al. 2006). There has also been some success with newer treatments such as the injection of fetal neuroblasts and neural precursors into the striatum of affected individuals, thereby replacing degenerated neurons (Bachoud-Levi, Remy, Nguyen et al. 2000; Rosser, Barker, Harrower et al. 2002; Bachoud-Levi, Gaura, Brugieres et al. 2006).

## **1.8 PURPOSE OF THIS STUDY**

It is important to be aware of the number of patients seen for genetic counselling and testing for HD at the Division so that appropriate services can be planned for and provided. By being aware of any changes in uptake, it may be possible to adjust the services accordingly, by training additional genetic counsellors, for example, or raising awareness of the services that are available at the Division. This study also has implications for the molecular testing service at the Division as they would need to be prepared for an increase in the number of samples if it is found that the number of tests performed is increasing. If the number is decreasing, however, it may be necessary to undertake research to determine the cause of this decrease.

Characterising the individuals who request predictive, prenatal or diagnostic HD testing is important as it provides insight into the patients and what their needs are. A better understanding of the patients who request these tests would be useful in the genetic counselling process and may help to improve the services offered. This study may also assist us in determining which sectors of the population make use of the services and which ones have limited access to the services.

Another aim of this study was to continue a previous study done by Kromberg et al. (1999) where the utilization of predictive, prenatal and diagnostic testing for HD was investigated in the same Division between 1975 and 1997. The continuation of the study will enable trends in patient numbers to be noted, as well as highlighting any changes in who is being seen for predictive, prenatal and diagnostic HD testing over the years.

## **1.9 AIM AND OBJECTIVES**

The aim of this study was to investigate the utilization and outcome of diagnostic, predictive and prenatal genetic testing for HD in Johannesburg from 1998 to 2006. The objectives were:

1. To determine the number of patients who were referred to or managed by the genetic counselling or laboratory services in the Division with regard to diagnostic, predictive and/or prenatal HD testing.

2. To characterise the individuals requesting predictive and diagnostic HD testing, including their gender, age, employment and relationship status, number of children, ethnicity, HD test results and motivations for undergoing predictive HD testing.
3. To assess the total number of patients who completed the HD predictive testing process and received a result and how long this took.
4. To compare the characteristics of those who completed the HD predictive testing process to those who did not.
5. To compare the characteristics of the patients who underwent diagnostic HD testing to those who went through the HD predictive testing process.
6. To characterise the individuals who requested prenatal testing for HD including at-risk/affected status, gender and age of the parent, gestational age and test result and to analyse the decisions made after HD prenatal testing results were given.
7. To compare the patient numbers and patient characteristics observed in this study to those from a previous study done by Kromberg et al. (1999) in the same Division between 1975 and 1997 to identify trends over time.

## **2 SUBJECTS AND METHODS**

### **2.1 SUBJECTS**

Subjects for this study included individuals who received genetic counselling for HD diagnostic, predictive or prenatal testing purposes and/or underwent genetic testing for HD through the Division of Human Genetics, National Health Laboratory Services, and the University of the Witwatersrand, Johannesburg, between January 1998 and December 2006. Patients may have had HD genetic counselling and/or genetic testing files and both were used, if available. Counselling files (see appendix A for an example) contain patient information, family history and counselling notes and a detailed patient report written to the referring doctor. HD genetic testing files contain limited patient information, test information and results. This study was retrospective and all files for these individuals were available in the Division.

Only files of patients counselled or tested for HD between January 1998 and December 2006 were included. The reason for selecting this time range was that a previous study was done in the same Division, with the aim of analysing the utilization of predictive, prenatal and diagnostic HD testing in Johannesburg and included patients counselled and/or tested for HD in Johannesburg between January 1975 and December 1998 (Kromberg et al. 1999). This study aimed to continue the study to the present and assess changing trends compared to the previous study.

Only individuals at risk of having inherited HD, or who were tested for the disorder were included in this study. Files for 287 individuals where diagnostic, predictive or prenatal HD testing was requested at the Division, from January 1998 until December 2006 were included in this study. There were 221 files where diagnostic HD testing had been requested, 57 where predictive HD testing was requested and 9 files for prenatal HD testing. All 221 individuals for whom diagnostic HD testing was requested had molecular testing files, and sixty-one (27.3%) of these individuals also had HD genetic counselling files. All individuals in the predictive HD testing group had genetic counselling files, and 38 individuals had molecular testing files for HD. All nine couples who requested prenatal HD testing had genetic counselling files and seven had HD prenatal molecular testing files.

All individuals of black or mixed ancestry who had diagnostic testing for HD were tested for both HD1 and HDL2, while white and Indian individuals were only tested for HD1. Individuals who requested predictive HD testing were tested for an expansion in either the *IT15* (HD1) or *JPH3* (HDL2) gene, whichever had been found in their affected family member/s.

## **2.2 DATA COLLECTION**

After an individual has been seen for genetic counselling, their name and the date of the session are written onto filing cards for each disorder and kept in a filing system. The names and date of testing of all individuals who have had molecular genetic testing for HD are stored in a separate database in the Molecular Genetics Laboratory and each family that has HD testing is allocated an HC (Huntington's chorea) number. Names and HC numbers of individuals who had genetic testing for HD between 1998 and 2006 were collected using the Molecular Genetics Laboratory database, while a list of patients who received genetic counselling for HD during this time period was obtained from the filing cards for HD. Molecular and counselling files for each of these patients were then drawn from the storage and filing facilities within the Division

For this study, each patient was randomly allocated a unique subject number which, along with the individual's name and HC number (if applicable), was typed into a master list. Only the researcher and her supervisors had access to the master list. Patients were then classified as predictive, diagnostic or prenatal, and information from their molecular and/or genetic counselling files was written onto the appropriate data collection sheets (refer to appendix B), along with their unique subject number that had been allocated earlier. To ensure anonymity, the data sheets did not contain any identifiable information such as patient names or HC numbers and patients were identified only by their subject number throughout the study. Individuals who had a genetic counselling file (and who may also have had a molecular testing file) were marked on the data collection sheets as having received genetic counselling, while those who had only molecular files were marked on the data collection sheets as not having received genetic counselling. All individuals who receive genetic counselling in the Division will have a genetic counselling file.

### **2.2.1 Information collected about individuals who had genetic counselling and/or testing for HD**

General information was collected for all patients. This information included their date of birth, gender, ethnicity (white, black, Indian, mixed ancestry), relationship status (single, divorced/separated, widowed, married/in relationship), employment status (employed, unemployed, retired, student), number of children, test result (positive for HD1, positive for HDL2, negative, not tested/no result). The ethnicity of the individuals was obtained from an existing HD patient database at the NHLS. This information is collected (where possible) by the laboratory as it is needed for accurate DNA testing. Other information was available from notes in the patients' counselling or molecular files. Where information such as ethnicity, relationship status, employment status and/or number of children was not written in the patient's file, the data sheet for that individual was marked as "unknown" for that characteristic.

### **2.2.2 Information collected about individuals who had diagnostic HD testing**

Individuals in the diagnostic HD testing category included patients referred for genetic testing for HD because they were already showing symptoms suggestive of this disorder. It also included patients who attended the Genetic Counselling Clinic for predictive HD testing and were found to be symptomatic by the psychiatrist or neurologist while going through the predictive testing protocol. Information about HD diagnostic patients who did not receive genetic counselling was limited, as the only information available was from their referral forms and test request forms.

In addition to the general information documented for all patients (section 2.2.1), the following information was also obtained from the files of patients who underwent diagnostic testing (where possible) and written onto data collection sheets:

- Date of the test (if done)
- Age at the time the test was done
- Reason for the test not completed (if applicable)

### **2.2.3 Information collected about individuals who had HD predictive genetic counselling and/or testing**

The predictive testing category included all individuals who began the HD predictive testing protocol by attending the first genetic counselling session. Where an individual initially attended a genetic counselling session wishing to undertake HD predictive testing but was then found to be symptomatic, they were reclassified as diagnostic. However, it was noted that they initially requested predictive testing.

In addition to information collected as described in section 2.2.1, the following additional information was obtained from the files of the HD predictive testing patients (where possible) and written onto data collection sheets:

- Date of first genetic counselling session
- Age at first genetic counselling session
- Date of entry into the predictive program
- Age at time of entry into the predictive program
- Date of result-giving session
- Age at result-giving session
- Motivation for undergoing HD predictive testing

### **2.2.4 Information collected about prenatal HD testing**

Prenatal testing involves an at-risk individual or an individual who has been confirmed to have HD requesting testing of a fetus during pregnancy. The fetal cells can be obtained by either CVS or by amniocentesis and are tested in order to determine whether or not the fetus is at risk of developing HD.

The following additional information was obtained from the files of patients who underwent prenatal testing and written onto data collection sheets:

- HD status of parent (i.e. affected or at 50% risk)
- Gender of affected/at risk parent

- Age of affected/at risk parent at time of test
- The year when the test was requested
- Gestational age at time CVS or amniocentesis was performed. If CVS/amniocentesis was not done, the gestational age at the time of attendance of the first genetic counselling session was used.
- Decision made after a positive HD result (i.e. termination or continuation of pregnancy)

### **2.3 DATA ANALYSIS**

This study involved retrospective analysis of data obtained from files of individuals who had diagnostic, predictive or prenatal HD testing through the Division between January 1998 and December 2006.

All information obtained from the data sheets was entered into Excel spreadsheets under the headings of diagnostic, predictive and prenatal HD testing.

Frequency calculations were used for each group (diagnostic, predictive and prenatal) to determine: the number of males and females; the number of individuals of white, black, Indian, mixed or unknown ancestry; the number of individuals in a relationship and the number not in a relationship; the number of employed and unemployed individuals; how many individuals had children and how many did not; the number of individuals who tested positive for HD1 and HDL2 and the number who tested negative for HD; the number of individuals within different age groups; and the number of tests done each year. Frequencies were reported as percentages (%) to one decimal place.

Excel was used to determine the median age of individuals in each group, as well as the median time taken to complete the HD predictive testing process. The “Median” and “Standard Deviation” functions in Excel were used for this. Standard deviation results were rounded to one decimal place.

Fisher’s exact and Mann-Whitney U tests were performed using statistical software available on the internet (Graphpad Quickcalcs and IFA Service Statistics respectively). The Fisher’s exact test

was used in this study to determine whether there was any association between two variables. The null hypothesis (H<sub>0</sub>) is that the variables are independent, and a p-value is generated to enable H<sub>0</sub> to be accepted or rejected (Raymond and Rousset 1995). It can be used for small population sizes (less than 5) rather than the similar  $\chi^2$  test, which is only accurate for larger sample groups. For Fisher's exact tests where the two variables being compared each had more than two values (i.e. not a 2x2 comparison using a 2x2 contingency table), a 2x5 contingency table Fisher's exact test was done using Simple Interactive Statistical Analysis (SISA) software which was also available online. The Mann-Whitney U test was used in this study to assess whether there was a significant difference between the medians from two sample groups. For both the Fisher's exact and the Mann-Whitney U test, a p value of less than 0.05 was considered to be statistically significant. A statistician at the University of the Witwatersrand was consulted to validate the results obtained and to ensure that the correct tests were used.

## **2.4 ETHICS**

Ethics clearance (protocol number M060945) was obtained from the Human Research Ethics Committee (Medical) on 29 September 2006 (See appendix C). Ethics clearance for this project was given subject to limiting data collection until October 2006. This limitation was appealed (appendix D) as the aim of this project was to assess the utilization and outcome of diagnostic, predictive and prenatal testing for HD in Johannesburg between 1 January 1998 and 31 December 2006.

### **3 RESULTS**

The number of patients who requested diagnostic, predictive and prenatal testing for HD between 1998 and 2006, as well as the number of tests that were requested each year in the Division, are presented in section 3.1.

The section on patients who had diagnostic testing gives an overview of the patient demographics, as well as a description of their test results analysed by gender, ethnicity and age. The demographics of patients who had predictive testing, their motivations for undergoing HD predictive testing and their test results are also shown. The section on prenatal testing describes the demographics of the individuals who requested prenatal testing for HD, as well as the results of any prenatal tests performed. The decisions parents made regarding termination or continuation of the pregnancy following a positive HD result are also presented.

A final section in this chapter includes two sets of comparisons. Firstly the demographics of the patients who had diagnostic and predictive HD testing are compared, and secondly the results found in this study are compared to those of a study done by Kromberg et al. (1999).

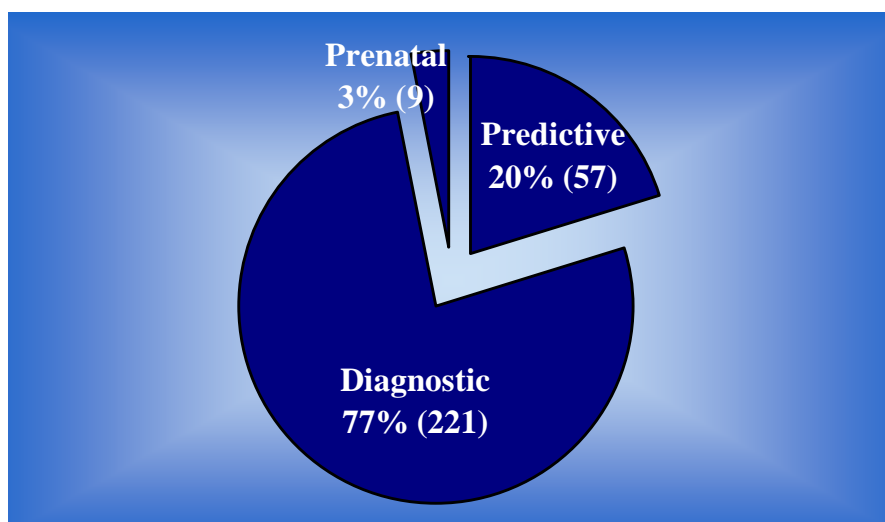
#### **3.1 PATIENT NUMBERS AND TESTS PER YEAR**

##### **3.1.1 Number of diagnostic, predictive and prenatal HD patients**

Files were available for 291 individuals for use in this study. These files were from individuals who requested predictive or prenatal testing for HD, as well as for individuals for whom diagnostic HD testing was requested. Four of these 291 files were excluded. One was excluded because it was a request for testing of an adopted baby who was potentially at-risk of having inherited HD. Another was excluded as it was a request by a woman to have her positive HD predictive testing result confirmed. She had been tested by a private laboratory and had not been through the predictive testing process. The remaining two files were excluded as they were requests for

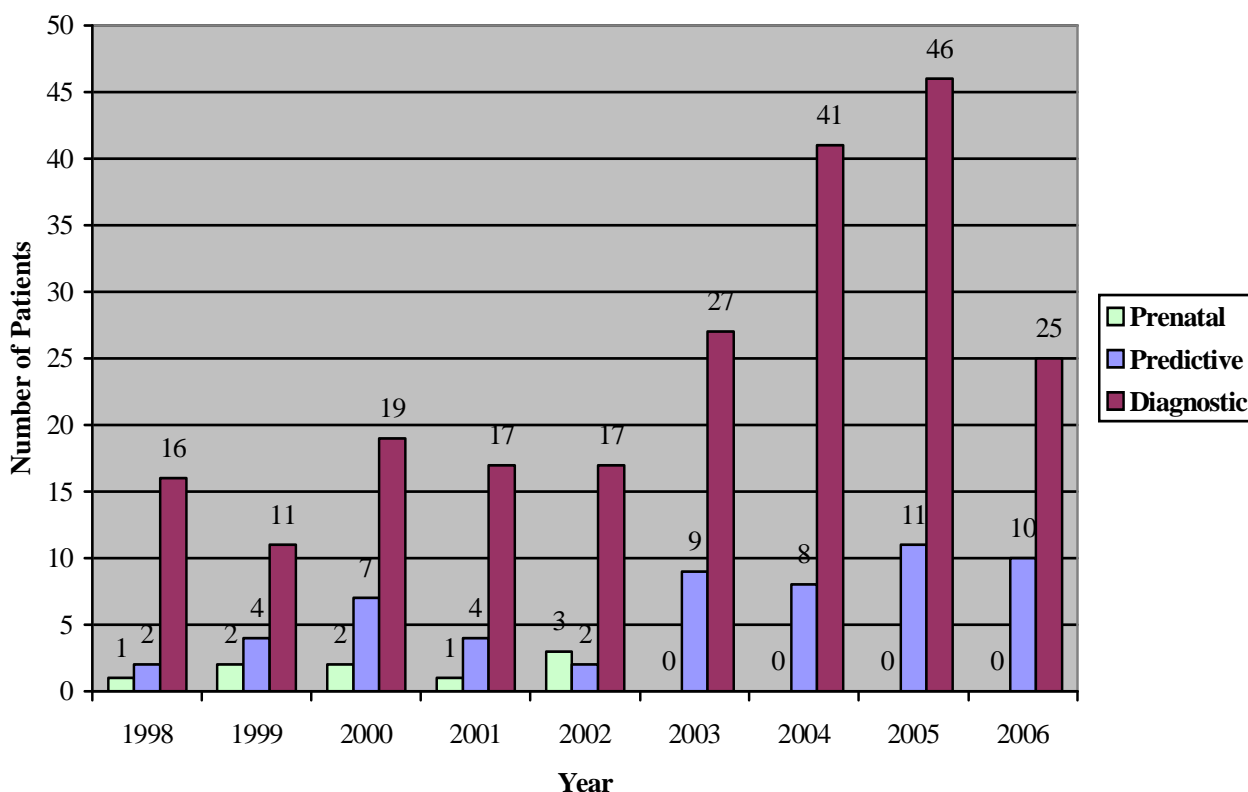
predictive testing but the individuals did not attend a genetic counselling session. The other 287 files were included in this study.

As can be seen from Figure 3-1, 77% of records obtained between 1998 and 2006 for HD were diagnostic. Predictive testing for HD was the next most requested service, comprising 20% of the sample group. Only a small proportion (3%) of at-risk or affected individuals requested prenatal testing and/or counselling for HD between 1998 and 2006.



**Figure 3-1: Number and percentage of diagnostic, predictive and prenatal records for individuals who had genetic counselling and/or genetic testing for HD between January 1998 and December 2006 by the Division.**

### 3.1.2 Number of diagnostic, predictive and prenatal HD tests requested per year between 1998 and 2006



**Figure 3-2: The number of patients who requested prenatal testing and/or counselling for HD (in green) , the number of patients who requested predictive HD testing and/or counselling (blue) as well as the number of patients for whom molecular genetic diagnostic testing for HD was requested (pink) at the Division each year from 1998 to 2006.**

As shown in Figure 3-2, there has been a general increase in the number of both predictive and diagnostic tests done for HD between 1998 and 2005. The increase has not been consistent and a decrease in the number of diagnostic tests performed in 2006 is evident.

The number of prenatal tests and/or prenatal HD counselling sessions requested each year has remained low with no prenatal HD tests or prenatal HD genetic counselling sessions being requested between 2003 and 2006. Of the nine prenatal HD genetic counselling sessions done, only five different couples were involved. Three couples received prenatal HD genetic counselling and/or testing for one pregnancy, one couple requested prenatal HD genetic testing for two pregnancies, while another couple had three pregnancies tested prenatally for HD (including a twin pregnancy).

### **3.2 DIAGNOSTIC TESTING FOR HD**

Diagnostic testing for HD was requested for a total of 221 individuals between January 1998 and December 2006. Five of these individuals had requested predictive testing but were found to have symptoms of HD after consultation with a neurologist during the predictive testing process, and were offered diagnostic testing. Sixty-one of the 221 individuals (27.3%) for whom diagnostic testing was requested received genetic counselling for HD. Of these 61 individuals who received genetic counselling for HD, 44 (72.1%) tested positive for HD1, three (4.9%) tested positive for HDL2, ten (16.4%) tested negative for HD and four (6.6%) were not tested.

#### **3.2.1 Characteristics of patients from whom diagnostic HD testing was required**

Gender, ethnicity, relationship status, employment status, number of children and age of individuals for whom diagnostic testing for HD was requested, were analysed in this study. These results are shown in Table 3-1 and Table 3-2.

**Table 3-1: Characteristics of the 221 individuals for whom diagnostic testing for HD was requested between January 1998 and December 2006.**

<b>Characteristic</b>	<b>Total</b>	<b>%</b>	<b>Characteristic</b>	<b>Total</b>	<b>%</b>
<b><u>Gender</u></b>			<b><u>Number of Children</u></b>		
Female	115	52	No children	24	10.8
Male	105	47.5	1 or more children	70	31.7
Unknown	1	0.5	Unknown	127	57.5
<b><u>Relationship Status</u></b>			<b><u>Age</u></b>		
Not in relationship <sup>a</sup>	25	11.3	Under 30	21	9.5
In a relationship/Married	68	30.8	30-39	35	15.8
Unknown	128	57.9	40-49	41	18.6
<b><u>Employment Status</u></b>			50-59	45	20.4
Employed	30	13.6	60-69	32	14.5
Not employed <sup>b</sup>	36	16.3	Over 70	12	5.4
Unknown	155	70.1	Unknown	35	15.8

<sup>a</sup> Included 14 single, 6 divorced and 5 widowed individuals

<sup>b</sup> Included 20 unemployed individuals, 8 students and 8 retired individuals

As shown in Table 3-1 the number of males and females in the diagnostic testing group was not statistically different from the expected population ratio of 48 males: 52 females ( $p=1.00$ ; Fisher's exact test). This ratio was calculated using data from the South African population census 2001, where 47.8% of individuals were male and 52.2% were female. The relationship status, employment status and number of children for many individuals who had diagnostic HD testing was unknown. Of the individuals whose relationship status was known, more than twice as many were in a relationship than not. The number of individuals employed and the number unemployed was not significantly different ( $p=0.16$ ; Fisher's exact test) from the general South African population, where 58.4% of individuals are employed and 41.6% are unemployed (Statistics South Africa 2001). Approximately 32% of individuals in the diagnostic testing group had one or more children compared to about 11% who had no children, although the number of children was unknown for 57.7% of the individuals.

The age range of individuals for whom diagnostic testing for HD was requested between 1998 and 2006 was 3 to 84 years, with a median age of 49 years ( $SD\pm 15.4$ ). Two individuals, one aged 20 and one aged 51, had already died when their tests were performed. Their ages at the time of death were noted, as this was the time at which their blood was taken.

**Table 3-2: South African population divided by ethnic groups according to the 2001 South African Population Census, and the observed number of patients for whom diagnostic testing for HD was requested between 1998 and 2006 divided by ethnic groups.**

<b>Population Group</b>	<b>% in population</b>	<b>Observed number of patients</b>	<b>Expected number of patients<sup>a</sup></b>
Black African	79%	93 (43.5%)	169.06 (79%)
White	9.6%	106 (49.5%)	20.54 (9.6%)
Coloured	8.9%	11 (5.1%)	19.05 (8.9%)
Indian or Asian	2.5%	4 (1.9%)	5.35 (2.5%)
Total	100%	214 <sup>b</sup>	214 <sup>b</sup>

<sup>a</sup> Expected values were calculated from the population percentages obtained from the South African Census 2001 data using the following formula: (diagnostic sample size of 214) x (proportion of individuals in each ethnic group). This was done to obtain the number of individuals of white, black, Indian and mixed ancestry in a sample of 214 individuals, based on the percentage of individuals in each ethnic group in the general South African population, expected to require HD diagnostic testing. Gene frequency has been assumed to be equal in all population groups, which may not be valid.

<sup>b</sup> Total used was 214 and not the total sample group of 221 because 7 individuals were of unknown ethnicity (3.2%).

The number of individuals in each ethnic group for whom HD diagnostic testing was requested was significantly different from the calculated expected numbers in each ethnic group based on the 2001 South African Population Census ( $p < 0.001$  using Fisher's exact test). The observed percentage of white individuals who had diagnostic testing (49.5%) was higher than the expected percentage of 9.6%. For the other three population groups (black, Indian and mixed ancestry), the observed percentage of individuals was lower than the expected percentages (Table 3-2)

**3.2.2 HD diagnostic test completion**

Thirteen of the 221 (5.9%) diagnostic tests requested were not completed. The reasons for this are shown in Table 3-3.

**Table 3-3: The reasons for HD diagnostic testing not being completed in thirteen cases, at the Division.**

<b>Reason for test not completed</b>	<b>Number of samples</b>
Incorrect tube	6
Test cancelled by doctor <sup>a</sup>	2
Forms incomplete <sup>b</sup>	2
Doctor wanted to confirm diagnosis clinically first <sup>c</sup>	2
Test requested by daughter <sup>d</sup>	1
<b>Total</b>	<b>13</b>

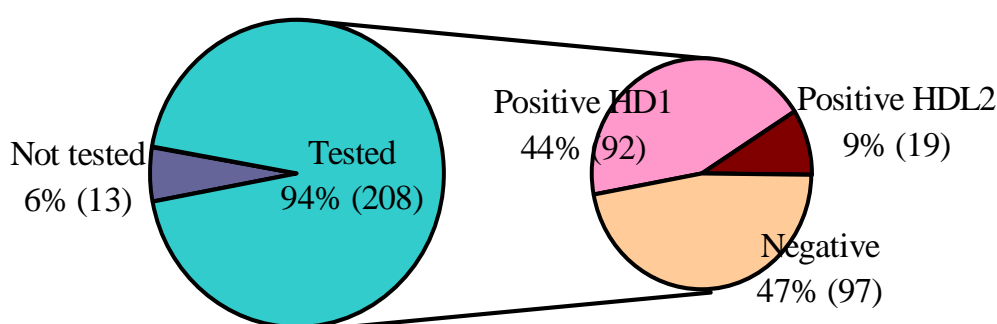
<sup>a</sup> Doctor no longer felt that the patient’s symptoms were due to HD

<sup>b</sup> Incomplete forms did not include patient details or did not include details of the test requested and the reason for the test being requested

<sup>c</sup> The doctor felt he wanted to first determine whether the patient’s symptoms were suggestive enough of HD before doing a diagnostic test

<sup>d</sup> Sample was rejected as it was sent in by a woman requesting that her father’s blood be tested for HD

**3.2.3 HD diagnostic test results**



**Figure 3-3: The outcomes of diagnostic testing for HD1 and HDL2 requested between January 1998 and December 2006 at the Division.**

As shown in Figure 3-3, HD diagnostic testing was completed for 208 patients (94%), and of these, 92 tested positive for HD1 and 19 tested positive for HDL2. All black and mixed ancestry individuals were tested for HDL2 as it has been found to occur in these population groups. HDL2 has not been found to occur in the white and Indian population groups, so individuals of these ethnicities were not tested for HDL2.

The number of children was unknown for 47 of the individuals who tested positive for HD (HD1 or HDL2), but was known for the other 64. These 64 HD positive individuals had 160 children between them, all of whom are at 50% risk of having inherited HD.

**Table 3-4: The number of individuals, by gender, who had HD diagnostic testing and tested either positive or negative for HD1 or HDL2.**

The total number tested is 208 rather than 221, as these results do not include the 13 individuals not tested for HD (see Table 3-3).

Gender	Total number tested	Positive			Negative (%)
		HD1	HDL2	Total (%)	
Male	100	45	8	53 (53.0)	47 (47.0)
Female	107	52	11	63 (58.9)	44 (41.1)
Unknown	1	0	0	0	1
<b>Total</b>	<b>208</b>	<b>97</b>	<b>19</b>	<b>116</b>	<b>92</b>

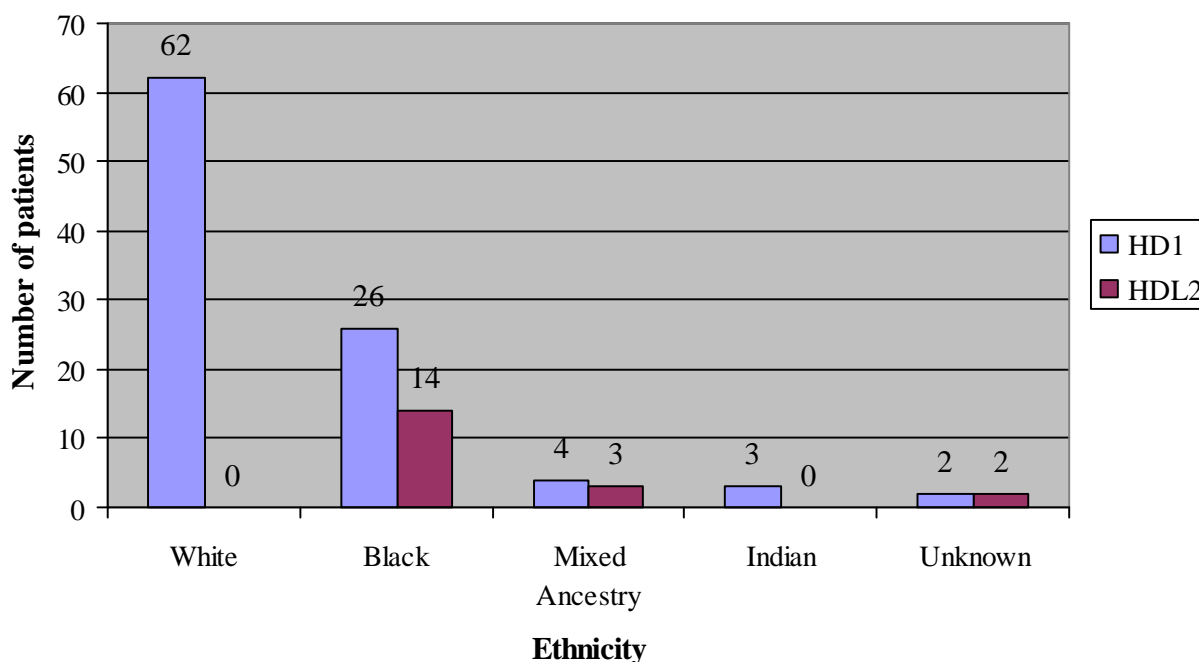
As seen in Table 3-4 the number of males and females receiving either a positive result or a negative result was similar ( $p=0.40$ ; Fisher's exact test). Gender did not also appear to have an effect on whether an individual tested positive for HD1 or HDL2 ( $p=0.80$ ; Fisher's exact test).

**Table 3-5 : The number of individuals, by ethnic group, who had HD diagnostic testing and tested either positive or negative for either HD1 or HDL2.**

The total number tested is 208 rather than 221, as these results do not include the 13 individuals not tested for HD (see Table 3-3).

Ethnicity	Total number tested	Positive (%) for HD1 or HDL2	Negative (%)
White	101	62 (61.4)	39 (38.6)
Black	87	40 (46.1)	47 (54.0)
Mixed Ancestry	11	7 (63.6)	4 (36.4)
Indian	4	3 (75.0)	1 (25.0)
Unknown	5	4 (80.0)	1 (20.0)
<b>Total</b>	<b>208</b>	<b>116</b>	<b>92</b>

As seen in Table 3-5, when comparing white individuals to black individuals, significantly more white individuals tested positive than negative while more black individuals tested negative than positive ( $p=0.04$ , Fisher’s exact test).



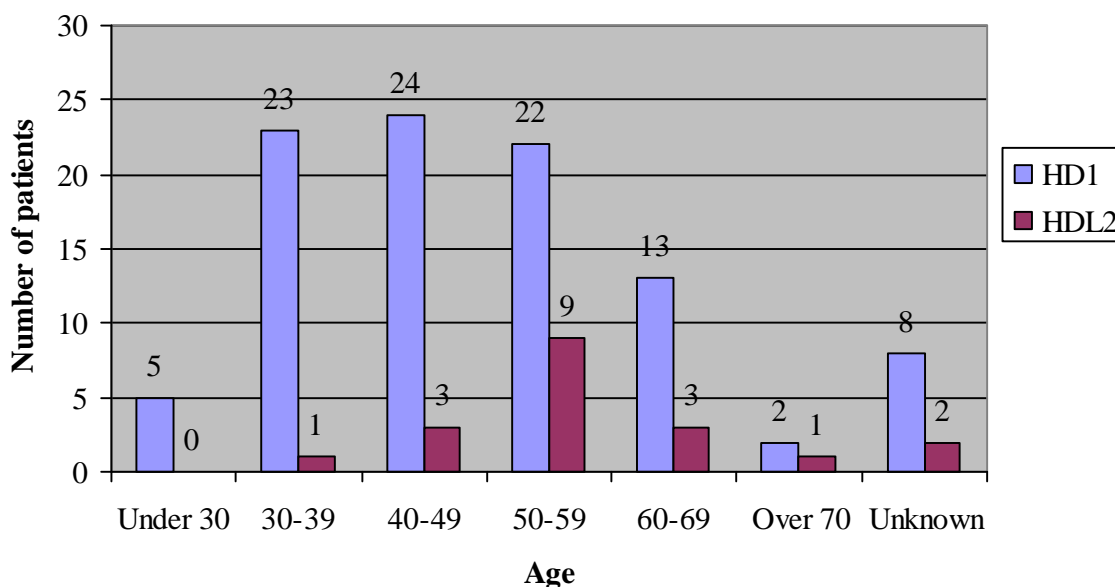
**Figure 3-4: The number of individuals in the diagnostic group who tested positive for either HD1 or HDL2 in each ethnic group.**

Individuals who tested positive for HDL2 were black, or of mixed ancestry, as can be seen from Figure 3-4. White individuals were not tested for HDL2 because HDL2 has not been found to occur in this population group. Amongst the black individuals in the diagnostic group, 35% of the individuals who tested positive for HD were positive for HDL2. In the mixed ancestry group, 43% of the HD positive individuals tested positive for HDL2. Overall, there was no significant difference between the number of black and mixed ancestry individuals testing positive for HD1 and the number testing positive for HDL2 ( $p=0.69$ ; Fisher's exact test).

**Table 3-6: The number of individuals, by age, who had HD diagnostic testing and tested positive for HD1 or HDL2 and the number who tested negative.**

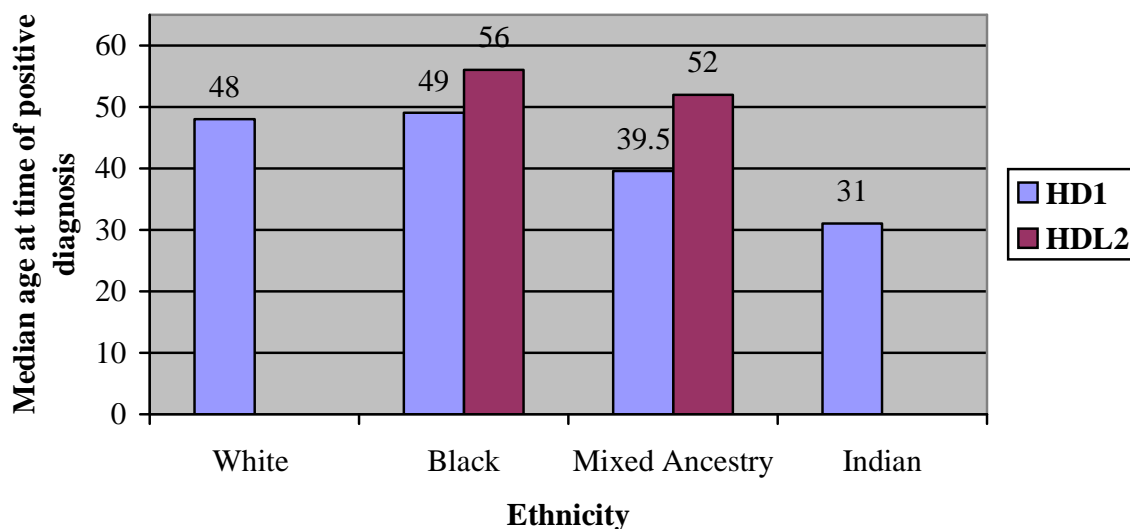
<b>Age</b>	<b>Total number tested (% of total)</b>	<b>Positive (%) for HD1 or HDL2</b>	<b>Negative (%)</b>
Under 30	21 (10.1)	5 (23.8)	16 (76.2)
30-39	37 (17.8)	24 (64.9)	13 (35.1)
40-49	39 (18.7)	27 (69.3)	12 (30.7)
50-59	44 (21.1)	31 (70.5)	13 (29.5)
60-69	17 (8.2)	16 (94.1)	1 (5.9)
Over 70	12 (5.8)	3 (25.0)	9 (75.0)
Unknown	38 (18.3)	10 (26.3)	28 (73.7)
<b>Total</b>	<b>208</b>	<b>116</b>	<b>92</b>

A positive test result (positive HD1 or HDL2) seemed to be dependent on age ( $p=0.047$ ; Fisher's exact test) with most individuals (64.9%) who tested positive for HD being between the ages of 30 and 69, as shown in Table 3-6. Individuals outside this age range were more likely to test negative for HD than positive. The most common age range during which individuals were tested for HD diagnostically was 50-59 years (21.2%), followed by 40-49 years (18.7%).



**Figure 3-5: The number of individuals in the diagnostic group who tested positive for HD1 and the number who tested positive for HDL2 in each ethnic group.**

The median age of individuals who tested positive for HDL2 was 55 years ( $SD \pm 9.3$ ), which was significantly older than the median age of 48 years ( $SD \pm 12.2$ ) for the individuals who tested positive for HD1 ( $p=0.03$ ; Fisher’s exact test). As seen in Figure 3-5, most individuals who tested positive for HDL2 were between the ages of 50 and 59 years, while most individuals who tested positive for HD1 were between 40 and 49 years of age.



**Figure 3-6: The median age of individuals at the time of either a positive HD1 or a positive HDL2 diagnosis, shown by ethnicity. No white or Indian individuals were tested for HDL2.**

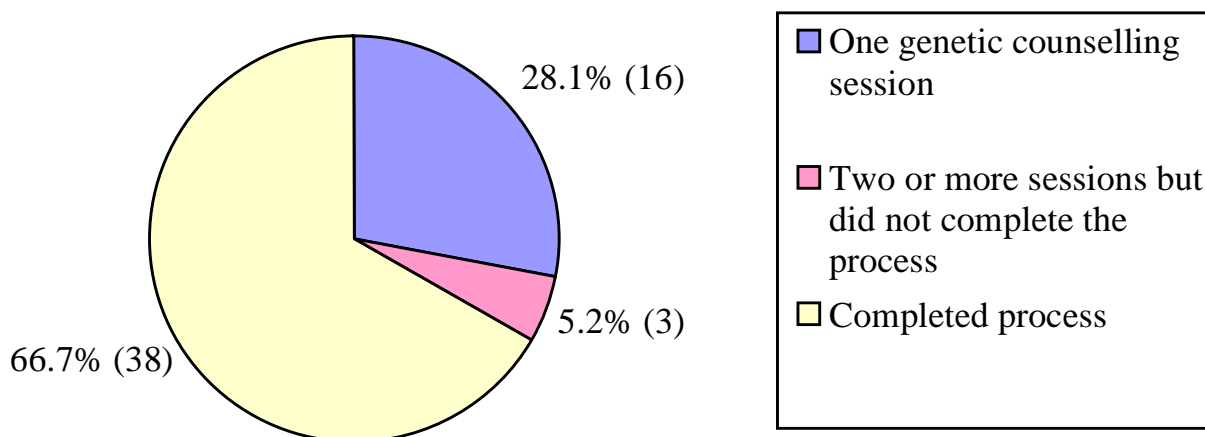
As seen in Figure 3-6, the median age of white individuals who tested positive for HD1 was 48 years ( $SD \pm 11.7$ ). The median age of black individuals testing positive for HD1 was 49 years ( $SD \pm 11.9$ ), and was 56 years for HDL2 ( $SD \pm 8.6$ ). Individuals of mixed ancestry who tested positive for HD1 had a median age of 39.5 years ( $SD \pm 17.4$ ) and those who tested positive for HDL2 had a median age of 52 years ( $SD \pm 13.1$ ). The median age at testing for Indian individuals was 31 years ( $SD \pm 2.7$ ). There were no significant differences found between the median ages at diagnosis in the different ethnic groups. The Indian sample size (3) is too small to draw significant conclusions.

### **3.3 PREDICTIVE TESTING FOR HD**

#### **3.3.1 Completion of HD predictive tests**

A total of 57 patients from 49 families requested predictive testing and/or attended a genetic counselling session regarding HD between January 1998 and December 2006. As shown in Figure 3-7, 38 of these individuals completed the predictive testing process by seeing a genetic counsellor at least twice, a neurologist, psychologist and psychiatrist and receiving a result. Three individuals attended an initial session with a neurologist, psychologist or psychiatrist after having a genetic

counselling session, but did not complete the predictive testing process. Sixteen individuals only attended one genetic counselling session and did not pursue predictive HD testing.



**Figure 3-7: Number of sessions attended by individuals who requested predictive testing for HD at the Division between 1998 and 2006.**

A “session” was defined as an appointment with a genetic counsellor, neurologist, psychologist or psychiatrist as part of the predictive testing process. All these professionals needed to be seen and a result received in order for an individual to complete the predictive testing process.

The number of days taken to complete the predictive HD testing process, from the initial session to the result giving session ranged from 17 days to 771 days (approximately 2 years and 2 months). The individual who took only 17 days to complete the process had already seen a neurologist, psychologist and psychiatrist just before attending the initial genetic counselling session and was not required to see these professionals again. The median time taken to complete the process was 125.5 days (approximately 4 months;  $SD \pm 156.6$ ), with only four of the 38 individuals who completed the process taking longer than 1 year to do so. One of these individuals lived in a country bordering South Africa, and experienced a delay in completing the predictive testing process due to travelling difficulties.

### **3.3.2 Characteristics of patients who requested predictive HD testing**

A number of characteristics of the individuals requesting predictive testing for HD were analysed in this study. These included gender, ethnicity, relationship status, employment status and number

of children (Table 3-7). None of these factors was found to significantly influence whether or not the HD predictive testing process was completed (Refer to Appendix E).

**Table 3-7: Characteristics of the 57 individuals who requested predictive testing for HD at the Division between 1998 and 2006.**

<b>Characteristic</b>	<b>Total</b>	<b>%</b>	<b>Characteristic</b>	<b>Total</b>	<b>%</b>
<b><u>Gender</u></b>			<b><u>Number of Children</u></b>		
Female	38	(66.7)	No children	31	(54.4)
Male	19	(33.3)	One or more children	25	(43.9)
			Unknown	1	(1.7)
<b><u>Relationship Status</u></b>			<b><u>Age</u></b>		
Not in relationship <sup>a</sup>	16	(28.1)	Under 20	3	(5.3)
In a relationship/Married	40	(70.2)	20-29	24	(42.1)
Unknown	1	(1.7)	30-39	20	(35.1)
			40-49	6	(10.5)
<b><u>Employment Status</u></b>			Over 50	4	(7.0)
Employed	42	(73.7)			
Not employed <sup>b</sup>	13	(22.8)			
Unknown	2	(3.5)			

<sup>a</sup> Included 13 single, 2 divorced and 1 widowed individual

<sup>b</sup> Included 10 unemployed individuals and 3 students

In the predictive testing group, approximately 67% of individuals who requested testing were female. This is significantly different from the expected ratio of 50% female to 50% male ( $p=0.02$ ; Fisher's exact test) for a dominant disorder. More than 70% of individuals in the predictive testing group were married or in a relationship, and 43.9% had one or more children. With regard to employment status, about 74% of individuals in the predictive testing group were employed, which is not significantly different ( $p=0.07$ ; Fisher's exact test) from the general South African population, where 58.4% of individuals are employed and 41.6% are unemployed (Statistics South Africa 2001). The age range of individuals who requested HD predictive testing was 18-56 years, with a median age of 30 years ( $SD\pm 8.88$ ).

**Table 3-8: South African population divided by ethnic groups according to the 2001 South African Population Census and the observed number of patients who requested predictive testing for HD between 1998 and 2006, divided by ethnic groups.**

Population Group	% in population	Observed number of patients requesting HD predictive testing	Expected number of patients <sup>a</sup>
Black African	79%	2 (3.5%)	45.03 (79%)
White	9.6%	52 (91.2%)	5.47 (9.6%)
Coloured	8.9%	2 (3.5%)	5.07 (8.9%)
Indian or Asian	2.5%	1 (1.8%)	1.43 (2.5%)
Total	100%	57 (100%)	57

<sup>a</sup> Expected values were calculated from the population percentages obtained from the South African 2001 Census data using the following formula: (predictive sample size of 57) x (proportion of individuals in each ethnic group). This was done to obtain the number of individuals of white, black, Indian and mixed ancestry in a sample of 57 individuals, based on the percentage of individuals in each ethnic group in the general South African population, who would be expected to request predictive testing for HD. This was calculated assuming an equal gene frequency in all population groups, which may be incorrect.

As shown in Table 3-8, more white individuals requested predictive testing than any of the three other groups (black, Indian and mixed ancestry). This was significantly different from the number of individuals in each ethnic group who would be expected to request HD predictive testing based on the make-up of the South African population (Statistics South Africa 2001) ( $p < 0.001$ ; Fisher's exact test). However, we have assumed equal gene frequency in the different ethnic groups, which may be incorrect. The calculation is also based on only 57 individuals requesting predictive testing for HD.

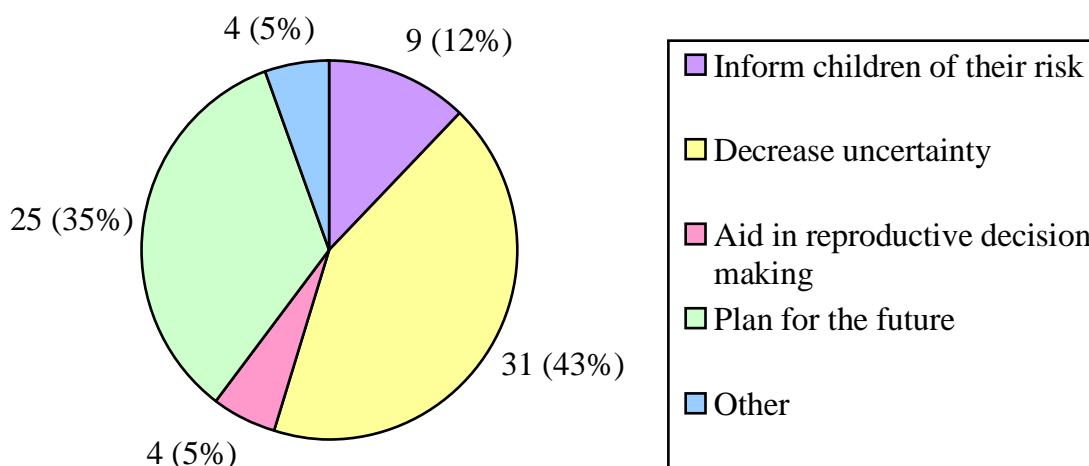
### **3.3.3 Motivation of individuals in the predictive HD testing group for entering the HD predictive testing process**

Four common reasons for entering the HD predictive testing process to find out whether or not one will develop HD have been described in the literature (Evers-Kiebooms, Swerts, Cassiman et al. 1989; Meissen, Mastromauro, Kiely et al. 1991; Quaid and Morris 1993; Lucas 1998). In this study, the same motivations were named by patients. These include:

- ❖ decrease uncertainty
- ❖ plan for the future (including financial, marital and career decisions)

- ❖ aid in reproductive decision-making
- ❖ inform children of their risks

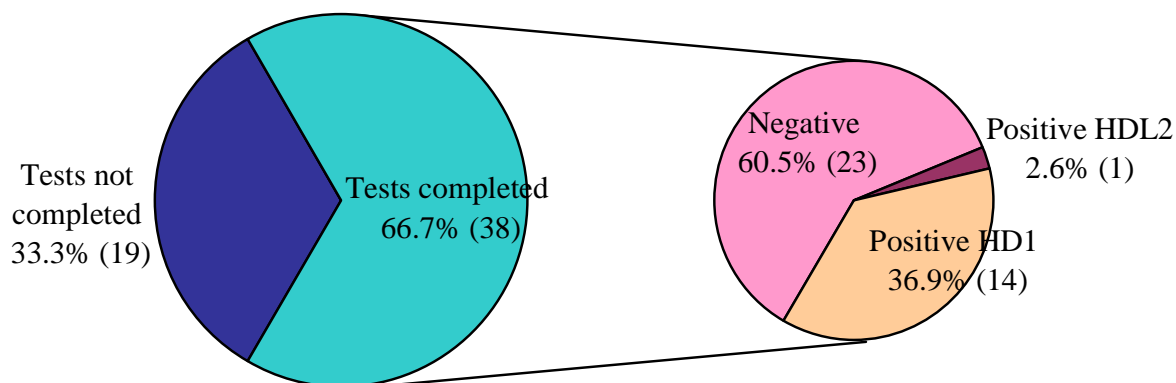
The motivation for entering the predictive testing program was obtained from notes written in the genetic counselling files for 53 of the 57 individuals who requested predictive testing for HD. Three of these 53 individuals were unsure if they wanted to be tested, and had requested genetic counselling to obtain more information, but did not enter the predictive testing program. Twenty-nine of the individuals who requested predictive testing gave only one main reason for doing so, nineteen gave two reasons and two individuals gave three reasons for requesting HD predictive testing. For this reason, the numbers in Figure 3-8 below exceed 50.



**Figure 3-8: Individuals who entered the predictive testing process had different motivations for doing so. These motivations, as well as the number of individuals who gave each motivation, are shown. Some individuals had more than one motivation for requesting HD predictive counselling and/or testing.**

Four of the individuals who requested predictive testing for HD gave other reasons for wanting to know their status and these included feeling that they had symptoms and wanting to be sure (3 individuals), and the test being requested by their pension or insurance fund (1 individual).

**3.3.4 HD predictive test results**



**Figure 3-9: The chart on the left shows the number and percentage of HD predictive tests that were completed between January 1998 and December 2006 at the Division. On the right are the results of the completed HD predictive tests, including the number of individuals who tested positive for HD1, the number who tested positive for HDL2 and the number who tested negative for HD.**

Of the total number of patients who requested predictive testing for HD, approximately 67% completed the process. As can be seen from Figure 3-9, 14 individuals tested positive for HD1, one patient tested positive for HDL2 (this was an individual of mixed ancestry who was a member of a large family confirmed to have HDL2 by diagnostic testing) and 23 individuals tested negative for HD (including one individual who tested negative for HDL2). All individuals who requested predictive testing for HD were at 50% risk so it was expected that 50% of the individuals tested would test negative for HD and 50% would test positive for HD. The results of this study were not significantly different from expected ( $p=0.50$ ; Fisher's exact test).

### 3.4 PRENATAL HD TESTING

Nine prenatal tests for HD were requested by five different couples between January 1998 and December 2006.

**Table 3-9: Characteristics of individuals who requested prenatal testing for HD, including their gestational age at the time of the test, the gender, age and HD status of the affected or at-risk parent, as well as the result of the prenatal HD test and the decision the parents took following a positive HD result.**

Couple	HD status of parent	Gender of at-risk parent	Age of at-risk parent	Year of pregnancy	Gestational age (weeks)	Prenatal result	Decision
1	50% risk	Female	24	1999	12	Not tested	Continued pregnancy
2	50% risk	Female	34	1999	12	Not tested	Continued pregnancy
3	Positive	Female	26	1998	Unknown	Negative	Continued pregnancy
			29	2000	10 (twin 1)	Positive	TOP <sup>b</sup>
			29	2000	10 (twin 2)	Positive	TOP <sup>b</sup>
			30	2002	10	Negative	Continued pregnancy
4	Positive	Male	31	2002	11	Positive	TOP <sup>b</sup>
			31	2002	11	Negative	Continued pregnancy
5	Positive	Male	41	2002	12	Negative (47XXX) <sup>a</sup>	Continued pregnancy

<sup>a</sup> Karyotype analysis is routinely done on all prenatal samples.

<sup>b</sup> TOP = Termination of pregnancy

As seen in Table 3-9, the gestational age at the time the HD prenatal test was requested was unknown for one patient, but for all others the tests were requested between 10 and 12 weeks gestation. Three of the five individuals requesting HD prenatal testing and/or prenatal HD genetic counselling were positive for HD and had been through the predictive HD testing process, while the other two individuals were at 50% risk. Two male and three female probands requested genetic

counselling to find out about HD prenatal testing. The age range of the individuals requesting HD prenatal testing was 24 - 41 years. All individuals who requested prenatal testing were white, married and employed (not shown in Table 3-9).

Two of the five couples who requested prenatal counselling for HD chose not to continue with a test on the pregnancy. In both cases, testing would have been performed by exclusion testing using linkage analysis, as the parents were at 50% risk of having inherited HD and had not been through the predictive HD testing process. Both fetuses were at 25% risk of having inherited the gene for HD, and both couples chose to continue their pregnancies. The other three couples chose to have prenatal HD testing and included an affected female parent and two affected male parents. For these three couples who decided to continue with HD prenatal testing, tests were done by chorionic villus sampling (CVS). Three of the seven fetuses (including a set of twins) tested positive for HD using direct mutation testing. Both couples chose to terminate their affected pregnancies. There were four negative results done by direct mutation testing, including one fetus which tested negative for HD but had a karyotype of 47, XXX. All of these pregnancies were continued.

### **3.5 COMPARISON OF INDIVIDUALS HAVING PREDICTIVE AND DIAGNOSTIC TESTS FOR HD**

This section compares the characteristics of individuals who had diagnostic and predictive testing for HD.

### 3.6.1 Comparison of the characteristics of individuals in the diagnostic HD testing group to those in the predictive HD testing group.

**Table 3-10: Comparison of characteristics of the individuals for whom diagnostic HD testing was requested to those who requested predictive counselling for HD between 1998 and 2006.**

	Predictive		Diagnostic		
	Total	(%)	Total	(%)	p-value <sup>a</sup>
<b><u>Gender</u></b>					
Female	38	(66.7)	115	(52)	p=0.045*
Male	19	(33.3)	105	(47.5)	
Unknown	0	(0)	1	(0.5)	
<b><u>Ethnicity</u></b>					
White	52	(91.2)	106	(48)	p<0.001*
Black	2	(3.5)	93	(42)	
Indian	1	(1.8)	4	(1.8)	
Mixed Ancestry	2	(3.5)	11	(5)	
Unknown	0		7	(3.2)	
<b><u>Relationship Status</u></b>					
Not in relationship	16	(28.1)	25	(11.3)	p=0.85
In a relationship/Married	40	(70.2)	68	(30.8)	
Unknown	1	(1.7)	128	(57.9)	
<b><u>Employment Status</u></b>					
Employed	42	(73.7)	30	(13.6)	p<0.001*
Not employed	13	(22.8)	36	(16.3)	
Unknown	2	(3.5)	155	(70.1)	
<b><u>Number of Children</u></b>					
No children	31	(54.4)	24	(10.8)	p<0.001*
One or more children	25	(43.9)	70	(31.7)	
Unknown	1	(1.7)	127	(57.5)	
<b><u>Age</u></b>					
Median (SD)	30 years (SD±8.88)		49 years (SD±15.38)		p<0.001*

<sup>a</sup> p-value is calculated using Fisher's exact test and is a comparison between the known variables only

\* Significant

When comparing the characteristics of the individuals who had diagnostic testing for HD to those who had predictive testing and/or predictive genetic counselling for HD, as shown in Table 3-10, the following were found to be significantly different between the two groups (unknowns were excluded from the calculations in all cases):

Gender ( $p=0.045$ ): In the HD predictive testing group, there were significantly more females requesting testing than males, compared to the diagnostic HD testing group, where the number of males and females who were tested was similar.

Ethnicity ( $p<0.001$ ): More white individuals requested predictive testing for HD than any other ethnic group whereas in the diagnostic testing group, the numbers of white and black individuals for whom HD testing was requested were similar. The ratio of white to black individuals in the HD predictive group (26:1) is much greater than the ratio of white to black individuals in the HD diagnostic group (1.1:1). In all ethnic groups, more individuals had diagnostic testing than predictive testing, but this was particularly obvious in the black population group.

Employment status ( $p<0.001$ ): Significantly more individuals in the HD predictive testing group were employed compared to the HD diagnostic testing group. In the predictive testing group, 73.3% of individuals were employed while of those whose employment status was known in the diagnostic testing group, 45% were employed. Employment status was known for all but 3.5% of individuals in the predictive testing group, but was unknown for 70.1% of individuals in the diagnostic testing group.

Number of children ( $p<0.001$ ): Significantly more individuals in the HD diagnostic testing group had one or more children compared to the HD predictive testing group. However, the number of children was unknown for 57.5% of individuals in the HD diagnostic testing group, while this was unknown for only 1.7% of individuals in the HD predictive testing group.

Age ( $p<0.001$ ): The median age (30 years) of the individuals who requested HD predictive testing was significantly lower than the median age (49 years) of individuals for whom HD diagnostic testing was requested.

### **3.6 COMPARISON OF RESULTS FROM THIS STUDY AND THOSE OF A STUDY BY Kromberg et al. (1999)**

One of the aims of the study was to compare the number of patients seen and the patient characteristics observed in this study (1998 to 2006 – 9 years) to the results obtained in a previous study done by Kromberg et al. (1999) to identify trends over time. Kromberg et al. (1999) studied the utilization of predictive, prenatal and diagnostic testing for HD in Johannesburg in the Division between 1975 and 1997 (22 years). Linkage analysis became available in the Division in 1989, and was used for predictive and prenatal HD testing. Samples for HD diagnostic testing were sent to the University of Cape Town for direct mutation analysis from 1994 until 2001 when direct mutation analysis became available at the Division for diagnostic, predictive and prenatal HD testing.

#### **3.6.1 Number of predictive and diagnostic HD tests done during the two different study periods**

**Table 3-11: Number and percentage of patients who had diagnostic and predictive testing for HD in this study and in the Kromberg et al. (1999) study.**

	<b>This study (1998-2006)</b>	<b>Kromberg et al. (1999) (1975-1997)</b>	<b>p-value</b>
<b>Diagnostic</b>	221 (79.5%)	52 (55.9%)	<i>p</i> <0.001
<b>Predictive</b>	57 (20.5%)	41 (44.1%)	
<b>Total</b>	278	93	

As seen in Table 3-11, almost 80% of tests completed between 1998 and 2006 were diagnostic HD tests, whereas diagnostic HD tests made up about 56% of all tests done between 1975 and 1997. The percentage of HD predictive tests decreased proportionately between the two studies. HD diagnostic tests made up significantly more ( $p < 0.001$ ; Fisher's exact test) of the total number of HD tests requested in this study compared to that in the Kromberg et al. (1999) study.

### **3.6.2 Number of tests done per year during the Kromberg et al. (1999) study and the number done per year during this study**

The Kromberg et al. (1999) study looked at patient numbers and characteristics over 22 years – from 1975 to 1997, while this study looked at a 9 year period – from 1998 to 2006. When calculating the average number of tests done per year, the year when testing first became available was taken into account. The number of HD diagnostic tests could not be calculated over a 22 year period, as diagnostic testing for HD could only be done from 1994, when the HD gene was cloned and direct mutation testing could be offered. This resulted in HD diagnostic tests only being done during a four year period during the Kromberg et al. (1999) study. For predictive and prenatal HD testing linkage analysis became available in 1989, so tests per year were calculated over a 9 year period (1989-1997). The average number of tests done per year in each of the two studies is shown below in Table 3-12.

**Table 3-12: The average number of diagnostic, predictive and prenatal HD tests done per year from the time testing became available during the Kromberg et al. (1999) study. Also shown is the average number of diagnostic, predictive and prenatal HD tests done per year during the 9 year time period of this study. Direct mutation testing for HD was only available from 1994.**

	This study – 9 years		Kromberg et al. (1999) – 22 years		
	Number of tests	Number of tests/year	Number of tests	Number of years during which testing was available	Number of tests/year
Diagnostic	221	24.56	52	4	13.00 <sup>a</sup>
Predictive	57	6.33	41	9	4.56 <sup>b</sup>
Prenatal	9	1.00	10	9	1.11 <sup>b</sup>

<sup>a</sup> As direct mutation analysis only became available in 1994, number of HD diagnostic tests per year for the Kromberg et al. (1999) study was calculated over a 4 year period (1994-1997).

<sup>b</sup> As linkage analysis only became available in 1989, the number of HD predictive tests per year and the number of HD prenatal tests per year for the Kromberg et al. (1999) study were calculated over a 9 year period (1989-1997).

More HD diagnostic and predictive tests were done per year during the 9-year period analysed in this study compared to the number done per year when testing was available during the Kromberg et al. (1999) study. The number of prenatal HD tests done per year has remained low and consistent.

### **3.6.3 Comparison of HD diagnostic testing between the two studies with regard to ethnicity and test result**

The study by Kromberg et al. (1999) showed HD diagnostic testing results by ethnicity (white/black) and by test result (positive/negative), so those characteristics were compared to the ethnicity and test results obtained in this study, as shown in Table 3-13.

**Table 3-13: Summary of the number of HD diagnostic tests completed by white and black individuals in this study compared to the number completed in the Kromberg et al. (1999) study.**

			<b>This study</b>	<b>Kromberg et al. (1999)</b>	<b>p-value</b>
<b>Number of tests requested</b>			221	52	
<b>Number of tests completed</b>			208 (94.1%)	52 (100%)	
<b>Tests completed</b>	Whites	Positive	62 (61.4%)	8 (47.1%)	$p=0.29$
		Negative	39 (38.6%)	9 (52.9%)	
	Blacks	Positive	40 (46.5%)	11 (55%)	$p=0.62$
		Negative	46 (53.5%)	9 (45%)	

There was no significant difference between the two studies with regard to the percentage of positive and negative HD results obtained in either the white ( $p=0.29$ , Fisher's exact test) or the black population ( $p=0.62$ ; Fisher's exact test).

**Table 3-14: Summary of the total number of white and black individuals who had HD diagnostic testing in this study compared to the Kromberg et al. (1999) study.**

	<b>This study</b>	<b>Kromberg et al. (1999)</b>	<b>p-value</b>
Total number of whites who had HD diagnostic testing	101 (54.0%)	17 (45.9%)	$p=0.38$
Total number of blacks who had HD diagnostic testing	86 (46.0%)	20 (54.1%)	

No significant difference was found between the percentage of white individuals and the percentage of black individuals who had HD diagnostic testing during the Kromberg et al. (1999) study compared to this study, as summarized in Table 3-14 ( $p=0.38$ ; Fisher's exact test).

### **3.6.4 Comparison of predictive testing for HD between the two studies with regard to gender**

Results of the Kromberg et al. (1999) study showed HD predictive testing results by gender.

**Table 3-15: Summary of the number of HD predictive tests completed by males and females in this study compared to the number completed in the Kromberg et al. (1999) study.**

		<b>This Study</b>	<b>Kromberg et al. (1999)</b>	<b>p-value</b>
<b>Number of tests requested</b>		57	41	$p=0.51$
<b>Number of tests completed</b>		38 (66.7%)	30 (73%)	
<b>Tests completed</b>	<b>Males</b>	15 (39.5%)	13 (43.3%)	$p=0.81$
	<b>Females</b>	23 (60.5%)	17 (56.7%)	

There was no significant difference between the number of HD predictive tests completed in this study and the number completed in the previous Kromberg et al. (1999) study ( $p=0.51$ ), as shown in Table 3-15. There was also no significant difference between the number of HD predictive tests completed by males and females in the two studies ( $p=0.81$ ), but in both studies there were more female patients than male patients.

### **3.6.5 Comparison between this study and the Kromberg et al. (1999) study with regard to the number of HD prenatal tests done and the test results obtained**

**Table 3-16: The number of prenatal tests requested during this study and the Kromberg et al. (1999) study as well as number of positive and negative results recorded during each study.**

		<b>This study</b>	<b>Kromberg et al. (1999)</b>
<b>Number of tests requested</b>		9 (5 couples)	10 (7 couples)
<b>Number of tests completed</b>		7 (77.8%)	10 (100%)
<b>Results</b>	Positive	3 (42.9%)	3 (30%)
	Negative	4 (57.1%)	7 (70%)

Results of the Kromberg et al. (1999) study showed HD prenatal testing results only (positive or negative for HD), and did not characterise the patients further. In both studies the number of

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prenatal tests requested was small, as shown in Table 3-16. No tests in this study were done using linkage analysis, while three tests in the Kromberg et al. (1999) study were performed using linkage analysis.

In both studies, all of the couples who received a positive HD prenatal diagnosis chose to terminate the pregnancy. One couple in the previous study received a risk of 48% of the fetus having inherited HD based on exclusion testing and chose to continue the pregnancy.

## **4 DISCUSSION**

### **4.1 NUMBER OF DIAGNOSTIC, PREDICTIVE AND PRENATAL TESTS FOR HD REQUESTED BETWEEN 1998 AND 2006**

The majority of HD tests (77%) requested between January 1998 and December 2006 at the Division were diagnostic tests. Predictive HD tests made up 20% of the study sample, while only 3% of the sample comprised prenatal HD tests.

#### **4.1.1 Utilization of the HD diagnostic testing service at the Division**

There has been an overall increase in the number of HD diagnostic tests requested since 1998, although the number decreased between 2005 and 2006. One reason for the increase in the number of HD diagnostic tests may be a greater recognition of symptoms suggestive of HD by doctors and specialists. This is an important reason, as most HD diagnostic tests are requested by specialists and doctors outside of the Division. There has possibly also been an increased awareness of the HD diagnostic testing service, particularly HDL2 testing which was set up in 2004 as part of a research project. HDL2 testing is now routinely done in the Division. Changes in the number of HD diagnostic tests done each year may also reflect changes within the Neurology Departments that refer patients to the Division. This may include, for example, the loss of staff that were aware of and utilized the services offered by the Division, thereby decreasing the number of HD diagnostic tests done, or the joining of new staff that have a good knowledge of HD and the testing services available for this disorder, leading to increased referrals from these professionals and an increase in the number of HD diagnostic tests done in the Division.

#### **4.1.2 Utilization of the HD predictive testing service at the Division**

There has been a general increase in the number of HD predictive tests requested since 1998, though the overall number of patients tested each year has remained relatively small. Between 1998 and 2002 an average of 3.8 HD predictive tests were done per year but in the last four years of this study (2003-2006) this has increased to an average of 9.5 per year, peaking at 11 patients in 2005. The upward trend in numbers could be due to an increase in the number of individuals who

have become aware of the HD predictive testing service, possibly from relatives, although we have no evidence for this. It seems likely that the number of HD predictive patients seen would increase when the number of diagnostic tests increases due to family members becoming aware of the condition in their family. However, the number of predictive tests done for HD has not increased proportionately, possibly due to poor referrals for HD genetic counselling by medical professionals.

#### **4.1.3 Utilization of HD diagnostic testing compared to HD predictive testing**

The number of HD diagnostic tests requested each year has remained higher than the number of HD predictive tests. One reason for this may be that HD predictive testing is a self-selection process as opposed to HD diagnostic testing where doctors can send samples straight to the laboratory for testing. It may also be that there is limited awareness of the HD predictive testing services offered at the Division or it could be due to a lack of awareness by individuals that they are at risk of having inherited HD. Evers-Kiebooms et al (1989) and Evers-Kiebooms, Nys, Harper et al (2002) suggested that fewer HD predictive tests may be requested out of fear of finding out one's status, a perceived inability to deal with a positive result or due to the lack of cure for HD (Taylor 2004). Although uptake of HD predictive testing was not determined in this study, as the number of at-risk individuals was not known, previous studies have shown various HD predictive testing uptake rates ranging from 4% to 24% (Reviewed in Richards and Rea 2005). A study by Creighton, Almqvist, MacGregor et al (2003) determined the rate of uptake of predictive testing for HD in Canada to be approximately 18%, while a study by Maat-Kievet, Vegter-van der Vlis, Zoetewij (2000) showed an uptake rate of 24% in the Netherlands. Uptake rates have been found to be much lower in Germany, Austria and Switzerland, estimated at below 3% to 4% (Laccone, Engel, Holinski-Feder et al. 1999). These researchers determined the uptake rates in their studies by using national or local registers containing the details of individuals with HD. No such register is available in South Africa so a calculation of the uptake rate could not be attempted in this study. Analysis of the number of at-risk individuals who request predictive testing for HD, out of the total number of at-risk individuals known to the Division, could possibly have been estimated by looking at the pedigrees of affected individuals. However, data were often limited as many individuals who had diagnostic HD testing in the Division did not have genetic counselling.

#### **4.1.4 Utilization of HD prenatal testing at the Division**

The uptake of HD prenatal testing was low, with only 9 prenatal tests being requested between 1998 and 2006. Between one and three tests were requested each year, with no tests being requested between 2003 and 2006. This is interesting, as it was during these four years (2003-2006) that the number of HD predictive and diagnostic tests requested increased, so it would be expected that more HD prenatal tests would have been requested.

A low uptake rate of HD prenatal testing has been found in other studies, with uptake rates estimated to be between 8% and 18% (reviewed in (Richards and Rea 2005; Robins Wahlin 2007). The low uptake of HD prenatal testing may be due to a lack of knowledge about this option amongst patients who do not receive genetic counselling for HD. Another factor may be that individuals choose not to have HD prenatal testing due to an objection to termination of an affected pregnancy (Maat-Kievit et al. 2000; Evers-Kiebooms et al. 2002; Richards and Rea 2005). It was also noted from this study that “aiding in reproductive decision making” was given as a reason for undergoing HD predictive testing by only four patients out of the 53 individuals (5%) who had HD predictive testing between 1998 and 2006. This suggests that most individuals who have predictive testing for HD may not consider HD prenatal testing to be very important, may hold hope for future cures or may decide not to have children if they test positive for HD. However, two couples in this study had more than one pregnancy tested for HD, which suggests that some individuals find the service beneficial.

HD prenatal testing uptake may also be low because HD is a dominant disorder, and therefore approximately 50% of at-risk individuals are expected to test negative. These individuals would no longer require prenatal testing for HD. In this study 59% of patients tested negative for HD which means that they would no longer need to consider prenatal diagnosis. Another reason may be that the individuals testing positive for HD (both symptomatic and asymptomatic individuals) may already have children and may not be planning further pregnancies. Some studies have shown that individuals are less likely to have further pregnancies after receiving a positive HD predictive test result (Meissen et al. 1991; Maat-Kievit et al. 2000; Tibben 2007). In this study, 44.1% of patients who had HD predictive testing had at least one child, while 52.5% did not. Individuals who test positive for HD may also consider other reproductive options (such as adoption, sperm/egg donors or PGD) and would then not require prenatal testing for HD. A study by Evers-Kiebooms et al.

(2002) found that a common reason for individuals declining HD prenatal testing was the hope of a future cure for their children as well as a fear of the tests used for prenatal diagnosis (CVS or amniocentesis).

## **4.2 GENETIC COUNSELLING FOR HD**

Genetic counselling is not a requirement for those having HD diagnostic testing, while all individuals who request predictive HD testing need to attend a genetic counselling session. This is one reason why only 27.6% of individuals for whom HD diagnostic testing was requested received genetic counselling. Also, many of these diagnostic requests come from doctors who want to confirm a diagnosis of HD in their patient. Although the laboratory results sent to the doctor have contact details for the genetic counselling service at the Division, doctors may not pass this information onto their patients and inform family members of their risks, possibly underestimating the value of genetic counselling for families. Samples for diagnostic HD testing are also sent in from all over South Africa, so the individuals being tested may not have easy access to the genetic counselling service in Johannesburg. It would be beneficial for HD diagnostic patients and their families to attend a genetic counselling session to learn more about the condition and about the at-risk status of other family members (Bernhardt, Biesecker and Mastromarino 2000). It would therefore be appropriate for all individuals testing positive for HD diagnostically, as well as their families, to be offered genetic counselling.

## **4.3 DEMOGRAPHICS OF PATIENTS WHO HAD DIAGNOSTIC, PREDICTIVE AND/OR PRENATAL TESTING FOR HD**

### **4.3.1 Demographics of individuals who had diagnostic testing for HD**

#### **4.3.1.1 Gender**

There was no significant difference between the number of males and females in the HD diagnostic testing group ( $p=0.71$ ; Fisher's exact test) and gender did not impact on the likelihood of receiving either a positive result (HD1 or HDL2) or a negative result ( $p=0.80$ ; Fisher's exact

test). This was expected, as HD is a dominantly inherited disorder which affects males and females equally. Other researchers have also found a ratio of approximately 1:1 male: female in their studies (Harper 1996; Creighton et al. 2003)

#### 4.3.1.2 Age, relationship status, number of children, and employment status

The majority of individuals for whom diagnostic HD testing was requested were between the ages of 40-49 years (18.7%) and 50-59 years (21.1%) (Table 3-6). This was expected as these are the age ranges during which HD symptoms typically present (Tibben 2007). HD symptoms usually start in the third or fourth decade of life (Young 2003; Landles and Bates 2004), so by the late 40's and early 50's, symptoms may have become severe and easier to detect, increasing the number of individuals tested diagnostically for HD.

The white individuals in this study who tested positive for HD were slightly younger (median 48 years) than the black (median 52 years) and mixed ancestry (median 50 years) individuals. Indian individuals who tested positive for HD had a median age of 31 years. The younger age of diagnosis of Indian individuals may suggest that they develop symptoms earlier, but the sample size (4 seemingly unrelated individuals) is too small to make assumptions about HD in this ethnic group as a whole. With regard to the later age of diagnosis of black individuals with HD, this may be due to the misconception that HD is rare in the black population group (Joubert and Botha 1988), causing some clinicians to be reluctant to consider it as a diagnosis. However, when an individual in a family is diagnosed with HD, it may enable other family members to be diagnosed earlier.

Although 13 individuals were under the age of 20 at the time of the test, only 1 of these individuals (7.7%) tested positive for HD. This was expected as juvenile HD is estimated to have a prevalence rate of about 4% in the South African white HD population and up to about 15.7% in the mixed ancestry HD population (Hayden et al. 1982). Although this makes juvenile HD relatively rare, these rates are much higher than those found elsewhere in the world, with a study by Laccone et al. (1999) reporting a 0.9% prevalence rate of juvenile HD in the German, Austrian and Swiss populations.

In the HD diagnostic testing group, more individuals were in a relationship (30.8%) than not (11.3%), and more individuals had children (31.7%) than did not (10.8%). This was expected due to the age of the individuals in this group, as more would be expected to be in a relationship and have had children by their 40's and 50's. However, the results may not accurately reflect what is happening in the HD diagnostic testing group, as the relationship status and number of children was unknown for the majority of individuals (57.9% and 57.5% respectively) in this group. This information is not requested on the test request forms and is usually only known if the patient receives genetic counselling. Not knowing the number of children of HD positive individuals means that this group, who are at 50% risk of having inherited HD from their affected parent, cannot be made aware of their risks (by HD information pamphlets/letters sent to them, for example) and offered genetic counselling as their details are not known.

There was no significant difference ( $p=0.16$ ) between the number of individuals in the HD diagnostic testing group who were known to be employed and the number known to be unemployed compared to the general South African population, where 58.4% of individuals are employed and 41.6% are unemployed (Statistics South Africa 2001). We would expect more unemployed individuals in the diagnostic testing group as they may be more likely to have had to stop working because of their HD symptoms. However, for a large proportion of the diagnostic testing group (70.1%) in this study, employment status was unknown. Harper (1996) reported that many individuals may be able to work up to 5 years after the start of symptoms, with a few maintaining their ability to work 10 years after symptoms start. It is often after the start of more severe symptoms that patients would present to a medical professional, when they have already had to stop working. It is possible that, in South Africa, the effect of HD on the ability of affected individuals to work may be obscured by the unemployment rate, as many of these individuals may be unemployed due to the unavailability of jobs, rather than due to HD symptoms preventing them from working.

#### 4.3.1.3 Ethnicity

With regard to ethnicity, a smaller proportion of black individuals underwent diagnostic testing for HD than would be expected based on population data from the 2001 South African Census. Also, a larger proportion of white individuals had HD diagnostic testing than was expected based on the

2001 South African Census data (Table 3-2). This may be due to a lack of awareness of this service among professionals treating black individuals with HD or a lack of consideration of HD as a diagnosis in this population group possibly due to a misconception that HD is rare in black individuals (Joubert and Botha 1988).

Another explanation is that HD may be less prevalent in the black population group. HD was first estimated to have a prevalence of 0.01 per 100,000 in the black and mixed ancestry populations by Hayden et al (1982), although this is now thought to be an underestimate. Other studies since then have provided HD prevalence rates of between 0.97 per 100,000 and 6.37 per 100,000 in the South African black population (Reviewed in Silber, Kromberg, Temlett et al. 1998). The prevalence of HD in the South African mixed ancestry population, however, seems to be similar to the prevalence in the South African white population (approximately 2.2 per 100,000) (Hayden, MacGregor and Beighton 1980) , with both HD1 and HDL2 occurring in the mixed ancestry group.

#### **4.3.2 Demographics of patients who requested predictive testing for HD**

##### 4.3.2.1 Gender

Significantly more females requested HD predictive testing than males ( $p=0.02$ ; Fisher's exact test). This has been found in numerous other studies (Tyler, Ball and Craufurd 1992; Harper et al. 2000; Maat-Kievit et al. 2000; Creighton et al. 2003; Richards and Rea 2005). Suggestions as to the reason for this include women being more likely to face health issues in themselves, as well as women's more active role in reproductive decision-making and childcare. Men may be less likely to want to deal with the emotional issues involved in predictive testing for HD and in reproductive decision-making (Harper et al. 2000; Maat-Kievit et al. 2000; Richards and Rea 2005)

##### 4.3.2.2 Age, relationship status, employment status and number of children

Most individuals requesting predictive testing for HD were between the ages of 20 and 29 years (42.1%), followed by those in the 30-39 year age group (35.1) (Table 3-7). The age range seen

here is similar to that found in studies by Tyler et al. (1992) and Creighton et al. (2003). These are the ages when individuals may start thinking about their future such as planning relationships, careers and/or finances and may want to know their HD status for this reason (Tibben 2007). Few individuals under the age of 20 would be expected to request predictive testing for HD as individuals usually need to be at least 18 years of age to enter the HD predictive testing program. Also, few individuals over the age of 40 would be expected to request predictive testing for HD, as at-risk individuals over this age are likely to have started showing symptoms already, if they have inherited the mutation, as symptoms of HD generally start in the 3<sup>rd</sup> or 4<sup>th</sup> decade of life (Young 2003; Landles and Bates 2004)

Most individuals in the HD predictive testing group were employed and in a relationship (73.7% and 70.2% respectively). This may be reflective of the age of the individuals requesting HD predictive testing (median 30 years), as many would be expected to have entered the working environment and be in a relationship by this time. Although not significantly different from the 58.4% employment rate among the general South African population ( $p=0.07$ ) (Statistics South Africa 2001), the higher employment rate of 73.7% seen in the HD predictive testing group may partly be due to the majority of individuals in the predictive testing group (91.2%) being white. Statistics from the South African Census 2001 show that out of the 41.6% of unemployed South Africans, only 6.9% are white, so in a mostly-white HD predictive testing group, a higher rate of employment can probably be expected, compared to the general South African population where 9.6% of individuals are white.

About 55% of individuals in the HD predictive testing group had children, while approximately 45% of individuals did not. These results were similar to a Belgian study by Decruyenaere et al. (2005) where approximately 47% of individuals had no children. Other studies, however, have found that more individuals have no children than have children. A study by Maat-Kievit et al. (2000) in the Netherlands, found that 56% of the individuals who had predictive testing for HD had no children, while 64% of individuals in a study by van der Steenstraten et al. (1994), also in the Netherlands, had no children. Richards and Rea (2006) looked at an Australian HD predictive testing group and found that 53% of these individuals had no children.

#### 4.3.2.3 Ethnicity

With regard to ethnicity, most of the individuals who requested predictive HD testing (91.2%) were white, with few black, Indian and mixed ancestry individuals requesting testing. The proportion of whites undergoing predictive testing (91.2%) is much higher than the proportion of whites expected to undergo predictive testing for HD (9.6%), based on South Africa population census data (Statistics South Africa 2001).

Reasons for this could include an increased awareness of and access to the HD predictive testing service by white individuals compared to those of other ethnicities. A study in the United States of America (USA) into the uptake of predictive testing for breast cancer found that less than 29% of at-risk black individuals were aware of genetic testing, compared to almost 40% of at-risk white individuals (Peters, Rose and Armstrong 2004). If affected black individuals are not adequately referred for HD genetic counselling, their relatives would not be aware of their at-risk status or of the services offered in the Division.

Another American study also showed that black individuals were less likely than white individuals to undergo genetic counselling and/or testing for the breast cancer susceptibility genes BRCA1 and BRCA2 (Armstrong, Micco, Carney et al. 2005). The authors suggest that this may be due to “health care related-distrust” which has been found to be higher among black individuals than white individuals in the USA (Armstrong et al. 2005). It is possible that this is also the case in South Africa, and may be one of the reasons fewer black individuals request predictive testing for HD compared to white individuals. A study by Ashton, Haidet, Paterniti et al. (2003) in the USA, also showed that a greater proportion of white than black individuals utilised health services. They suggested that this may be due to a bias on the part of the doctors treating black individuals, resulting in them being less likely to refer black individuals to other health care professionals. The lower proportion of black individuals utilizing health services in the USA may also be due to the preferences of the black individuals not to seek treatment, or may be due to difficulties in communication and decision-making between the patient and the health care professional (Ashton et al. 2003).

It is also possible that black individuals are seeking help from traditional healers for HD rather than attending a hospital or clinic. A study by Ngoma, Prince and Mann (2003) involving a Swahili

population in Tanzania, showed that 48% of individuals visiting traditional healers did so for common mental disorders, compared to 24% of individuals visiting primary health clinics. Individuals may seek help from traditional healers owing to their availability compared to primary health clinics, or due to cultural beliefs, such as mental illness being the result of ancestral or evil spirits being used to inflict misfortune (Ngoma et al. 2003). It is possible that in South Africa, as in Tanzania, black individuals who are experiencing psychiatric symptoms, such as those associated with HD, are not seeking medical care from primary health centres, but rather from traditional healers, which may be one reason for the small number of black individuals in the HD predictive testing group.

Another reason for the small number of black individuals requested HD predictive testing, may be that HD is possibly less prevalent in the black population compared to the white population (Hayden, MacGregor and Beighton 1980; Hayden et al. 1982; Silber et al. 1998).

#### 4.3.2.4 Demographics of the individuals who completed the HD predictive testing process and those who did not complete it

Gender, ethnicity, relationship status, employment status and number of children were not found to significantly influence whether or not the HD predictive testing process was completed (Refer to Appendix E). This is in keeping with a study in the Netherlands by Maat-Kievit (2000) who found no difference with regard to age, gender and number of children between the individuals who completed the HD predictive testing program and those who did not complete it.

There may be other factors which influence the decision to complete the HD predictive testing process such as fear about the test result and its meaning for the individual, fear of discrimination and stigmatization (regarding insurance, medical cover, job opportunities, etc), financial issues, family influence and external factors out of their control (Taylor 2004). A study by van der Steenstraten, Tibben, Roos et al. (1994) in the Netherlands found that the most common reason for not participating in the HD predictive program was “fear of confirmation of getting HD”, followed by “fear of adverse effects after receiving an increased risk”. Other common reasons given were that the individuals felt they were coping well with their current uncertainty about whether or not they would develop HD, they did not want to shatter their hope or that they did not want to have testing when there is currently no treatment available for HD (van der Steenstraten et al. 1994).

However, reasons individuals did not complete the HD predictive testing process were not assessed in this study.

#### **4.3.3 Demographics of the individuals for whom HD diagnostic testing was requested compared to the demographics of the individuals who requested HD predictive testing**

Significantly more females than males requested HD predictive testing, while an approximately equal number of males and females requested HD diagnostic testing. This is reflective of the expected 1:1 male to female ratio in the HD diagnostic group due to the autosomal dominant nature of HD. However, this does not hold true for HD predictive testing, as this is a self-selection process, where females seem to be more likely than males to request testing, for reasons discussed in section 4.3.2.1.

The median age of individuals in the HD predictive testing group was significantly lower (30 years) than the median age of individuals in the HD diagnostic group (49 years) ( $p=0.03$ ; Fisher's exact test). This was not surprising, as it was expected that individuals who request HD predictive testing would be younger than those in the HD diagnostic testing group, where individuals have already started showing symptoms of HD. As stated earlier, symptoms of HD generally start in the 3<sup>rd</sup> or 4<sup>th</sup> decade of life (Young 2003; Landles and Bates 2004).

The older age of individuals in the HD diagnostic group may account for the lower rate of employment seen in the HD diagnostic testing group than in the HD predictive testing group, possibly due to these individuals having symptoms of HD. The lower rate of employment seen in this group may also be due to the larger percentage of black individuals in the diagnostic testing group (42%), compared to the predictive testing group (3.5%). Statistics available from the 2001 South African Census show that 31.1% of black individuals in South Africa are unemployed compared to 6.9% of white individuals (Statistics South Africa, 2001). The higher unemployment rate seen in the HD diagnostic testing group may be reflective of the general unemployment rate among the black population in South Africa, as this population group is better represented in the HD diagnostic testing group than the HD predictive testing group. Age may also be the reason why more individuals in the HD diagnostic group had children compared to individuals in the HD

predictive testing group ( $p < 0.001$ ; Fisher's exact test). Relationship status was similar in both the HD diagnostic and predictive testing groups, with most individuals being in a relationship.

In both the HD diagnostic and predictive testing groups, more white individuals had testing than was expected based on the data from the South African 200 Census, but this was particularly obvious in the HD predictive testing group. As discussed earlier, this may be due to lack of awareness of HD in population groups other than the white population or may be due to a decreased incidence of HD in other population groups (reviewed in Silber et al. 1998) or may be due to cultural factors, as discussed in section 4.3.2.3.

#### **4.3.4 Demographics of the individuals who requested prenatal testing for HD**

All individuals in this study who requested prenatal testing for HD were white and in a relationship. Most individuals requesting prenatal counselling and/or testing for HD were between the ages of 24 and 34, with one individual being over the age 40. Three of the individuals who requested HD prenatal testing were female and two were male. Other studies have found that more females than males request prenatal testing for HD, but the sample size of this study is too small to make comparisons (Richards and Rea, 2005). It has been suggested that males may be reluctant to ask their female partners to undergo prenatal testing and consider termination of pregnancy (Richards and Rea, 2005).

All 7 tests that were completed were for individuals who already knew their status. The two individuals who were at 50% risk of having inherited HD and would have needed exclusion testing opted not to have prenatal HD testing. This was also seen in a study by Richards and Rea (2005) where individuals preferred to know their own status before testing a pregnancy. Individuals may prefer to be sure about whether or not their child is actually at risk of having inherited HD before placing the pregnancy at risk by undergoing amniocentesis or CVS.

## **4.4 TEST COMPLETION, RESULTS AND MOTIVATIONS**

### **4.4.1 Diagnostic testing for HD**

#### 4.4.1.1 Test completion

In this study, 13 of the 227 (5.7%) diagnostic tests requested were not completed. The most common reason for this (46.2% of uncompleted cases) was blood being sent in the incorrect tube, so it could not be processed. This suggests that in some cases doctors may not be aware of the correct procedures required by the Division when sending in samples for HD diagnostic testing.

#### 4.4.1.2 HD diagnostic test results

All individuals who had diagnostic testing for HD would have had symptoms suggestive of a neurodegenerative disorder, though these symptoms may not have been entirely suggestive of HD. A review of black HD negative individuals by Professor Amanda Krause, Head of the Clinical Section at the Division showed that only a few of these HD negative individuals actually had symptoms suggestive of HD (A. Krause, personal communication, November 2007). This may account for the approximately 45% of individuals in the HD diagnostic testing group whose HD tests results were negative.

HD symptoms may also closely resemble other disorders such as chorea-acanthocytosis and spinocerebellar ataxia 17 and may be a differential diagnosis (Gold, Shifteh, Bello et al. 2006; Schneider, van de Warrenburg, Hughes et al. 2006). There is also the small possibility that a third, as yet unidentified, locus may be the cause of HD in some of the individuals who tested negative for HD1 and HDL2.

Receiving either a positive result (HD1 or HDL2) or a negative HD result appeared to be dependent on ethnicity ( $p < 0.001$ ; Fishers exact test). Individuals of white, mixed or Indian ancestry were more likely to test positive for HD than negative, while black individuals were equally likely to test positive or negative for HD. This may suggest increased accuracy in the identification of symptoms of HD in the white, mixed ancestry and Indian population groups than in the black population group. It may also suggest that disorders mimicking HD are less common

in population groups other than the black population, so individuals from the white, mixed ancestry or Indian population groups who present with symptoms suggestive of HD are more likely to have HD than some other condition.

In the HD diagnostic testing group, 35% of black individuals who tested positive for HD were positive for HDL2. This result is similar to information obtained from Prof. Amanda Krause, Head of the Clinical Section at the Division, that approximately one third of black South African HD patients have HDL2 (A. Krause, personal communication, November 2007). Approximately 43% of individuals of mixed ancestry who tested positive for HD were positive for HDL2, suggesting that HDL2 is almost as common as HD1 in this population group.

Children of individuals testing positive for HD are at 50% risk of having inherited the disorder. The number of children was known for 64 of the 111 individuals (57.7%) in the diagnostic testing group who tested positive for HD1 or HDL2, and totalled 160 individuals. These individuals may or may not be aware of their risk of having inherited HD so it is important that these individuals are informed of this and have access to genetic counselling for HD. In August 2007 it was decided by the Division to attempt to do this by posting a HD newsletter to the last known address of individuals who tested positive for HD, providing them with support and information, as well as contact details for the genetic counselling unit at the Division. Also included was a form inviting individuals to update their contact details if they wished to receive further correspondence. Thirty-three letters have been sent out to date, and 10 responses (all requesting further information) have been received so far.

#### **4.4.2 Predictive testing for HD**

##### **4.4.2.1 Motivation of individuals in this study for requesting HD predictive testing**

The results show that 50.9% of individuals in this study who requested predictive testing for HD had one main reason for doing so, while some individuals had two or more reasons. The most common reason given was to decrease anxiety, which is similar to results of other studies (Evers-Kiebooms et al. 2002). Knowing one's status may help to relieve stress and enable the individual to think about their future to some extent (Robins Wahlin 2007). This is closely linked to the second most commonly given reason for finding out one's status - planning for the future. In a

study by Lucas (1998), “planning for the future” was the most commonly given reason for entering the HD predictive testing process. Planning for the future usually involves financial planning to ensure enough money is available for care of an affected individual and for their family (Evers-Kiebooms et al, 2002), looking for appropriate medical and life insurance plans, deciding on issues relating to relationships and reproduction and deciding on careers and employment. Lucas (1998) found that individuals considered planning for the future to include planning a family, making financial and residential decisions for the future, deciding whether to marry and/or making changes in their employment status.

Only four of the individuals in this study cited “aiding in reproductive decision making” as one of their reasons for entering the HD predictive testing process. This is much lower than the rates given by other researchers such as Evers-Kiebooms et al. (2002) who reported that 38% of individuals requesting predictive testing gave “reproductive decision-making” as a main reason. The low rates reported in this study may be that many individuals in this study could have considered “aiding in reproductive decision-making” to be a way of “planning for the future”, as was seen in the study by Lucas (1998). Information regarding motivations for entering the predictive testing program were obtained from notes written in the patient’s genetic counselling file rather than by direct questioning, so it was not known what each individual considered to be part of “planning for the future” when providing this as their motivation. Another reason why “aiding in reproductive decision making” was only given as a motivation by four individuals, may be that many individuals in this study already had children (43.9%) and did not require the service. Other individuals may not have been concerned with child-bearing at that time.

Only 12% of individuals in this study reported wanting to know their HD status to inform their children of their new risk. This is lower than the rate found by Evers-Kiebooms et al (2002) who reported that 28% of individuals in their study said they wanted to know their HD status to inform their children of their risks.

#### 4.4.2.2 HD predictive test completion

Approximately 67% percent of individuals in this study who attended an initial genetic counselling session for HD predictive testing completed the process. A study by Mandich, Jacopini, Di Maria

et al. (1998) in Italy showed that more than half of the individuals who entered their predictive testing program withdrew after counselling once they had had time to contemplate what a positive HD test result may mean for them in their lives. Reasons for not entering the HD predictive program or not completing the HD predictive testing process may include fear of receiving an increased-risk result and not being able to cope with it, knowing there is no available treatment for the disorder, fear of the future and what will happen to them if the results show they will develop HD, fear of discrimination such as loss of insurance, the financial costs of completing the HD predictive testing program, and just preferring not to know (Reviewed in Maat-Kievit et al, 2000; Evers-Kiebooms et al, 2002). However, it is not easy to study the individuals who do not enter the HD predictive testing process, as it may be difficult to obtain contact details for them and, if contacted, these individuals may be reluctant and/or feel pressurised to participate in studies regarding HD.

The median time taken to complete the HD predictive testing process was 125.5 days (approximately 4 months;  $SD \pm 156.6$ ). Time taken to complete the HD predictive testing process has not been reported in other studies, so comparative data are not available. The time taken to complete the process reflects the number of professionals who need to be seen before a result is given. Individuals who completed the program in a shorter time period may have been very anxious and have wanted to complete the process quickly while individuals who took longer may not have been ready to receive a result or may, like some of the individuals in this study, have lived in bordering countries or far away from Johannesburg, with transport problems hampering the process.

#### 4.4.2.3 Result of HD predictive testing

Of the individuals who had predictive testing for HD, 39.5% tested positive (for HD1 or HDL2) while 60.5% tested negative. This did not differ significantly from the 50% positive rate ( $p=0.50$ ; Fisher's exact test) expected for autosomal dominant disorders, such as HD. Other studies have also shown an increased number of negative test results, although also not significantly increased, with a ratio of approximately 40% positive/60% negative (Benjamin, Adam, Wiggins et al. 1994; Almqvist et al. 1999; Harper et al. 2000; Maat-Kievit et al. 2000; Creighton et al. 2003). Maat-Kievit et al. (2000) suggested that this may be due to the age range of individuals requesting

predictive testing (usually 30-39 years) which would reduce the age-related risk from 50% to between 47.6% and 42.5% because a percentage of individuals would have already started showing symptoms (Harper et al. 2000). They also suggest that the self-selection process for HD predictive testing may play a role. Individuals may feel they are asymptomatic and feel confident of receiving a negative result so request HD predictive testing. Alternatively, early cognitive symptoms in HD carriers may cause them to be less motivated to request predictive testing, thereby lowering the number of positive test results seen (Tyler et al, 1992; Maat-Kievit et al. 2000). A larger study sample will be needed to confirm the apparent increased number of negative results seen in this and other studies.

Only one individual tested positive for HDL2 in the predictive testing group. This is due to the small number of black (two) and mixed ancestry (two) individuals requesting HD predictive testing in this study. HDL2 has not been found to occur in white individuals and this population group made up the majority of the HD predictive testing group in this study.

#### **4.4.3 HD diagnostic testing compared to HD predictive testing with regards to test completion**

Most HD diagnostic tests were completed (94.7%), while only 66.7% of the individuals who attended a genetic counselling session regarding HD predictive testing completed the predictive testing process. This is due to the self-selection process involved in HD predictive testing, where individuals can choose whether or not to continue with the HD predictive protocol, while with HD diagnostic testing, samples are usually sent in by a doctor.

#### **4.4.4 Prenatal testing results**

Seven fetuses (including a set of twins) were tested for HD. Three fetuses tested positive for HD (including both twins) and these pregnancies were terminated. The other four pregnancies tested negative, and were continued. Individuals who choose to terminate an affected pregnancy may have decided they did not want to have a child who will eventually develop HD. Other studies have also found that individuals who have a pregnancy tested for HD choose to terminate the pregnancy if the fetus tests positive (Reviewed in Richards and Rea, 2005).

#### **4.5 COMPARISON OF THE UTILIZATION OF HD TESTING SERVICES BETWEEN THIS STUDY AND THAT OF A PREVIOUS STUDY DONE BY Kromberg et al. (1999)**

##### **4.5.1 Comparison between the two studies with regard to the utilization of the HD diagnostic testing service offered by the Division**

Compared to the Kromberg et al. (1999) study, there has been an increase in both the number of HD diagnostic tests done per year (from 13.00 per year to 24.56 per year), as well as the percentage of diagnostic tests out of all HD tests done. This may be due to an increased awareness of the service by medical professionals and an increased awareness of and diagnosis of HD, particularly in the local system, due to talks, research and genetic counselling. The number of tests performed may also have increased due to the development of direct mutation analysis for HD, and its implementation at the Division, making testing more easily available in Gauteng.

##### **4.5.2 Comparison between the two studies with regard to the utilization of the HD predictive testing service offered by the Division**

More HD predictive tests have been done per year between 1998 and 2006 (6.33 per year) than the number done per year between 1989 and 1997 (4.56 per year). Although the Kromberg et al. (1999) study looked at testing from 1975 to 1997, linkage analysis only became available in 1989. The increase in the number of HD predictive tests done may be due to the implementation of the predictive testing program, as well as the development and availability of direct mutation testing for HD. This would eliminate the need for extensive family study, and would provide a more definitive result than linkage analysis. A Scottish study also showed an increase in the number of HD predictive tests requested following the development of direct mutation testing for HD, and the researchers suggested that this was due to the definitive results obtained by this method (Holloway, Porteous, Fitzpatrick et al. 1998). Awareness of the HD predictive testing program may also have increased, perhaps from outreach projects, such as presentations to professionals, or by word of mouth within families.

#### **4.5.3 Comparison between the two studies with regard to the utilization of the HD prenatal testing service offered by the Division**

The number of HD prenatal tests done per year has remained low, with 1.11 tests being done per year between 1989 and 1997 and one being done per year between 1998 and 2006. The lack of an increase in the number of HD prenatal tests performed over time may suggest a continuing lack of awareness of the service, most probably by individuals who have tested positive for HD diagnostically rather than those who completed the HD predictive testing process. There may also perhaps be a lack of interest in the service. The reasons for possible lack of interest in the service have been discussed in section 4.1.4.

Decisions taken after a positive HD result have remained consistent, with all couples choosing to terminate the pregnancy. Direct mutation testing has replaced linkage analysis, as this test is more definitive. Similar findings with regard to terminating affected pregnancies, and a shift towards direct mutation testing, have been shown in other studies (Reviewed in Richards and Rea, 2005)

#### **4.6 LIMITATIONS OF THE STUDY DESIGN AND DATA COLLECTION**

A limitation of this study included lack of information such as missing files or missing information in files. Information used in this study was obtained from patient records from the Division and relied on information written by genetic counsellors and clinicians, as well as laboratory test results. One area where this may have been a major limitation is with regard to patients' motivations for entering the HD predictive testing program. Many individuals may have given "planning for the future" as their reason for requesting HD predictive testing, which would have been written into the file, but what they meant by this may not have been fully documented (for example, financial planning, aiding in reproductive decision-making, planning relationships, careers and employment).

Missing information in files may have resulted in inaccurate reflections about the cohort of HD diagnostic patients. Missing information included ages, ethnicity, number of children, relationship status, employment status and gender, which was often not reported in the files of HD diagnostic patients.

#### **4.7 RECOMMENDATIONS**

From the results of this study, it appears that there has been an increase in the number of individuals requesting HD predictive testing, as well as an increase in the number of HD diagnostic tests being performed. The service still seems to be under-utilised, though. It is recommended that the current service be continued, but that provisions are made for an increasing number of individuals who may start utilising this service. This may include training more genetic counsellors to offer predictive testing for HD, and increasing laboratory staff numbers over time to ensure that the HD diagnostic service continues to run smoothly.

It is also important to continue with publicity awareness because it seems that there is still a lack of awareness of the services available. This can include giving talks to educate medical professionals about the indications for HD testing, and talking to doctors after their patients receive a positive HD result to encourage them to refer these patients for genetic counselling. Publicity awareness can also include providing information for family members of individuals with HD as well as involvement in HD support groups. One way in which patients are being educated about HD is by posting newsletters to affected patients with up-to-date HD information, which they can pass onto their families. Information can also be made available to the public to educate them about HD and inform them of the services offered, either by way of pamphlets or by hosting seminars.

#### **4.8 FUTURE RESEARCH**

Future research could include continuing this study into the future to assess and follow trends further over time. It may also be important to extend this study to include patients seen for HD diagnostic, predictive and prenatal testing in other areas of South Africa. The laboratories at the NHLS in Johannesburg and in Cape Town, process the majority of HD samples in South Africa. There are also private laboratories in South Africa which offer HD testing. Individuals tested by other laboratories in South Africa could be included in a study, and results of a broader study could give a better overview of HD in South Africa.

Future research could include further studies into the prevalence of HD within the different ethnic groups, as well as reasons for any discrepancies found. It may also be interesting to look at the

## Discussion

motivations of South African individuals not to enter the HD predictive testing program, and reasons why individuals do not complete the process. Other areas of interest which could be researched include the number of individuals who decide to have children following a positive HD result, whether these individuals choose an alternative reproductive method such as adoption, egg/sperm donation or PGD, and how many individuals who do not choose alternative reproductive options have prenatal HD testing.

## **5 CONCLUSION**

The majority of HD tests done by the Division were diagnostic tests (77%), followed by predictive tests (20%), then prenatal tests (3%). One of the overall findings of this study is that there has been an increase in diagnostic and predictive testing for HD, with prenatal testing remaining consistently low. It is, therefore, necessary to ensure that there are adequate resources available and sufficient numbers of trained laboratory technicians, clinicians and genetic counsellors to cope with the increasing number of HD tests being requested.

Another finding of this study was that all individuals who requested prenatal and predictive HD testing received genetic counselling, but only 27.6% of individuals who had HD diagnostic testing received genetic counselling. This study therefore highlights the need for education of affected and at-risk individuals and their families about HD and the creation of a greater awareness of the HD genetic counselling and testing services among professionals and the public.

This study has also provided an insight into the characteristics and motivations of the individuals who had genetic counselling and/or testing for HD. Most individuals in the HD diagnostic testing group were between the ages of 40 and 59 years, while those in the HD predictive testing group were between 20 and 29 years of age. In the HD predictive testing group, most individuals were white, suggesting an increased awareness of the HD predictive testing service among at-risk individuals in this population group. In the HD diagnostic testing group, the black population was still under-represented compared to the white population but to a lesser extent than in the HD predictive testing group. The most common motivation given by individuals who requested predictive testing for HD was to “decrease uncertainty” followed by “planning for the future”. The information obtained from this study regarding patient characteristics may be helpful in future genetic counselling sessions, and in patient care.

This study has also highlighted areas where more information is needed, for example, regarding the frequency of HD in different ethnic groups, the low uptake rate of HD prenatal testing and reasons for individuals not entering the HD predictive testing program. A lot of information has been generated from this study, and can be used as a starting point for future research.

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**APPENDIX A**  
**Genetic Counselling Facesheet**

**APPENDIX B**  
**DATA COLLECTION SHEET 1**

SUBJECT CODE: \_\_\_\_\_.

**PREDICTIVE TESTING**

1. Date of Birth: \_\_\_\_\_.

2. Gender:

1. Female

2. Male

3. Ethnicity:

1. White

2. Black

3. Indian

4. Mixed Ancestry

5. Unknown

4. Relationship status:

1. Single

2. Divorced/Separated

3. Widowed

4. Married/ in relationship

5. Unknown/Uncertain

5. Occupation Status:

1. Employed

2. Unemployed

6. Number of children:

0

1

2

3

4

5

6

7 Unknown

7. Date of first genetic counselling session: \_\_\_\_\_.

7.1 Age at time: \_\_\_\_\_.

8. Date of entry into the predictive testing process: \_\_\_\_\_.

8.1 Age at time: \_\_\_\_\_.

9. Date of result-giving session: \_\_\_\_\_.

9.1 Age at time: \_\_\_\_\_.

9.2 Not applicable

10. Test Result:

1.1 Positive HD1

1.2 Positive HD2

2. Negative

3. Not tested/No result

11. Motivation for undergoing testing:

1. To decrease uncertainty

2. To plan for the future

3. To aid in reproductive decision-making

4. To inform children

5. Other: \_\_\_\_\_.

**APPENDIX B (Cont.)**  
**DATA COLLECTION SHEET 2**

SUBJECT CODE: \_\_\_\_\_.

**DIAGNOSTIC TESTING**

1. Date of Birth: \_\_\_\_\_.

2. Gender:

1. Female

2. Male

3. Ethnicity:

1. White

2. Black

3. Indian

4. Mixed Ancestry

5. Unknown

4. Relationship status:

1. Single

2. Divorced/Separated

3. Widowed

4. Married/ in relationship

5. Unknown/Uncertain

5. Occupation Status:

1. Employed

2. Unemployed

6. Number of children:

0

1

2

3

4

5

6

7 Unknown

7. Date of test: \_\_\_\_\_.

7.1. Age at time of test: \_\_\_\_\_.

8. Test Result:

1.1 Positive HD1

1.2 Positive HD2

2. Negative

3. Not tested/No result

8. Reasons for test not being completed (if applicable):

1. Test cancelled by requesting doctor

2. Test failed

3. Incorrect collection tube used

4. Other: \_\_\_\_\_.

**APPENDIX B (Cont.)**  
**DATA COLLECTION SHEET 3**

SUBJECT CODE: \_\_\_\_\_.

**PRENATAL TESTING**

1. Gestational Age:\_\_\_\_\_.

2. Gender of fetus:

1. Female

2. Male

3. Unknown

3. Status of parent:

1. Affected

2. At 50% risk

4. Gender of affected/at risk parent:

1. Female

2. Male

5. Age of affected parent at time of test:\_\_\_\_\_.

6. Number of other children in the family:

0  1  2  3  4  5  6  7 Unknown

7. Test Result:

1.1 Positive HD1

1.2 Positive HD2

2. Negative

3. Not tested/No result

8. Number of CAG repeats:\_\_\_\_\_.

9. Decision made following a positive result:

1. Termination of pregnancy

2. Continuation of pregnancy

3. Not Known

**APPENDIX C**  
**Ethics Clearance Certificate**

**APPENDIX D**

**Letter of appeal regarding ethics clearance conditions**

**APPENDIX E****Table of characteristics of the individuals who completed the HD predictive testing program and those who did not**

Table summarising the characteristics of the individuals who requested predictive testing for HD between January 1998 and December 2006. The table also shows a comparison between the individuals who completed the predictive testing process and those who did not.

	<b><u>Total</u></b>	<b><u>Completed</u></b>	<b><u>Not Completed</u></b>
<b><u>Gender</u></b>			
Female	38 (66.7)	23	15
Male	19 (33.3)	15	4
<b>p-value</b>		$p=0.21$ (Not significant)	
<b><u>Ethnicity</u></b>			
White	52 (91.2)	36	16
Black	2 (3.5)	0	2
Indian	1 (1.8)	0	1
Mixed Ancestry	2 (3.5)	2	0
<b>p-value</b>		$p=0.07$ (not significant)	
<b><u>Relationship Status</u></b>			
Not in relationship	16 (28.1)	9	7
In a relationship/Married	40 (70.2)	29	11
Unknown	1 (1.7)	0	1
<b>p-value</b>		$p=0.34$ (not significant)	
<b><u>Employment Status</u></b>			
Employed	42 (73.7)	31	11
Not employed	13 (22.8)	7	6
Unknown	2 (3.5)	0	2
<b>p-value</b>		$p=0.19$ (not significant)	
<b><u>Number of Children</u></b>			
0 children	31 (54.4)	21	10
1 or more children	25 (43.9)	17	8
Unknown	1 (1.7)	0	1
<b>p-value</b>		$p=1.0$ (not significant)	

<b>Age</b>				
Under 20	3	(5.3)	2	1
20-29	24	(42.1)	14	10
30-39	20	(35.1)	16	4
40-49	6	(10.5)	4	2
Over 50	4	(7.0)	2	2
<b>p-value</b>			<i>p</i> =0.53 (not significant)	