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A review of Spinal Muscular Atrophy (SMA) in Black South African paediatric patients.

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Project Title:

A review of Spinal Muscular Atrophy (SMA) in Black South African paediatric patients.

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Declaration

This is a declaration that the submitted work is my own and materials consulted have been appropriately referenced. This work has been submitted for evaluation for the degree of Masters in medicine Paediatrics at the University of the Witwatersrand and has not been submitted at any other university.

Signed: K C Flack

14 day of Decmeber 2021 in Parkwood, JHB

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A special thank you to my mother Dr. P.S. Flack for her patience and words of encouragement. Thank you to the rest of my family for their unwavering support despite the physical distance between us.

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Table of contents

Declaration 2

Acknowledgements: 3

List of Abbreviations 5

List of tables 5

Table 1: Age of onset of symptoms based on history 5

Table 2: Clinical features of SMA and distribution across SMA types 5

Table 3: Muscle biopsy and or genetics results 5

Chapter 1: 6

Submissable Paper: 6

Introduction 7

Methods 9

Results 11

Discussion 14

Conclusion 16

References 16

CHAPTER 2: 18

Protocol 18

CHAPTER 3: 28

Appendix 1: Author Guidelines 28

Appendix 2:Ethics clearance certificate 40

Appendix 3:Plagiarism Deceleration: 41

Appendix 4:Turn it in report 43

List of Abbreviations

SMA- Spinal Muscular Atrophy

CHBAH- Chris Hani Baragwanath Academic Hospital

CMJAH – Charlotte Maxeke Johannesburg Academic Hospital

NHLS- National Health Laboratory Service

SMN1- Survival of Motor Neuron 1- telemetric gene that encodes functional full size SMN protein

SMN2- Survival of Motor Neuron 2- centromeric gene that encodes a partially functional SMN protein

PCR- Polymerase chain reaction

DNA- Deoxyribonucleic acid

MLPA- Multiplex ligation-dependent probe amplification

List of tables

Table 1: Age of onset of symptoms based on history

Table 2: Clinical features of SMA and distribution across SMA types

Table 3: Muscle biopsy and or genetics results

Chapter 1:

Submitted to South African Journal of Child Health

Submissable Paper:

A review of Spinal Muscular Atrophy (SMA) in black South African paediatric patients.

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Key words: SMA, neurology, SMN, genetics

Abstract

Background: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder that is present in all populations and results in muscle weakness due to anterior horn cell degeneration. SMA is divided into three clinical subtypes and is an important genetic cause of morbidity and mortality but has not been well studied in sub-Saharan Africa.

Objective: The aim of this study is to describe the clinical features and genetic findings in black patients with SMA presenting to the Division of Paediatric Neurology at Chris Hani Baragwanath Academic Hospital (CHBAH) over a 30-year period.

Method: This study was a retrospective review of patient records. The study population was black paediatric neurology patients with clinical SMA, who attended CHBAH Neurology clinic between 1988 and 2018. Patients were categorized into SMA type 1, 2 or 3 based on the neurology assessment and clinical features were recorded.

Results: The clinical findings in the black South African patients with SMA were similar to those found in international studies. There were 131 patients fulfilling the inclusion criteria seen over a 30-year period at CHBAH, 86 of who had genetic testing. 84.8% of the genetic results for these patients were positive, which is significantly higher than previously reported in SA. 23.6% of patients had facial involvement.
Conclusions: This study adds to the limited body of research on SMA in sub-Saharan Africa and highlights the lower frequency of a homozygous deletion seen in the black South African population when compared to the expected 95% worldwide.

Word count: 248

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder, which results in progressive and symmetrical muscle weakness due to irreversible anterior horn cell degeneration [1]. The incidence of SMA in the United States is estimated at 1 in 6000 to 1 in 11000 live births with a carrier frequency estimated at 1:38 [2] thus making it the second most common neuromuscular disorder after Duchenne muscular dystrophy [3]. SMA is present in all populations but Verhaart et al suggested a slightly lower incidence in patients of sub-Saharan ancestry of 1 in 18808[4]. However a South African study suggested that SMA is more common than previously thought with an incidence of 1 in 3574 in the black population and 1 in 1945 in the white population[3].

SMA is divided into three clinical subtypes that represent a spectrum of clinical severity. Type 1 SMA, usually presents before 6 months of age and patients never sit unsupported [5]. It is the most common type, affecting up to 50% of all SMA patients. As a result of the swallowing difficulty patients often fail to thrive. Type 1 SMA patients usually develop respiratory failure within the first 2 years of life and less than 65% survive beyond this age [3]. Outcome is dependent on respiratory involvement.

Type 2 SMA or “sitters” achieve the gross motor milestone of sitting unsupported but are unable to stand or walk. The age of onset is usually between 6 and 12 months. This intermediate form of SMA is generally slower to progress with a variable prognosis [5]. Most children with type 2 SMA survive beyond the age of 5 years and survival thereafter is dependent on the development of complications involving the respiratory system [3].

Type 3 SMA describes a group clinically defined as “walkers”. The onset of observable symptoms in this group is usually after age 12 to 18 months. This is considered a mild chronic form of SMA with little effect on patient lifespan. These patients are able to walk but over time develop proximal weakness involving the lower limbs more than the upper limbs [6].These patients have a normal life expectancy.

Two extremes of these subtypes have also been described, Type 0 SMA and Type 4 SMA. Type 0 SMA is a rare type on the extreme of the clinical spectrum and develops in utero [7]. Mothers often give a history of reduced fetal movements in the antenatal period. Patients with type 0 SMA can be born with severe respiratory distress and this form is usually fatal by age 6 months [8]. Type 4 SMA is the mildest form of disease and the onset of symptoms is usually in the third decade of life. As the symptoms predominantly start in adulthood, this group is not described further in this study.

SMA is a leading genetic cause of infant mortality[7] and has autosomal recessive inheritance caused by a homozygous deletion or mutation of the survival motor neuron (*SMN1*) gene on chromosome 5q13 in 95% of patients worldwide [3]. However, there have been studies in South Africa that propose a possible different genetic cause with only 51% of South African Black patients having the homozygous deletion of the *SMN1* gene. One such study was conducted at CHBAH [3] Another study done in the Western Cape concluded that the genetic findings in their patients were in keeping with those that are internationally expected. This group included only 12 black patients and was therefore much smaller than the CHBAH study sample size[9][10].

The most common form of SMA is autosomal recessive SMA but X linked and autosomal dominant cases have been described [11]. The genetic defect was identified in 1995 and a wide range of clinical severity was noted [12] . It was discovered that 2 forms of the SMN gene exist, *SMN1* and *SMN2*. The *SMN2* gene is identical to the *SMN1* gene except for a single nucleotide change at exon 7. The resulting protein produced by transcription at *SMN2* is shorter, less functional and degrades faster than the protein produced at *SMN1*. Only about 10% of the protein produced by the *SMN2* gene is fully functional. As patients with SMA have deletion of *SMN1* they rely on the SMN protein produced at *SMN2* for survival. A correlation between disease severity and *SMN2* copy number has been found, in that a patient with more *SMN2* copies has a less severe clinical phenotype of SMA[13].

The aim of this study is to describe the clinical features and genetic findings in black South African patients with spinal muscular atrophy presenting to the Division of Paediatric Neurology at Chris Hani Baragwanath Academic Hospital over a 30-year period and compare results to two previous studies from this site as well as other South African publications. The focus of the study was on black South African patients as previous studies suggested there could be a different genetic basis for SMA in this group [3]. There are no studies with a comparable size available and there is an overall paucity of data from sub–Saharan Africa. We know that other diseases have been found to have different disease manifestations as a result of a different mutation than that seen in the Caucasian population, for example cystic fibrosis, galactosaemia and Fanconi's anaemia. [3][110] . This therefore provides the rationale for the study.

Methods

Chris Hani Baragwanath Academic Hospital is the only large public hospital in Soweto, Johannesburg. The paediatric neurology clinic receives referrals not only from the surrounding area but also from the rest of the province of Gauteng as well as other provinces and Southern African countries where specialist expertise is lacking.

This study was a retrospective review of the patient records captured by the paediatric neurology team at CHBAH during the period 1988-2018.All the files of patients with a diagnosis of SMA were reviewed.

The study population included all black paediatric neurology patients with clinical, genetic and/or histological diagnosis of SMA who attended CHBAH Neurology clinic from 1988-2018.

All patients presenting to the Division of Paediatric Neurology at CHBAH with clinical features in keeping with the diagnosis of SMA type 1, 2 or 3 as determined by the paediatric neurologists were included. This group also included all patients presenting with a confirmed genetic result or with a confirmed muscle biopsy result. Genetic testing for the homozygous *SMN1* deletion became available at the National Health Laboratory Service in 1996. Patients who presented before this time had muscle biopsy done. A small number of these biopsy patients later had genetic testing done as part of a study. MLPA testing is not done as a routine diagnostic study for SMA but has also been used in research projects to assess for heterozygous deletions and *SMN* copy numbers.

Patients attending paediatric neurology clinic where the diagnosis of SMA was in doubt were excluded.

Data was entered into a confidential and secure Microsoft Excel spreadsheet. Each patient file was reviewed and multiple variables were entered for each patient. The age at first presentation to neurology clinic was captured and the age of onset based on history was recorded as either less than 6 months, 6 to 18 months or greater than 18 months. The grade of SMA at diagnosis, type 1, 2 or 3 was determined from the initial neurologist assessment and the clinical features present at the first assessment were captured. Files were reviewed further for possible genetic or muscle biopsy results.

The resulting spreadsheet was exported into STATA for analysis. Comparative tables were generated and p values determined using either Fischer's exact test or the Chi square test.

Ethics approval

This study was approved unconditionally by the Human Research Ethics Committee of the University of the Witwatersrand. Certificate number M190317.

Results

A total number of 145 patients classified as SMA were seen at CHBAH from1988-2018. The focus of this study was SMA in black South African patients in particular and therefore 14 patients of European, Asian or Mixed race ancestries were excluded. The total number of patient files at CHBAH that were then analyzed was 131.

The patient files contained information on clinical findings at initial presentation to neurology clinic and biopsy or genetic results. Only 45 patient files also contained information on progression of clinical features and therefore for the purposes of this study only clinical features at the first visit were reviewed.

The median age of presentation of all SMA patients to CHBAH paediatric neurology clinic was 15 months (interquartile range: 6-34). The median age at presentation of type 1 SMA patients was 7 months (4-11), type 2 SMA 24 months (16-32) and type 3 was 56 months (36- 72).

Table 1: Age of onset of symptoms based on history

SMA type	Age <6m	6-18m	>18m	Total
1	60 (98.3%)	11 (25.0%)	0 (0%)	71 (54.2%)
2	1 (1.7%)	28 (63.6)%)	3 (11.5%)	32 (24.4%)
3	0 (0 %)	5 (11.4%)	22 (88.5%)	27 (21.4)
total	61	44	25	131

Paediatric neurologists working at CHBAH made the diagnosis of SMA clinically and later confirmed with muscle biopsy (prior to the availability of genetic testing) or genetic testing. SMA affected males and females equally with 51.2% males and 48.8% females affected in our study group. Most of the patients who gave a history of symptoms starting before age 6 months had SMA type 1 (98.3 %). Similarly the majority of patients had onset of symptoms between age 6 and 18 months had SMA

type 2 (63.6%). Most of the patients who developed symptoms after the age of 18 months were classified as Type 3 SMA (88.4%).

Table 2: Clinical features of SMA and distribution across SMA types

SMA	Total (n=131)	Type 1 (n=71)	Type 2 (n=32)	Type 3 (n=28)	P value
Male	67 (51.2%)	36 (53.7%)	15 (22.4%)	16 (23.9%)	0.725
Hypotonia	128 (97.7%)	71 (100%)	32 (100%)	25 (89.2%)	0.009
Tongue fasciculations	105 (80.2%)	64 (90.1%)	30 (93.7%)	11 (39.3%)	0.000
Areflexia	109 (83.2%)	69 (97.2%)	28 (87.5%)	12 (42.8%)	0.000
Polyminimyoclonus	57 (43.5%)	15 (21.1%)	23 (71.8%)	19 (67.9%)	0.000
Facial involvement	31 (23.6%)	22 (30.9%)	5 (15.6%)	4 (14.3%)	0.121
Respiratory compromise	50 (38.2%)	37 (52.1%)	9 (28.1%)	4 (14.3%)	0.001
Contractures	26 (19.8%)	11 (15.4%)	10 (31.2%)	5 (17.8%)	0.199
Scoliosis	9 (6.9%)	3 (4.2%)	4 (12.5%)	2 (7.1%)	0.33

Table 2 shows the presenting clinical features and gender across all SMA types.
The primary clinical features documented were hypotonia (97.7% of patients), absent or diminished reflexes (83.2%), and tongue fasciculation (80.2%).
Hypotonia was present in 100% of the type 1 and type 2 patients and 89% of the type 3 patients. Absent lower limb reflexes were found in 98% of patients, 22 patients (16.8%) had preserved upper limb reflexes and only 2 patients (1.5%) having all reflexes elicited at presentation. Both of these patients were type 3 SMA, with one having a homozygous deletion of SMN 1 and the other having a negative genetic result but positive biopsy result. More than 90% of type 1 SMA patients had fasciculation of the tongue whilst fasciculations were only present in 39% of type 3 SMA patients. Further analysis showed that 48 (36.6%) patients had both tongue fasciculation and polyminimyoclonus whilst just 13 (9.9%) had polyminimyoclonus and no tongue fasciculations and 61 (46.5%) had only tongue fasciculations with no polyminimyoclonus documented.

Closer review of our data showed 31 (23.6%) of 131 patients with facial involvement. Multiple different clinicians made notes over the study period and some referred to "expressionless" and "myopathic" faces. Unfortunately nomenclature and extent of involvement was not consistent across all patient files. Of these 11 (35.4%) had a positive genetic result and 15 (48.3%) had a positive muscle biopsy confirming the diagnosis of SMA.

Some patients had already developed complications of the disease at the time of presentation and contractures, scoliosis and respiratory compromise were also reviewed. 37% of the patients had respiratory compromise at presentation. Scoliosis and contractures were the least common findings.

Table 3: Muscle biopsy and/ or genetics results

SMA type	Positive results	Type 1	Type 2	Type 3	P value
Muscle Biopsy N=48	47 (97.9%)	21 (43.7%)	13 (27.0%)	13 (27.0%)	0.563
Genetics N=86	73* (84.8%)	40 (46.5%)	17 (19.7%)	16 (18.6%)	0.466

* Positive genetic results including heterozygous deletions

Table 3 shows the muscle biopsy and genetic results. Most of the patients in the study population had either a muscle biopsy or genetic testing done. 97.9% of the 48 muscle biopsies were positive. The only patient with a negative biopsy showed non-specific histological changes but had a confirmed homozygous deletion of the *SMN1* gene. 84.8% of the 86 patients who had genetic testing had a positive result. Amongst the patients with positive genetics 2 had a heterozygous deletion i.e. only a single deletion of *SMN1* and a presumed mutation not detected by traditional genetic testing. Both of these patients had positive muscle biopsies.

17 patients had both genetic testing and a muscle biopsy. 5 patients had a biopsy performed after genetic testing and of these patients, 2 were heterozygous deletions, 2

had no deletions and 1 result was inconclusive. All 5 of the biopsies done confirmed SMA.

5 patients had neither a biopsy nor genetic testing. 4 of these patients had planned biopsies but were lost to follow up and one patient family refused biopsy. All 5 of these patients were seen before genetic testing was available.

Discussion

Spinal muscular atrophy is an important genetic cause of infant mortality [1] and has not been well studied in sub Saharan Africa[14] .In this study males and females were equally affected and the median age of presentation across all types of SMA was 15 months (6-34 months IQR). SMA type 1 is the commonest form (54.2% of the SMA patients) with patients typically presenting by 7 months of age and onset of symptoms before age 6 months in 98.3%. The results are similar to previously published international data [5]. Although the majority of type 2 SMA patients had onset of symptoms between ages 6-18 months most only presented at around 24 months of age. In the South African public health care system delay in presentation is a common occurrence due to multiple factors such as access to health care, cultural beliefs and level of patient education.

This study focused on a single racial group as previous studies from South Africa suggested that there might be a different genetic basis for SMA in black South Africans [3]. The main finding was that 73/86 (84.8%) of the genetic results for patients were positive with the majority being homozygous deletion positive (82.5%). This result is significantly higher than what had previously been reported in a study at CHBAH where only 51% of the patients had the expected *SMN1* deletion [3]. This result may be attributed to more widespread access to genetic testing over the last two decades.

The result of this study also differs from what has been reported at Red Cross War Memorial Children's Hospital in Cape Town [10]. This study features a large sample size of 131 black patients, compared to 12 black patients in the Cape Town study. The study performed in Cape Town strictly excluded all patients with facial involvement

[10] but our study results suggest that in doing so up to 1 in 4 patients (23.6%) with confirmed SMA could potentially be missed.

Prior to access to genetic testing, the mainstay of diagnosis was muscle biopsy. 97.9% of patients with available muscle biopsy results confirmed the diagnosis of SMA highlighting a strong correlation with clinical diagnosis. Despite this muscle biopsy is not the preferred initial test for SMA. The procedure is invasive and painful for patients [12]. There are additional associated risks for example general anesthetic in a patient who may already have respiratory compromise. Muscle biopsy cannot give a genetic profile and assist with further genetic counseling for parents and patients therefore genetic testing is still the preferred first line test. In patients where there is a strong clinical suspicion of SMA despite a negative genetic test and inconclusive muscle biopsy, MLPA is available as an option to look for alternate diagnoses.

A recent publication showed that MLPA is not a suitable test to determine carrier status in black South Africans, as up to 50% have multiple *SMN1* copy numbers. This has the potential to mask a heterozygous deletion and will not pick up a carrier state in these cases[15]. The same study reported only 8.3% of the clinically suggestive patients had a heterozygous deletion which is lower than previously reported but higher than what was found in our study [15] [3].

As genetic testing evolves and gene-based therapies become available, a further understanding of the genetic basis for disease in black South Africans is needed. Clinical diagnosis is the first step and early intervention as a team, including paediatric neurologists and rehabilitation specialists, may improve quality of life for these patients.

Study limitations

A limitation of this study is that it is retrospective and requires reviewing medical records compiled by multiple different clinicians. The study is further limited as it was carried out at a single center study with a single ethnic group. Review of results at other sites with comparable patient volumes may be warranted.

Conclusion

This study adds to the limited body of research on SMA in sub- Saharan Africa. The main finding from this study, that 82.5% of the genetic results for patients were homozygous deletion positive, is significantly higher than what had previously been reported in South Africa. This is still lower than what is expected internationally and what is found in Caucasian patients. This review also showed that the clinical presentation of this population group is comparable to what has been internationally reported. Facial involvement is an important clinical sign noted in up to 25% of patients. The focus on a single racial group is justified as previous studies from South Africa suggested that there might be a different genetic basis for SMA in Black South Africans. Ongoing research in this area is needed.

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KF, MH, LGS: write up and editing
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CHAPTER 2:

Protocol

Project Title:
A review of Spinal Muscular Atrophy (SMA) in Black South African paediatric patients.

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Table of Contents: Protocol

List of Abbreviations **19**
Introduction **20**
Aim **22**
Methods **23**
Limitations **24**
Ethics **24**
Proposed time line: **24**
Funding: **25**
References **25**
Data capture sheet: **27**
43

List of Abbreviations

SMA- Spinal Muscular Atrophy
CHBAH- Chris Hani Baragwanath Academic Hospital
NHLS- National Health Laboratory Service
SMN1- Survival of Motor Neuron 1- telemetric gene that encodes functional full size SMN protein
SMN2- Survival of Motor Neuron 2- centromeric gene that encodes a partially functional SMN protein
PCR- Polymerase chain reaction
DNA- Deoxyribonucleic acid
MLPA- multiplex ligation-dependent probe amplification

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder. It results in progressive and symmetrical muscle weakness due to irreversible anterior horn cell degeneration(1). The incidence of SMA in the United States is estimated at 1 in 6000 to 1 in 11000 live births with a carrier frequency estimated at 1:38(2) thus making it the second most common neuromuscular disease after Duchenne Muscular dystrophy(3). SMA is present in all populations and one study suggested a slightly lower incidence in patients of sub Saharan ancestry of 1 in 18808(4). However a South African study suggested that SMA is more common in South Africa with a birth incidence of 1 in 3574 in the Black population and 1 in 1945 in the White population(3).

SMA has typically been divided into three clinical subtypes, which represent a spectrum of clinical severity. Clinical guidelines describe a maximal functional status achieved across the three subtypes(1).
Type 1, the most severe form, classifies patients as "non sitters;" these are weak infants unable to sit unsupported. Type 2 patients are defined as "sitters" who are non ambulant but able to sit independently. Type 3 SMA patients are ambulatory and are called "walkers"(5). Two extremes have also been described: Type 0, which manifests in the antenatal period, and Type 4, which is adult onset and the mildest form of SMA. As this study will be describing paediatric neurology patients attending Chris Hani Baragwanath Academic Hospital (CHBAH), type 4 will not be discussed further.

Patients with Type 1 SMA, previously known as Werdnig- Hoffman disease, present before the age of 6 months and never sit unsupported. It is the most common type, affecting up to 50% of all SMA patients(6). Patients present with typical clinical features such as: hypotonia, symmetrical flaccid paralysis and lack of head control. Muscle weakness is symmetrical, proximal more than distal and affects the lower limbs more than the upper limbs, resulting in "frog leg" posture. Type 1 SMA patients have absent or diminished deep tendon reflexes(7). Patients usually develop a bell shaped chest deformity due to weakness of the intercostal muscles with relative sparing of the diaphragm, which results in paradoxical abdominal breathing. A classic feature in these patients is fasciculation of the tongue, seen in most patients in this group. Facial weakness may also be present but usually develops later in the course of disease progression. Eventually difficulty in swallowing secondary to bulbar motor neuron involvement develops and patients are at increased risk of aspiration. As a result of the swallowing difficulty patients often fail to thrive. Type 1 SMA patients usually develop respiratory failure within the first 2 years of life and less than 65% survive beyond this age. Outcome is dependent on respiratory involvement. These patients typically have normal cognition(8).

Type 2 SMA or "sitters" achieve the gross motor milestone of sitting unsupported but are unable to stand or walk. The age of onset is usually between 6 and 12 months. Type 2 patients also have hypotonia, a progressive proximal weakness affecting the legs more than arms and absent or diminished deep tendon reflexes. This intermediate form of SMA is generally slower to progress with a variable prognosis. Most children with type 2 SMA survive beyond the age of 5 years and survival thereafter is dependent on the development of complications involving the respiratory system.

This includes a progressive scoliosis and weak intercostal muscles contributing to restrictive lung disease. Other clinical features in this group include fasciculation of the fingers as well as tongue. Patients may also develop joint contractures as in type 1 SMA. Patients in this group lose the ability to sit in their teens, with approximately 68% survival at age 25 years(1)(8).

Type 3 SMA, previously known as Kugelber-Welander disease, describes a group clinically defined as "walkers". The onset of symptoms in this group usually starts after age 12 to 18 months. This is considered a mild chronic form of SMA with little effect on patient lifespan. These patients are able to walk but over time develop proximal weakness affecting the legs more than arms.(8) As a result these patients are at increased risk of falling and may have trouble going up and down stairs by age 3 years(6).

Type 0 SMA or very severe SMA is a rare phenotype on the extreme of the clinical spectrum(9). This rare form of SMA develops in utero with mothers reporting reduced fetal movements in the antenatal period. Patients with this type of SMA can be born with severe respiratory distress. They will have generalized hypotonia, absent gag and suck reflex and absent deep tendon reflexes. These patients may also have developed contractures in utero. This extreme form is universally fatal in most patients by age 6 months(8).

SMA is a leading genetic cause of infant mortality(10). SMA has an autosomal recessive inheritance and is caused by a homozygous deletion or mutation of the survival motor neuron (SMN1) gene on chromosome 5q13 in 95% of patients worldwide(3). However, there have been studies in South Africa, which propose a possible different genetic cause with only 51% of South African Black patients having the homozygous deletion of the SMN1 gene. One such study was conducted at CHBAH(3) (11). Another study done in the Western Cape concluded that the genetic findings in their patients were in keeping with those that are internationally expected. This group however did not include any patients with facial involvement and the sample size was much smaller than the CHBAH study sample size(12).

The most common form of SMA is autosomal recessive SMA but X linked and autosomal dominant cases have been described(13). The genetic defect was identified in 1995 and a wide range of clinical severity was noted(14). It was discovered that 2 forms of the SMN gene exist, SMN 1 and SMN 2. The SMN 2 gene is identical to the SMN 1 gene except for a single nucleotide change at exon 7. The resulting protein produced by transcription at SMN2 is shorter, less functional and degrades faster than the protein produced at SMN1. Only about 10% of the protein produced by the SMN2 gene is fully functional. As patients with SMA have deletion of SMN1 they rely on the SMN protein produced at SMN 2 for survival. A correlation between disease severity and SMN2 copy number has been found, in that a patient with more SMN2 copies has a less severe clinical phenotype of SMA(15).

The function of the SMN protein is not fully known. It is present in all cells but appears to have a higher rate of expression in motor neurons when compared with other tissues. Complete elimination of the SMN protein in mice was found to be lethal.

Current standard NHLS testing is able to test only for the homozygous deletion of the SMN1 gene. This test has been available since 1996 and at present multiple research projects are currently ongoing to develop a possible alternate diagnostic test in our setting.

The standard test uses polymerase chain reaction (PCR) and restriction enzyme analysis. First genomic DNA is isolated from peripheral blood using the salting out method. (3)The sample is amplified using polymerase chain reaction (PCR) amplifying SMN1 and SMN2 simultaneously. PCR primers were added on exon 7 and exon 8 of the SMN gene. A restriction enzyme Hinf 1 is added to help distinguish between SMN1 and SMN2 and the resulting solution is added to a 3% aragose gel using gel electrophoresis. SMN1 and SMN 2 are then distinguished by their different sizes. This test is limiting, as it is only able to detect homozygous deletions the SMN 1 gene.

In recent years MLPA and quantitative PCR testing has become available. These tests have assisted in the detection of heterozygote SMN1 deletions, which can assist with carrier testing. It is not used as standard but can be done on special request with the NHLS genetics division. MLPA looks at the specific nucleotide location where SMN1 differs from SMN2 c840 C>T. MLPA uses fluorescent labeled probes and resulting copy numbers are compared to reference probes and control samples. (16) MLPA is considered the gold standard test for determining exon deletion or duplication detection.(16)

Overall neuromuscular disease has not been widely studied in sub-Saharan Africa and more population based studies are needed(17). In a systematic review of data published up until 2015 only 28 studies about SMA, amyotrophic lateral sclerosis or progressive muscular atrophy were found from sub-Saharan Africa. Only 10 of these discussed SMA and 5 of these studies were conducted in South Africa (17).

Aim

The aim of this study is to describe the clinical features and genetic findings in Black South African patients with spinal muscular atrophy, presenting to the Paediatric Neurology Department at Chris Hani Baragwanath Academic Hospital over a 30-year period.

Objectives

1.Describe the clinical features found in different SMA subtypes in black patients at CHBAH and the median age of presentation.
2. Describe the relative frequency of SMA subtypes in our population and compare it with published data.
3 Compare the genetic results in the study group with previously described data sets
4. Compare the clinical features in the genetically confirmed subtypes with previously reported findings

Methods

Study design:
A retrospective review of the patient records captured by the paediatric neurology team at CHBAH during the period 1988-2018.

Chris Hani Baragwanath Academic Hospital is the only public hospital in Soweto. The paediatric neurology clinic receives referrals not only from the surrounding area but also from the rest of the province as well as other provinces and countries where specialist expertise is lacking.

All paediatric neurology patient files are kept in a separate filing room and colour coded according to diagnosis. All the files of patients with a diagnosis of SMA will be reviewed.

Study population:
Paediatric neurology patients with: clinical, genetic and/or histological diagnosis of SMA that attended CHBAH Neurology clinic from 1988-2018.

Inclusion criteria:
1. All patients presenting to the Division of Paediatric Neurology at CHBAH with clinical features in keeping with the diagnosis of SMA type 1, 2 or 3 as determined by the paediatric neurologists
2. All patients presenting to the CHBAH Division of Paediatric Neurology with a confirmed genetic result.
3. All patients presenting to the CHBAH Division of Paediatric Neurology with a confirmed muscle biopsy result.

Exclusion criteria
1. Patients attending paediatric neurology clinic where the diagnosis of SMA was in doubt.
2. Patients, with whom, the paediatric neurologists did not reach a consensus on the diagnosis.
3. Patients without any clinical features of SMA

Data capture sheet
The proposed data capture sheet will be used to capture relevant information from each patient file.
This includes, race, gender, age at presentation, genetic results, clinical features, a review of progression of symptoms, types of SMA using the most severe clinical subtype, admissions relating to SMA, family history and when patient was last seen at follow up.

Data analysis
This is a descriptive study. The data set will be reviewed and the SMA patients will be described, with particular focus on clinical features found in this population group. The genetic and or muscle biopsy findings will also be described per patient subtypes. This will then be compared to existing data.

After data collection, the data quality will be assessed. It can be assumed that not all records will be complete and only information that is available will be analyzed. The average age at presentation to neurology can be drawn from the date first seen at clinic. The clinical features of each patient will be noted at each visit and progression of symptoms can be seen from these records. A paedatric neurologist determined the type of SMA for each patient and the relative frequencies of each subtype, type 1,2 or 3 will be reviewed and compared to previous data.

Limitations

Advantages

The main advantages of such a study:

1. The study is relatively inexpensive
2. The study uses existing records
3. This study allows for the study of rare diseases in an infrequently studied population.

Disadvantages

1. This study relies on the accuracy of diagnosis made by the paediatric neurology team
2. The data that can be collected is dependent on completeness of written records.

Ethics

This protocol will be submitted for approval to the Human Research Ethics Committee at the University of the Witwatersrand. Special permission will be obtained from the Head of the Department of Paediatrics at CHBAH to access their patient records. A letter requesting permission to conduct this study has also been addressed to the CEO of CHBAH.

As this is a retrospective record review, there will be no direct patient contact. All data will be anonymous; patients will be allocated a unique study number to maintain anonymity and the principal investigator will remove names and surnames from the database prior to data analysis. This will ensure patient identity is protected. Only the principal investigator will have access to patient identity.
All have a unique hospital number assigned to them when they become a CHBAH patient, which will only be used by the principal investigator with the names and surnames when cross checking genetic results with NHLS.

Proposed time line:

	August 2018	September 2018	October 2018	November 2018	April 2019	March/ April 2019	June 2019	June 2019	July 2019
Literature review									
Protocol write up									

Protocol submission									
Post grad committee clearance									
Ethical clearance									
Data collection									
Data analysis and write up									

Funding:

Estimated budget for the study: R1500
1.Petrol costs to and from CHBAH for data collection: R500
2.Printing: estimated 50c/page black and white printing
Total cost approximately R750
3.Binding: estimated R15 per copy
Total cost approximately R300.
The total cost of the study will be born by the principle investigator

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Appendix 1:

Data capture sheet:

Patient study number:		
Age at presentation		
Gender	M/F	
Race		
Age at onset of symptoms		
Clinical features present at first assessment		
	Hypotonia	Y / N
	Fasciculation	Y / N
	Absent reflexes	Y / N
	Scoliosis	Y / N
	Contractures	Y / N
	Facial involvement	Y / N
	Respiratory compromise	Y / N
	Feeding difficulty	Y / N
Type of SMA at presentation		
Type 1		
Type 2		
Type 3		
Genetics	Done: Y/N	Result:
Muscle biopsy	Done: Y/N	Result:
Progression		
	Hypotonia	Y / N
	Fasciculation	Y / N
	Absent reflexes	Y / N
	Scoliosis	Y / N
	Contractures	Y / N
	Facial involvement	Y / N
	Respiratory compromise	Y / N
	Feeding difficulty	Y / N
Family History	Siblings involved	Y/N
Admission	Reason for admission Number of admissions	
Age at death / last seen at clinic		

CHAPTER 3:

Appendices:

Appendix1: SAJCH Author Guidelines

Please view the Author Tutorial for guidance on how to submit on Editorial Manager.

To submit a manuscript, please proceed to the *SAJCH* Editorial Manager website: Editorial Manager

There is currently a backlog of articles in production. Authors submitting new articles should note that the earliest date for publication will be in the second quarter of 2022.

To access and submit an article already in production, please see the SAJCH author guidelines here.

Please take the time to familiarise yourself with the policies and processes below. If you still have any questions, please do not hesitate to ask our editorial staff (tel.: +27 (0)21 532 1281, email: submissions@hmpg.co.za).

Article Processing Charges

All articles published in the South African Journal of Child Health are open access and freely available online upon publication. This is made possible by applying a business model to offset the costs of peer review management, copyediting, design and production, by charging an article-processing charge (APC) of R3 275.40 (ex Vat) for all articles published. The charge applies only to Research articles submitted after 1 Jan 2019. The APC is standard and does not vary based on length, colour, figures, or other elements.

When submitting a Research article to the SAJCH, the submitting author must agree to pay the APC should the article be accepted for publication. The APC is payable when your manuscript is editorially accepted and before production commences for publication. The submitting author will be notified that payment is due and given details on the available methods of payment. Prompt payment is advised; the article will not enter into production until payment is received.

Queries can be directed to claudian@hmpg.co.za.

Please refer to the section on 'Sponsored Supplements' regarding the publication of supplements, where a charge is applicable. Queries can be directed to dianes@hmpg.co.za or claudian@hmpg.co.za

Authorship

Named authors must consent to publication. Authorship should be based on: (*i*) substantial contribution to conceptualisation, design, analysis and interpretation of data; (*ii*) drafting or critical revision of important scientific content; or (*iii*) approval of the version to be published. These conditions must all be met for an individual to be included as an author (uniform requirements for manuscripts submitted to biomedical journals; refer to www.icmje.org)

If authors' names are added or deleted after submission of an article, or the order of the names is changed, all authors must agree to this in writing.

Please note that co-authors will be requested to verify their contribution upon submission. Non-verification may lead to delays in the processing of submissions.

Author contributions should be listed/described in the manuscript.

Conflicts of interest

Conflicts of interest can derive from any kind of relationship or association that may influence authors' or reviewers' opinions about the subject matter of a paper. The existence of a conflict – whether actual, perceived or potential – does not preclude publication of an article. However, we aim to ensure that, in such cases, readers have all the information they need to enable them to make an informed assessment about a publication's message and conclusions. We require that both authors and reviewers declare all sources of support for their research, any personal or financial relationships (including honoraria, speaking fees, gifts received, etc) with relevant individuals or organisations connected to the topic of the paper, and any association with a product or subject that may constitute a real, perceived or potential conflict of interest. If you are unsure whether a specific relationship constitutes a conflict, please contact the editorial team for advice. If a conflict remains undisclosed and is later brought to the attention of the editorial team, it will be considered a serious issue prompting an investigation with the possibility of retraction.

Research ethics committee approval

Authors must provide evidence of Research Ethics Committee approval of the research where relevant. Ensure the correct, full ethics committee name and reference number is included in the manuscript.

If the study was carried out using data from provincial healthcare facilities, or required active data collection through facility visits or staff interviews, approval should be sought from the relevant provincial authorities. For South African authors, please refer to the guidelines for submission to the National Health Research Database. Research involving human subjects must be conducted according to the principles outlined in the Declaration of Helsinki. Please refer to the National Department of Health's guideline on Ethics in Health research: principles, processes and structures to ensure that the appropriate requirements for conducting research have been met, and that the HPCSA's General Ethical Guidelines for Health Researchers have been adhered to.

Clinical trials

Since 1st December 2005, all clinical trials conducted in South Africa have been required to be registered in the South African National Clinical Trials Register. The *SAJCH* therefore requires that clinical trials be registered in the relevant public trials registry at or before the time of first patient enrollment as a condition for publication. The trial registry name and registration number must be included in the manuscript.

Protection of rights to privacy

Patient

Information that would enable identification of individual patients should not be published in written descriptions, photographs, radiographs and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) has given informed written consent for publication and distribution. We further recommend that the published article is disseminated not only to the involved researchers but also to the patients/participants from whom the data was drawn. Refer to Protection of Research Participants. The signed consent form should be submitted with the manuscript to enable verification by the editorial team.

Other individuals

Any individual who is identifiable in an image must provide written agreement that the image may be used in that context in the *SAJCH*.

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Privacy statement

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Ethnic/race classification

Use of racial or ethnicity classifications in research is fraught with problems. If you choose to use a research design that involves classification of participants based on race or ethnicity, or discuss issues with reference to such classifications, please ensure that you include a detailed rationale for doing so, ensure that the categories you describe are carefully defined, and that socioeconomic, cultural and lifestyle variables that may underlie perceived racial disparities are appropriately controlled for. Please also clearly specify whether race or ethnicity is classified as reported by the patient (self-identifying) or as perceived by the investigators. Please note thatit is not appropriate to use self-reported or investigator-assigned racial or ethnic categories for genetic studies.

Continuing Professional Development (CPD)

SAJCH is an HPCSA-accredited service provider of CPD materials. Principal authors can earn up to 15 CPD continuing education units (CEUs) for publishing an article; co-authors are eligible to earn up to 5 CEUs; and reviewers of articles can earn 3 CEUs. Each month, *SAJCH* also publishes a CPD-accredited questionnaire relating to the academic content of the journal. Successful completion of the questionnaire with a pass rate of 70% will earn the reader 3 CEUs. Administration of our CPD

programme is managed by Medical Practice Consulting. To complete questionnaires and obtain certificates, please visit MRP Consulting

Manuscript preparation

Preparing an article for anonymous review

To ensure a fair and unbiased review process, all submissions are to include an anonymised version of the manuscript. The exceptions to this requirement are Editorials, Correspondence, Book reviews and Obituary submissions.

Submitting a manuscript that needs additional blinding can slow down your review process, so please be sure to follow these simple guidelines as much as possible:

- An anonymous version should not contain any author, affiliation or particular institutional details that will enable identification.
- Please remove title page, acknowledgements, contact details, funding grants to a named person, and any running headers of author names.
- Mask self-citations by referring to your own work in third person.

General article format/layout

Submitted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction prior to being sent for review, which will delay publication.

General:

- Manuscripts must be written in UK English (this includes spelling).
- The manuscript must be in Microsoft Word or RTF document format. Text must be 1.5 line spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes). Pages and lines should be numbered consecutively.
- Please make your article concise, even if it is below the word limit.
- Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.
- Please be sure to insert proper symbols e.g. µ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.

If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

SAJCH is a Journal on child health, therefore for articles involving genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.
- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.

** NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'
- Use the latest approved gene or protein symbol as appropriate:

- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

Preparation notes by article type

Research

Guideline word limit: 3 000 words (excluding abstract and bibliography)

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Where appropriate, sample size calculations should be included to demonstrate that the study is not underpowered. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

- May include up to 3 illustrations or tables.
- A max of 20 - 25references

Structured abstract

- This should be no more than 250words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.

- **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
- **Conclusion:** must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors. It should be able to be intelligible to the reader without referral to the main body of the article.
- Do not include any references in the abstracts.

***Scientific letters*/short reports**

These include case reports, side effects of drugs and brief or negative research findings.

Guideline word limit: 1500 words

- Abstract: unstructured, of about 100-150 words
- May include only one illustration or table
- A maximum of 6 references

Editorials

Guideline word limit: 1 000 words

These opinion or comment articles are usually commissioned but we are happy to consider and peer review unsolicited editorials. Editorials should be accessible and interesting to readers without specialist knowledge of the subject under discussion and should have an element of topicality (why is a comment on this issue relevant now?) There should be a clear message to the piece, supported by evidence.

Please make clear the type of evidence that supports each key statement, e.g.:

- expert opinion
- personal clinical experience
- observational studies
- trials
- systematic reviews.

Review articles

Review articlesshould always be discussed with the Editor prior to submission.

Guideline word limit: 4 000 words

These are welcome, but should be either commissioned or discussed with the Editor before submission. A review article should provide a clear, up-to-date account of the topic and be aimed at non-specialist hospital doctors and general practitioners. They should be aligned to practice in South and/or sub-Saharan Africa and not a precis of reviews published in the international literature

Please ensure that your article includes:

- Abstract: unstructured, of about 100-150 words, explaining the review and why it is important
- Methods: Outline the sources and selection methods, including search strategy and keywords used for identifying references from online bibliographic databases. Discuss the quality of evidence.
- When writing: clarify the evidence you used for key statements and the strength of the evidence. Do not present statements or opinions without such evidence, or if you

have to, say that there is little or no evidence and that this is opinion. Avoid specialist jargon and abbreviations, and provide advice specific to southern Africa.
- Personal details: Please supply your qualifications, position and affiliations and MP number (used for CPD points); address, telephone number and fax number, and your e-mail address; and a short personal profile (50 words) and a few words about your current fields of interest.

Correspondence (Letters to the Editor)

Guideline word limit: 400 words

Letters to the editor should relate either to a paper or article published by the SAJCH or to a topical issue of particular relevance to the journal's readership

- May include only one illustration or table
- Must include a correspondence address.

Obituaries

Guideline word limit: 400 words

Should be offered within the first year of the practitioner's death, and may be accompanied by a photograph.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide evidence of consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.
- Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
- All illustrations (graphs, diagrams, charts, etc.) must be in PDF form.
- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
- Scans/photos showing a specific feature e.g. *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author.
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) consecutively as they are referred to in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings, and include units where necessary.

- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:

Each row of data must have its own proper row:

Do not: use separate columns for *n* and %:

Rather:

Combine into one column, *n* (%):

Do not: have overlapping categories, e.g.:

Rather:

Use <> symbols or numbers that don't overlap:

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. If reference manager software is used, the reference list and citations in text are to be unformatted to plain text before submitting..*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:
  - On the Crossref homepage, paste the article title into the 'Metadata search' box.
  - Look for the correct, matching article in the list of results.
  - Click Actions > Cite
  - Alongside 'url =' copy the URL between { }.
  - Provide as follows, e.g.: https://doi.org/10.7196/07294.937.98x

Some examples:

- *Journal references:* Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. http://dx/doi.org/10.1000/hgjr.182
- *Book references:* Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.
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  Rex v Jopp and Another: Name of the parties concerned

  1949: Date of decision (or when the case was heard)

  (4): Volume number

  SA: SA Law Reports

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  (N): In this case Natal - where the case was heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.

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Appendix 2:

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CLEARANCE CERTIFICATE NO. M190317

NAME: (Principal Investigator)	Dr Katherine Flack
DEPARTMENT:	Paediatrics Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	A review of spinal muscular atrophy in Black South African paediatric patients
DATE CONSIDERED:	29/03/2019
DECISION:	Approved Unconditionally
CONDITIONS:	
SUPERVISOR:	Prof G Scher and Dr Marc Hauptfleisch
APPROVED BY:	CB Penny Doctor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	05/04/2019

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

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Appendix 3

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Word count: 3748
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Project Title:

A review of Spinal Muscular Atrophy (SMA) in Black South African paediatric patients.

Investigator:

Name: Dr. Katherine Flack

BScEngSci BME (Wits), MBBCh (Wits), DCH (SA), Dip HIVMan (SA)

Student number: 0512395N

Degree: MMed (Paediatrics)

Supervisors:

Dr. MPK Hauptfleisch (Paediatric Neurologist CHBAH and University of the Witwatersrand)

Prof. LG Scher (Paediatric Neurologist CMJAH and University of the Witwatersrand)

Declaration

This is a declaration that the submitted work is my own and materials consulted have been appropriately referenced. This work has been submitted for evaluation for the degree of Masters in medicine Paediatrics at the University of the Witwatersrand and has not been submitted at any other university.

Signed:

_____day of_____20_____in_____

Acknowledgements:

A special thank you to my mother Dr. P.S. Flack for her patience and words of encouragement. Thank you to the rest of my family for their unwavering support despite the physical distance between us.

Thank you to both of my supervisors Dr. M Hauptfleisch and Prof G Scher for their support and patience while navigating a global pandemic

Table of contents

Declaration 22

Acknowledgements: 33

List of Abbreviations 55

List of tables 55

Abstract 66

Introduction 77

Methods 99

Results 1010

Table 1: Age of onset of symptoms based on history and age patient presented ... 1111

Table 2 :Clinical features of SMA and distribution across SMA types..... 1111

Table 3: Muscle biopsy and or genetics results available for patients seen with SMA at CHBAH from 1988-2018..... 1212

Discussion 1313

Conclusion 1515

References 1616

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List of Abbreviations

SMA- Spinal Muscular Atrophy

CHBAH- Chris Hani Baragwanath Academic Hospital

CMJAH – Charlotte Maxeke Johannesburg Academic Hospital

NHLS- National Health Laboratory Service

SMN1- Survival of Motor Neuron 1- telemetric gene that encodes functional full size SMN protein

SMN2- Survival of Motor Neuron 2- centromeric gene that encodes a partially functional SMN protein

PCR- Polymerase chain reaction

DNA- Deoxyribonucleic acid

MLPA- Multiplex ligation-dependent probe amplification

List of tables

Table 1: Age of onset of symptoms based on history and age patient presented

Table 2: Clinical features of SMA and distribution across SMA types

Table 3: Muscle biopsy and or genetics results available for patients seen with SMA at CHBAH from 1988-2018

Submissable Paper:

Abstract

Background: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder that results in muscle weakness due to anterior horn cell degeneration. SMA is present in all populations and is divided into three clinical subtypes. SMA is an important genetic cause of morbidity and mortality and has not been well studied in sub- Saharan Africa.

Objective: The aim of this study is to describe the clinical features and genetic findings in Black South African patients with SMA presenting to the Division of Paediatric Neurology at Chris Hani Baragwanath Academic Hospital (CHBAH) over a 30-year period.

Method: This study was a retrospective review of patient records. The study population was all the Black paediatric neurology patients with clinical SMA, who attended CHBAH Neurology clinic between 1988 and 2018. Patients were categorized into SMA type 1, 2 or 3 based on the neurology assessment and clinical features were recorded.

Results: The clinical findings in the Black South African patients with SMA were similar to those found in international studies. There were 132 Black South African patients seen over a 30-year period at CHBAH, 86 of which had genetic testing. 84.8% of the genetic results for these patients were positive, which is significantly higher than what had previously been reported. 24.2% of patients had facial involvement.

Conclusions: This study adds to the limited body of research on SMA in sub-Saharan Africa and highlights the lower frequency of a homozygous deletion seen in the black South African population.

Word count: 245

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder which results in progressive and symmetrical muscle weakness due to irreversible anterior horn cell degeneration(1). The incidence of SMA in the United States is estimated at 1 in 6000 to 1 in 11000 live births with a carrier frequency estimated at 1:38(2) thus making it the second most common neuromuscular disease after Duchenne muscular dystrophy(3). SMA is present in all populations but Verhaart et al suggested a slightly lower incidence in patients of sub Saharan ancestry of 1 in 18808(4). However a South African study suggested that SMA is more common than previously thought with an incidence of 1 in 3574 in the Black population and 1 in 1945 in the White population(3).

SMA is divided into three clinical subtypes that represent a spectrum of clinical severity.

Type 1 SMA, usually presents before 6 months of age and patients never sit unsupported (5). It is the most common type, affecting up to 50% of all SMA patients. As a result of the swallowing difficulty patients often fail to thrive. Type 1 SMA patients usually develop respiratory failure within the first 2 years of life and less than 65% survive beyond this age (3). Outcome is dependent on respiratory involvement.

Type 2 SMA or “sitters” achieve the gross motor milestone of sitting unsupported but are unable to stand or walk. The age of onset is usually between 6 and 12 months. This intermediate form of SMA is generally slower to progress with a variable prognosis (5). Most children with type 2 SMA survive beyond the age of 5 years and survival thereafter is dependent on the development of complications involving the respiratory system (3).

Type 3 SMA describes a group clinically defined as “walkers”. The onset of observable symptoms in this group usually starts after age 12 to 18 months. This is considered a mild chronic form of SMA with little effect on patient lifespan. These patients are able to walk but over time develop proximal weakness with the lower

limbs being more affected than the upper limbs(6). These patients have a normal life expectancy.

SMA is a leading genetic cause of infant mortality(7) and has autosomal recessive inheritance caused by a homozygous deletion or mutation of the survival motor neuron (*SMN1*) gene on chromosome 5q13 in 95% of patients worldwide(3). However, there have been studies in South Africa that propose a possible different genetic cause with only 51% of South African Black patients having the homozygous deletion of the *SMN1* gene. One such study was conducted at CHBAH(3) Another study done in the Western Cape concluded that the genetic findings in their patients were in keeping with those that are internationally expected. This group included only 12 black patients and was therefore much smaller than the CHBAH study sample size(8).

The most common form of SMA is autosomal recessive SMA but X linked and autosomal dominant cases have been described(9). The genetic defect was identified in 1995 and a wide range of clinical severity was noted (10). It was discovered that 2 forms of the SMN gene exist, *SMN1* and *SMN2*. The *SMN2* gene is identical to the *SMN1* gene except for a single nucleotide change at exon 7. The resulting protein produced by transcription at *SMN2* is shorter, less functional and degrades faster than the protein produced at *SMN1*. Only about 10% of the protein produced by the *SMN 2* gene is fully functional. As patients with SMA have deletion of *SMN1* they rely on the SMN protein produced at *SMN2* for survival. A correlation between disease severity and *SMN2* copy number has been found, in that a patient with more *SMN2* copies has a less severe clinical phenotype of SMA(11).

The aim of this study is to describe the clinical features and genetic findings in Black South African patients with spinal muscular atrophy presenting to the Division of Paediatric Neurology at Chris Hani Baragwanath Academic Hospital over a 30-year period and compare results to two previous studies from this site as well as other South African publications. The focus of the study was on Black South African patients as previous studies suggested there could be a different genetic basis for SMA in this group (3). There are no studies with a comparable size available and there is an overall paucity of data from sub –Saharan Africa. We know that other

diseases have been found to have different disease manifestations as a result of a different mutation than that seen in the caucasian population, for example cystic fibrosis, galactosaemia and Fanconis anaemia. (3)(10) This therefore provides the rationale for the study.

Methods

Chris Hani Baragwanath Academic Hospital is the only large public hospital in Soweto, Johanneseburg. The paediatric neurology clinic receives referrals not only from the surrounding area but also from the rest of the province of Gauteng as well as other provinces and southern African countries where specialist expertise is lacking.

This study was a retrospective review of the patient records captured by the paediatric neurology team at CHBAH during the period 1988-2018.All the files of patients with a diagnosis of SMA were reviewed.

The study population was all black paediatric neurology patients with: clinical, genetic and / or histological diagnosis of SMA who attended CHBAH Neurology clinic from 1988-2018.

All patients presenting to the Division of Paediatric Neurology at CHBAH with clinical features in keeping with the diagnosis of SMA type 1, 2 or 3 as determined by the paediatric neurologists were included. This group also included all patients presenting with a confirmed genetic result or with a confirmed muscle biopsy result. Genetic testing for the homozygous *SMN1* deletion became available at the National Health Laboratory Service in 1996. Patients who presented before this time had muscle biopsy done. A small number of these biopsy patients later had genetic testing done as part of a study. MLPA testing is not done as a routine diagnostic study for SMA but has also been used in research projects to assess for heterozygous deletions and *SMN* copy numbers.

Patients attending paediatric neurology clinic where the diagnosis of SMA was in doubt were excluded.

Data was entered into a confidential and secure Microsoft excel spreadsheet. Each patient file was reviewed and multiple variables were entered for each patient. The age at first presentation to neurology clinic was captured. Age of onset based on history was categorized as either less than 6 months, 6 to 18 months or greater than 18 months. SMA type at diagnosis either type 1, 2 or 3 was determined from the initial neurologist assessment. Clinical features present at the first assessment were captured. Files were reviewed further for possible genetic or muscle biopsy results

The resulting spreadsheet was exported into STATA for analysis. Comparative tables were generated and p values determined using either Fischer's exact test or the Chi square test.

Ethics approval

This study was approved unconditionally by the Human Research Ethics Committee of the University of the Witwatersrand. Certificate number M190317.

Results

A total number of 145 patients classified as SMA were seen at CHBAH from1988-2018. The focus of this study was SMA in Black South African patients in particular and therefore 13 patients of White, Asian or mixed race ancestries were excluded. The total number of Black SMA patient files at CHBAH that were then analyzed was 132

The patient files contained information on clinical findings at initial presentation to neurology clinic and biopsy or genetic results. Only 45 patient files also contained information on progression of clinical features and therefore for the purposes of this study only clinical features at the first visit were reviewed and compared.

The median age of presentation of all SMA patients to CHBAH paediatric neurology clinic was 15 months (interquartile range: 6-34). Type 1 SMA patients median age at presentation was age 7 months (4-11), Type 2 SMA by age 24 months (16-32) and type 3 by 56 months (36- 72).

Table 1: Age of onset of symptoms based on history and age patient presented

SMA type	Age <6m	6-18m	>18m	Total
1	59 (95.1%)	11 (15.7%)	0 (0)	70 (53.0%)
2	3 (4.8%)	28 (82.3%)	3 (11.5%)	34 (25.7%)
3	0 (0 %)	5 (17.8%)	23 (88.5%)	28 (21.2%)
total	62	44	26	132

Paediatric neurologists working at CHBAH made the diagnosis of SMA clinically and later confirmed with muscle biopsy (prior to the availability of genetic testing) or genetic testing. SMA affected males and females equally with 50.7% males and 49.2% females affected in our study group with no statistically significant difference between the two genders. Most of the SMA type 1 (95.1 %) patients gave a history of symptoms starting before age 6 months. Similarly the majority of SMA type 2 (82.3%) patients had onset of symptoms between age 6 and 18 months. Most of the Type 3 SMA (88.5%) patients developed symptoms after the age of 18 months.

Table 2: Clinical features of SMA and distribution across SMA types

SMA	Total (n=132)	Type 1 (n=70)	Type 2 (n=34)	Type 3 (n=28)	P value
Male	67 (50.7%)	35 (50%)	16 (47%)	16 (57.1 %)	0.719
Female	65 (49.2%)	35 (50%)	18 (52.9%)	12 (42.8 %)	
Hypotonia	129 (97.7%)	70 (100%)	34 (100%)	25 (89.2 %)	0.009
Tongue fasciculations	105 (79.5%)	64 (91.4%)	30 (88.2%)	11 (39.2%)	0.000
Areflexia	110 (83.3%)	68 (97.1%)	30 (88.2%)	12 (42.8%)	0.000
Polyminimyoclonus	57 (43.1%)	15 (21.4%)	23 (67.6%)	19 (67.8%)	0.000
Facial involvement	32 (24.2%)	22 (31.4%)	6 (17.6%)	4 (14.2%)	0.153
Respiratory compromise	50 (37.8%)	36 (51.4%)	10 (29.4%)	4 (14.2%)	0.001
Contractures	22 (16.6%)	9 (12.8%)	9 (26.4%)	4 (14.2%)	0.263
Scoliosis	9 (6.81%)	3 (4.3%)	4 (11.7%)	2 (7.1%)	0.336

Table 2 shows the presenting clinical features and gender across all SMA types.

The clinical features present in our study population all suggest SMA as the most likely diagnosis. The primary clinical features documented were hypotonia (97% of patients), absent or diminished reflexes (83%), and tongue fasciculation (79%).

Hypotonia was present in 100% of the type 1 and type 2 patients and 89% of the type 3 patients. Absent reflexes were found in 83.3% of patients and in all patients with absent lower limb reflexes, the percentage is closer to 98%. Twenty-two patients had preserved upper limb reflexes with only two patients having all reflexes present at the time of presentation. Both of these patients were type 3 SMA, with one having a homozygous deletion of SMN 1 and the other having a negative genetic result but positive biopsy result. More than 90 % of type 1 SMA patients had fasciculation of the tongue whilst fasciculation were only present in 39% of type 3 SMA patients.

Closer review of our data showed 32 (24.2%) of 132 patients with facial involvement. Of these 11 (34.3%) had a positive genetic result and 16 (50%) had a positive muscle biopsy confirming the diagnosis of SMA.

Some patients had already developed complications of the disease at the time of presentation and contractures, scoliosis and respiratory compromise were also reviewed. 37% of the patients had respiratory compromise at presentation. Scoliosis and contractures were the least common findings.

Table 3: Muscle biopsy and or genetics results available for patients seen with SMA at CHBAH from 1988-2018

SMA type	Total positive results	Type 1	Type 2	Type 3	P value
Muscle Biopsy N=49	48 (97.9%)	20 (40.8%)	15 (30.6%)	13 (26.5%)	0.54
Genetics N=86	73 (84.8%)	40 (46.5%)	17 (66.2%)	16 (18.6%)	0.46

Table 3 shows the available muscle biopsy and genetic results. Most of the patients in the study population had either a muscle biopsy or genetic testing done over the period of 1988- 2018. At total of 49 muscle biopsies were done 97% of which were positive. The only patient with a negative biopsy showed non-specific histological changes but had a confirmed homozygous deletion of the *SMN1* gene. Of 86 patients who had genetic testing done 84.8% had a positive result. Two of the genetic positive patients were heterozygous positive i.e. only a single deletion of *SMN1* and a presumed mutation not detected by traditional genetic testing. Both of these patients had positive muscle biopsies.

A total of 17 patients had both genetic testing done and a muscle biopsy. Of these only 5 had a biopsy sent after genetic testing was done. Two of these were heterozygous deletions, two had no deletions and one result was inconclusive. All five of the biopsies done on these patients were positive for SMA.

Only 5/132 patients had neither a biopsy nor genetic testing done. Four of these patients had planned biopsies but were lost to follow up and one patient family refused biopsy. All five of these patients were seen before genetic testing was available.

Discussion

2 Spinal muscular atrophy is an important genetic cause of morbidity and mortality and has not been well studied in sub Saharan Africa. (13) In this study males and females were equally affected and the median age of presentation across all types of SMA was 15 months (6-34 months IQR). SMA type 1 is the commonest form (53% of the SMA patients) with patients typically presenting by 7 months of age and onset of symptoms before age 6 months in 84%. The results are similar to previously published international data (5). Although the majority of type 2 SMA patients had onset of symptoms between ages 6-18 months most only presented at around 24 months of age. In the South African public health care system delay in presentation is a common occurrence due to multiple factors such as access to health care, cultural beliefs and level of patient education.

This study focused on a single racial group as previous studies from South Africa suggested that there might be a different genetic basis for SMA in black South Africans(3). The main finding was that 73/86 (84.8%) of the genetic results for patients were positive with the majority being homozygous deletion positive (82.5%). This result is significantly higher than what had previously been reported in a study at CHBAH where only 51% of the patients had the expected *SMN1* deletion(3). This result may be attributed to more widespread access to genetic testing over the last two decades.

The result of this study also differs from what has been reported at Red Cross War Memorial Children's Hospital in Cape Town (10). This study features a large sample size of 132 Black patients, compared to 12 Black patients in the Cape Town study. The study performed in Cape Town strictly excluded all patients with facial involvement (10) but our study results suggest that in doing so up to 1 in 4 patients with confirmed SMA could potentially be missed.

Prior to access to genetic testing, the mainstay of diagnosis was muscle biopsy. 97% of patients with available muscle biopsy results confirmed the diagnosis of SMA highlighting a strong correlation with clinical diagnosis. In patients where there is a strong clinical suspicion of SMA despite a negative genetic test and inconclusive muscle biopsy, MLPA is available as an option to look for alternate diagnoses.

A recent publication showed that MLPA is not a suitable test to determine carrier status in Black South Africans as up to 50 % have multiple *SMN1* copy numbers. This has the potential to mask a heterozygous deletion and will not pick up a carrier state in these cases.(14) The same study reported only 8.3% of the clinically suggestive patients had a heterozygous deletion which is lower than previously reported but higher than what was found in our study (14) (3)

As genetic testing evolves and gene-based therapies become available, a further understanding of the genetic basis for disease in Black South Africans is needed.

Clinical diagnosis is the first step and early intervention as team involvement including paediatric neurologists and rehabilitation specialists may improve quality of life for these patients

Study limitations

A limitation of this study is that it is retrospective and requires reviewing medical records compiled by multiple different clinicians. The study is further limited as it was carried out at a single center study with a single ethnic group. Review of results at other sites with comparable patient volumes may be warranted.

Conclusion

This study adds to the limited body of research on SMA in sub- Saharan Africa. The main finding from this study, that 84.8% of the genetic results for patients were positive, is significantly higher than what had previously been reported. This review also showed that the clinical presentation of this population group is comparable to what has been internationally reported. Facial involvement is an important clinical sign noted in up to 25% of patients. The focus on a single racial group is justified as previous studies from South Africa suggested that there might be a different genetic basis for SMA in Black South Africans.

Word count: 2802

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