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Dravet syndrome in South African infants: Tools for an early diagnosis

Alina I. Esterhuizen[a,b,*], Heather C. Mefford[c], Rajkumar S. Ramesar[a,b], Shuyu Wang[d], Gemma L. Carvill[e], Jo M. Wilmshurst[f,g]

[a] Division of Human Genetics, Institute of Infectious Diseases and Molecular Medicine, Department of Pathology, University of Cape Town, Cape Town, South Africa
[b] National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa
[c] Department of Pediatrics, Division of Genetic Medicine, University of Washington, Seattle, WA, USA
[d] Department of General Medicine, Alfred Health, Victoria, Australia
[e] Ken and Ruth Davee Department of Neurology, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA
[f] Paediatric Neurology and Neurophysiology, Red Cross Children's War Memorial Hospital, Cape Town, South Africa
[g] School of Child and Adolescent Health, University of Cape Town, South Africa

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ABSTRACT

Purpose: Dravet syndrome (DS) is a well-described, severe genetic epileptic encephalopathy with an increased risk of SUDEP. The incidence and genetic architecture of DS in African patients is virtually unknown, largely due to lack of awareness and unavailability of genetic testing. The clinical benefits of the available precision medicine approaches to treatment emphasise the importance of an early, correct diagnosis. We investigated the genetic causes and clinical features of DS in South African children to develop protocols for early, cost-effective diagnosis in the local setting.

Method: We selected 22 South African children provisionally diagnosed with clinical DS for targeted re-sequencing of DS-associated genes. We sought to identify the clinical features most strongly associated with *SCN1A*-related DS, using the DS risk score and clinical co-variates under various statistical models.

Results: Disease-causing variants were identified in 10 of the 22 children: nine *SCN1A* and one *PCDH19*. Moreover, we showed that seizure onset before 6 months of age and a clinical DS risk score of > 6 are highly predictive of *SCN1A*-associated DS. Clinical reassessment resulted in a revised diagnosis in 10 of the 12 variant-negative children.

Conclusion: This first genetic study of DS in Africa confirms that *de novo SCN1A* variants underlie disease in the majority of South African patients. Affirming the predictive value of seizure onset before 6 months of age and a clinical DS risk score of > 6 has significant practical implications for the resource-limited setting, presenting simple diagnostic criteria which can facilitate early correct treatment, specialist consultation and genetic testing.

1. Introduction

Dravet syndrome (DS) (OMIM 607208), previously described as severe myoclonic epilepsy of infancy (SMEI) is a severe genetic epilepsy with associated encephalopathy [1]. Early clinical presentation of DS is characterised by the onset of prolonged, febrile and afebrile generalized clonic or hemiclonic seizures in an otherwise normally developing infant. Seizures are usually resistant to typically prescribed anti-epileptic drugs (AEDs) and evolve with the disease progression to include myoclonic, atypical absences and focal seizures. After this initial phase, the clinical presentation becomes less distinctive and the opportunity to recognize the condition early may be missed. Life-threatening episodes of status epilepticus (SE), seizure-related accidents and sudden unexpected death in epilepsy (SUDEP), all contribute towards a significantly increased premature mortality among individuals with DS [2,3]. An important reason for early recognition of DS, is the contraindication of treatment with sodium channel inhibitors (e.g. carbamazepine, oxcarbazepine, lamotrigine), as this may worsen the condition [4,5]. Other contraindications include chronic use of benzodiazepine (BZ), which may facilitate encephalopathy and resistance to BZ administered for status epilepticus (SE) [6].

DS progresses in three stages: the first diagnostic "febrile stage" is marked by frequent, prolonged febrile seizures in the first year of life; the second "worsening stage" occurs between the ages of 1 and 5 years with frequent seizures and episodes of status epilepticus, behavioral deterioration and neurological signs, followed by the third

* Corresponding author at: National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa.
E-mail addresses: alina.esterhuizen@uct.ac.za (A.I. Esterhuizen), hmefford@uw.edu (H.C. Mefford), raj.ramesar@uct.ac.za (R.S. Ramesar), sh.wang@alfred.org.au (S. Wang), gemma.carvill@northwestern.edu (G.L. Carvill), jo.wilmshurst@uct.ac.za (J.M. Wilmshurst).

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"stabilization stage" characterized by a decrease in convulsive seizures which occur mainly during sleep. During this last stage, seizures continue to impact on the child's quality of life, though myoclonic and absence seizures may disappear. Neurological development may improve but a variable degree of cognitive impairment persists, often with challenging behavioural issues. Ataxia and gait problems become a major concern [1]. At present, the realistic objective of treatment is cessation of prolonged seizures, reduced seizure frequency and cognitive and motor sequelea [6]. The degree of success however, is wholly dependent on early correct diagnosis and appropriate intervention.

Over 80% of DS cases are associated with *de novo* variants in the *SCN1A* gene (OMIM 182389*),* which encodes the alpha subunit of the sodium ion channel [1]. The majority of the remaining DS patients do not carry currently identifiable variants, though some children harbour pathogenic variants in other ion and non-ion channel genes [7]. This relative genetic homogeneity holds DS apart from most other EEs, which are highly genetically heterogeneous [8,9]. Careful clinical correlation is important, as *SCN1A* variants are also found in other severe epilepsies (e.g. epilepsy of infancy with migrating focal seizures (EIMFS) [10], as well as less severe epilepsy phenotypes such as familial febrile seizures (FS) [11], or genetic epilepsy with febrile seizures plus (GEFS+) [12]. The factors predicting long-term developmental outcome remain unclear but an early diagnosis and seizure control may delay or prevent the onset of EE and mitigate the outcomes [5,13].

The incidence of DS in high income countries (HICs) is estimated to range between 1 in 15 700 and 1 in 40 900 live births [1]. At present, the incidence of DS in Africa is unknown, due to virtual absence of genetic testing or epilepsy research. However, given that most *SCN1A* variants arise *de novo*, we expect the incidence of DS in Africa to reflect that of international studies. Whilst Africa, particularly sub-Saharan Africa (SSA), bears the highest burden of epilepsy in the world [14], genetic epilepsy is among the most underdiagnosed and under-investigated disorders on the continent. In a setting where seizures are frequently a result of endemic parasitic disease, central nervous system (CNS) infections, traumatic brain injury or perinatal insults, a diagnosis of a genetic epilepsy is rarely considered. Acute symptomatic seizures and febrile seizures are frequently assumed to be due to malaria, limiting the search for other causes [15]. Lack of awareness, limited specialist expertise, suboptimal health infrastructure and unavailability of diagnostic testing all contribute towards this void in knowledge and medical care. The extensive evidence of the genetic contribution to many epilepsy phenotypes, and the clinical utility of testing, especially relevant to early-life epilepsies, has informed the diagnostic laboratory protocols of many HICs. [16–18]. It is important to ensure that African patients also benefit from this knowledge and that new knowledge is gained through research on the highly genetically diverse populations of Africa [19].

In this study, we collected clinical and clinico-electrical information and performed genetic testing on a cohort of 22 South African infants diagnosed with Dravet or Dravet-like syndromes. Our main aim was characterization of the genetic landscape of DS in South Africa (SA) for the purpose of drawing up clinical and molecular diagnostic protocols for early, cost-effective diagnosis of DS in the local setting, including retrospective cascade counselling and patient follow-up. To our knowledge, this is the first report correlating phenotypic and genetic aspects of DS in Africa.

2. Methodology

2.1. Cohort recruitment

Infants with provisional clinical diagnoses of DS were recruited over a period of six months by clinicians affiliated to or working in the Paediatric Neurology service at the Red Cross Children's War Memorial Hospital (RCCWMH) in Cape Town. The patients were referred to the Epilepsy Clinic at the RCCWMH, which is the only specialist paediatric epilepsy clinic in sub-Saharan Africa. The cohort comprised of 22 unrelated infants (12 males and 10 females) of European (n = 5); Indigenous Black African (n = 10); and Mixed (n = 7) ancestries, as per parental self-classification. Inclusion was based on a history of infantile-onset (before two years of age), recurrent complex febrile seizures (FS) with prior normal development [20]. Structural or metabolic causes were previously excluded. The inclusion criteria were purposefully broad and simple to enhance recruitment. Peripheral blood was drawn from the infants and parents, after obtaining parental written consent. The study was approved by the Human Research Ethics Committee of the University of Cape Town (HREC REF: 232/2015).

2.2. Genetic analysis

Genomic DNA was isolated from peripheral blood (2–5 ml) of the probands and parents (where available). DNA isolation and the integrity checks were performed using standard methods (NanoDrop™1000 and, Qubit® dsDNA HS (High Sensitivity) Assay, ThermoFisher Scientific, USA).

The cohort was initially tested locally (Division of Human Genetics, University of Cape Town (UCT)), where the Ion Torrent™ PGM platform (ThermoFisher Scientific) was used to resequence six genes previously reported to carry pathogenic variants in children with DS (*SCN1A, GABRA1, GABRG2, STXBP1, HCN1* and *PCDH19*) (refs). The AmpliSeq™ Designer Software v4.47 (ThermoFisher Scientific, USA) was used to design two pools of primers for a total of 161 amplicons, predicted to capture 9908% of all the coding exons (with 100% capture for the *SCN1A* gene specifically), each flanked by ten bases of intronic sequence (RefSeq. hg19 build), to be sequenced at a minimum 100x depth of coverage. The NGS library was prepared on the Chef DL8 using the Ion AmpliSeq™ Kit (ThermoFisher Scientific, USA) according to the manufacturer's protocol. Basic NGS quality assessment, read alignment, variant identification, annotation, prioritisation, and filtering was performed by the Ion Reporter™ cloud-based software (ThermoFisher Scientific, USA). The VCF files were then used for further manual variant filtering and prioritisation. Only nonsynonymous, splice-site and frameshift variants not found in the ExAC v0.3, ESP6500 or 1000 Genomes databases were assessed further [21–23]. All putative variants were confirmed by Sanger sequencing. Segregation analysis was done in all cases where parental samples were available. All variant-negative samples were tested with the multiple ligase-dependent probe amplification (MLPA) assay for exonic deletions/duplications in the *SCN1A* gene (P137-B2 probe mix, MRC-Holland).

To validate our findings on the Ion Torrent™PGM system, the DS cohort was re-tested on the Illumina HiSeq™ platform at the University of Washington (Seattle, USA), as part of a larger project investigating the genetic causes of EEs in South African patients (ongoing). The single molecule Molecular Inversion Probe (smMIP) technology was employed as previously described [24] to capture all exons and intron-exon boundaries (5-bp flanking sequences) of the target genes at capture, including *SCN1A, GABRA1, GABRG2, STXBP1, HCN1* and *PCDH19* (RefSeq, hg19 build) [25]. Sequencing was performed at 98% capture and 40X minimum depth of coverage. NGS quality assessment, read alignment, depth of coverage, variant identification, annotation, prioritisation, and filtering was also performed using previously published methods [25–27]. The VCF files were then subject to further manual variant filtering and prioritisation.

2.3. Pathogenicity assessment of variants

Only nonsynonymous, splice-site and frameshift changes were considered for pathogenicity assessments (Table 2). Variants were classified according to the interpretation guidelines from the American College of Medical Genetics and Genomics–Association for Molecular Pathology (ACMG–AMP) [28]. Briefly, a variant was classified as likely/pathogenic if it arose *de novo* (or from a somatic mosaic parent)

and was not found in the publically available control datasets (ExAC v0.31, ESP6500, 5000 Genomes, gnomAD r2.0.2) [21–23]. In cases where DNA from both parents was unavailable for segregation analysis, likely/pathogenicity was inferred on the basis of (1) the variant type (truncations and splice variants were seen as likely pathogenic), (2) recurrence (previously recorded as disease-causing in the literature or disease databases (3) analysis with *in silico* pathogenicity prediction tools (CADD, PolyPhen-2, and GERP), where all outputs had to be in agreement (CADD > 25, PolyPhen-2 > 0.9, and GERP > 5). Microsatellite analysis (Authentifiler™ PCR Amplification kit, ThermoFisher Scientific) was performed on of all parents of probands with a *de novo* variants to confirm parentage.

2.4. Clinical data assessment

Clinical demographics, seizure semiology, seizure evolution and treatment history were collected both prospectively and retrospectively by clinical assessment, parent/guardian interview and review of patient records (Table 3). A clinical risk score for progression to DS after an initial complex febrile seizure described by Hattori et al., was determined for each patient [29]. The score takes into account the age at seizure onset, total number of seizures before one year of age, total number of prolonged seizures (longer than 10 min), and the seizure type and trigger (Table 1). The clinical score was then compared to the clinician's level of confidence in the diagnosis of DS: definitely compatible with DS or possible DS. It was also correlated with the presence/ absence of an *SCN1A* variant.

2.5. Statistical analysis

Statistical comparison of the clinical demographics, seizure semiology, seizure evolution and treatment history was made between the group of patients with *SCN1A* variants and the group with no identified variants (Table 3), using *R* [30]. This was intended to highlight any possible statistically significant associations between specific clinical features, and the presence/ absence of an *SCN1A* variant. Fisher's exact test was used where there were two nominal variables. To permit nonparametric analysis of the two groups without assuming normal distribution of values the Mann-Whitney U test was used for other parameters.

3. Results

3.1. Genetic analysis

Pathogenic changes were found in 10 out of 22 patients: nine carried *SCN1A* variants (four missense, three frameshift and two nonsense) and one female carried a heterozygous nonsense variant in the *PCDH19 gene*. The specific coverage achieved for the coding region of the *SCN1A* gene (26 exons) was 100% capture and a > 100X depth of coverage on Ion Torrent and > 40X unique capture with smMIPs. *De novo* variants could be shown in only five patients, as DNA from both parents was not available in the remaining cases (Table 2).

Table 1
Predictive risk scoring for an early diagnosis of DS, proposed by Hattori et al. [29]. A total cumulative score of ≥6 strongly increases the risk of DS [29].

Predictive risk factors	Risk score
Age of febrile seizure onset ≤ 7 months	2
A total number of seizures ≥ 5	3
Prolonged seizures lasting > 10 min	3
Hemiconvulsions	3
Focal-onset seizures	1
Myoclonic seizures	1
Hot water–induced seizures	2

3.2. Statistical analysis

The key findings in the two main groups, namely, the *SCN1A*-positive group (n = 9) and the variant-negative group (n = 12), are summarised in Table 3. Median age at the time of the last clinic review was 24 months (range 19–51.75) for variant-negative patients and 75 months (range 25–103) for the *SCN1A* variant-positive patients. The analysis revealed a number of significant differences, the most notable of which were the DS clinical score and the age at seizure onset (AAO). The high DS clinical risk score among the *SCN1A*-positive group (median score 9.00, range 8.00–11.00) was consistent with the level of confidence in the diagnosis of DS among the *SCN1A*-positive patients (definitely DS in 6/9 (68%)), compared to the variant-negative patients (definitely DS in 2/12 (17%)). Age at first seizure was shown to be markedly younger in the *SCN1A*-positive group, with a median of four months (range 3–6) months, compared to 12 months (range 8.75–13.25) in the variant-negative group. The *SCN1A*-positive group were also more likely to have suffered prolonged febrile seizures (> 10 min) or febrile SE. Despite a range of seizure types described in the study cohort, significance was only found for myoclonic and focal seizures in the *SCN1A*-positve group.

Regarding interventions, the *SCN1A*-postitive group was more likely to receive a combination of AEDs (eight out of nine *SCN1A*-positive patients), whilst 11 out of 12 variant-negative children were managed effectively with monotherapy. No significant differences between the two groups were noted in the median number of AEDs trialed or the degree of seizure control achieved. Whilst there was no difference in the developmental function before seizure onset, developmental delay was more likely in the *SCN1A*-positive group after seizures onset. Similar findings were noted for subsequent speech, behaviour and features of the Autism Spectrum Disorder (ASD), based on neurodevelopmental assessments. The *SCN1A*-positive children were significantly more likely than the variant-negative group to require ancillary support and to be placed in special-needs schools. They were also better attendees to the Neurology service, with more frequent hospital visits related to the challenges of managing intractable seizures and the associated complications.

Long term follow-up enabled clinical reassessment of the variant-negative group with a revised diagnosis in ten patients: seven were re-diagnosed with febrile seizures plus (FS+) and one with early onset epileptic encephalopathy (EOEE). Perinatal insult and moyamoya disease were determined as the cause of seizures in the remaining two cases. It was also noted that out of 11 Indigenous Black African children included in our study (45% of the cohort), only one carried a *SCN1A* variant (LRG_8t1(SCN1A):c.5314 G > A, p.(Ala1772Thr), with the other *SCN1A* variants detected in children of European (four) and Mixed Ancestry (four). The diagnosis of DS was subsequently revised for eight of the nine variant-negative black patients (six FS+, one perinatal insult and one moyamoya disease). Also, closer scrutiny of the clinical demographics showed that the median clinical score among the variant-negative indigenous black African children was six (range 0–8), and the median age of onset was 12 months (range 3–17 months) (not included in Table 3).

4. Discussion

We have described the results of the first genetic study of DS in Africa. Despite the small cohort size, our findings carry significant implications for the diagnosis and management of children with DS in SA, and perhaps more broadly in Africa. Although the clinical features and genetic underpinnings of DS in our cohort were not novel, identification of nine patients carrying pathogenic or likely pathogenic *SCN1A* variants (41% of the cohort) and one female patient with a pathogenic *PCDH19* variant, confirmed that the genetic aetiology of DS

Table 2
Pathogenic variants identified by targeted NGS analysis of known Dravet syndrome genes.

#	Sex	GENE	cDNA level[a]	Protein level	Variant type	Detection method	Novel / known	Variant classification [28]	SIFT/ PolyPhen	GERP	Segrega-tion	Score [29]	AAO (mths)	EXaC MAF [22]
1	F	*SCN1A*	c.5314 G > A	Ala1772Thr	missense	Ion Torrent PGM and smMIPS Illumina	known rs121917980	Pathogenic	0/1	5,69	only maternal DNA available	10	2	none
2	F	*SCN1A*	c.3007delA	Ile1003fs	frameshift	Ion Torrent PGM and Sanger Sequencing[b]	*Novel*	Likely Pathogenic	–	–	no parental DNA	7	4	none
3	M	*SCN1A*	c.664C > T	Arg222Ter	nonsense	Ion Torrent PGM and smMIPS Illumina	known rs121918624	Pathogenic	0/1	5,77	*de novo*	15	5	none
4	M	*SCN1A*	del exons 5–8 (c.(602 + 1_603-1)_ (1170 + 1_1171-1)del)	Tyr202Hisfs*10	frameshift	smMIPS Illumina and MLPA[c]	*Novel*	Pathogenic	–	4,73	*de novo*	7	8	none
5	F	*SCN1A*	c.2552 G > C	Arg851Pro	missense	Ion Torrent PGM and smMIPS Illumina	*Novel* [d]	Likely Pathogenic	0/0.999	4,75	*de novo*	11	3	none
6	F	*SCN1A*	c.4016 T > G	Val1339Gly	missense	Ion Torrent PGM and smMIPS Illumina	known (HGMD, Meng et al., 2015)	Pathogenic	0/0.993	4,25	*de novo*	9	6	none
7	F	*SCN1A*	c.5236 G > T	Gly1746Trp	missense	Ion Torrent PGM and smMIPS Illumina	*novel*	Likely Pathogenic	0/1	5,69	no parental DNA	12	6	none
8	F	*SCN1A*	c.4352_4357 delACTTTG	Tyr1451fs	frameshift	Ion Torrent PGM and smMIPS Illumina	*novel*	Likely Pathogenic	–	5,3	no parental DNA	8	4	none
9	M	*SCN1A*	c.1129C > T	Arg377Ter	nonsense	Ion Torrent PGM and smMIPS Illumina	known rs794726799	Pathogenic	–	3,34	only maternal DNA available	9	2	none
10	F	*PCDH19*	c.2371C > T	Gln791Ter	nonsense	Ion Torrent PGM and smMIPS Illumina	*novel*	Likely Pathogenic	–	5,64	de novo	9	7	none

[a] *SCN1A* RefSeq NM_006920.4, LRG_8t1; *PCDH19* RefSeq NM_020766.2.
[b] failed on smMIPS.
[c] confirmed with MLPA.
[d] previously reported pathogenic variant in this position: p.Arg851Gln (rs121918785); AAO: age at seizure onset.

Table 3
Statistical comparison of the clinical demographics between the variant-negative and the *SCN1A* variant-positive patient groups.

Clinical and demographic features	No variant (N = 12)	*SCN1A* variant (N = 9)	p-value	test	Odds ratio (95% CI)
DS Risk Score (median, IQR)	**5.50 (3.75, 6.00)**	**9.00 (8.00, 11.00)**	**0,001**	**Mann-Whitney-Wilcoxon**	
Certainty of DS diagnosis					
Possibly	10	3	0,032	Fisher exact	0.12 (0.01,1.06)
Definitely	**2**	**6**	**0,032**	**Fisher exact**	**8.73(0.94,135.76)**
Sex	5F:7M	4F:5M	1,000	Fisher exact	1.11(0.14,8.46)
Age at onset (median, IQR in mths)	**12.00 (8.75, 13.25)**	**4.00 (3.00, 6.00)**	**0,003**	**Mann-Whitney-Wilcoxon**	
Age last seen (median, IQR in mths)	**24.50 (19.00, 51.75)**	**75.00 (25.00, 103.00)**	**0,036**	**Mann-Whitney-Wilcoxon**	
Seizure History					
No of prolonged (> 10 min)	**0.00 (0.00, 1.25)**	**4.00 (2.00, 5.00)**	**0,012**	**Mann-Whitney-Wilcoxon**	
Status epilepticus	**0.00 (0.00, 1.25)**	**2.00 (1.00, 5.00)**	**0,058**	**Mann-Whitney-Wilcoxon**	
Seizure type					
Hemiclonic	0	2	0,171	Fisher exact	_
Focal	**3**	**6**	**0,087**	**Fisher exact**	**5.44(0.66,60.59)**
Myoclonic	**3**	**7**	**0,030**	**Fisher exact**	**9.14(1.01,140.65)**
Atypical abs	3	0	0,229	Fisher exact	_
Typical abs	0	3	0,063	Fisher exact	_
Tonic	2	2	1,000	Fisher exact	1.40(0.08,23.88)
GTCS	10	7	1,000	Fisher exact	0.71(0.04,12.07)
Atonic	2	3	0,611	Fisher exact	2.39(0.21,36.66)
Seizure triggers					
Fever	12	9	1,000	Fisher exact	_
Hot water	**0**	**3**	**0,063**	**Fisher exact**	**_**
Light	0	3	0,063	Fisher exact	_
Family history	5	3	1,000	Fisher exact	0.71(0.08,5.73)
Peri or postnatal complications	2	1	1,000	Fisher exact	0.64(0.01,14.44)
Investigations (abnormal)					
Metabolic	0	0	1,000	Fisher exact	_
Neuroimaging	4	2	0,659	Fisher exact	0.59(0.04,5.70)
EEG	6	5	1,000	Fisher exact	1.24(0.16,9.89)
Antiepileptic drugs					
Monotherapy	11	1	0,0004	Fisher exact	0.02(0.0003,0.28)
2+ agents	**1**	**8**	**0,0004**	**Fisher exact**	**55.66(3.54,4052.99)**
KD	0	3	0,063	Fisher exact	_
Surgery	0	0	1,000	Fisher exact	_
Seizure control					
Controlled	4	1	0,338	Fisher exact	0.27(0.005,3.53)
Partial control	8	5	0,673	Fisher exact	0.64(0.8,5.23)
Poor control	0	3	0,063	Fisher exact	0.27(0.01,3.53)
Maximum trialed AEDs	2.00 (1.00, 2.25)	3.00 (2.00, 3.00)	0,083	Mann-Whitney-Wilcoxon	
Development (n = abnormal)					
Before seizure onset	1	1	1,000	Fisher exact	1.35(0.02,117.49)
Post seizure onset	**4**	**9**	**0,005**	**Fisher exact**	**_**
Specific concerns					
Gait	**2**	**5**	**0,159**	**Fisher exact**	**5.66(0.61,83.65)**
Speech	**3**	**7**	**0,030**	**Fisher exact**	**9.14(1.012,140.65)**
Sleep	0	3	0,063	Fisher exact	_
Behaviour	**3**	**7**	**0,030**	**Fisher exact**	**9.14(1.01,140.65)**
ASD	0	1	0,429	Fisher exact	_
Interventions					
Physiotherapy	**3**	**6**	**0,087**	**Fisher exact**	**5.44(0.66,60.6)**
Occupational therapy	**2**	**7**	**0,009**	**Fisher exact**	**14.46(1.44,259.45)**
Speech therapy	**2**	**6**	**0,032**	**Fisher exact**	**8.73(0.94,135.76)**
Median IQ/DQ					
Normal / Mild	7	2	0,184	Fisher exact	0.22(0.16,1.88)
Moderate/Severe	1	1	1,000	Fisher exact	1.35(0.02,117.49)
Special school	**2**	**5**	**0,159**	**Fisher exact**	**5.65(0.61,83.66)**
Ancestry					
European	0	4	0,021	Fisher exact	_
Indigenous Black	10	1	0,002	Fisher exact	0.03(0.001,0.43)
Mixed	2	4	0,331	Fisher exact	3.72(0.38,55.00)

Key: IQR = interquartile range; Peri or postnatal complications: premature, respiratory distress, intrauterine growth retardation, hypoxic ischaemic encephalitis (HIE), trauma; Neuroimaging = MRI (in all) and / or CT: very minor non-specific changes; atrophy, asymmetry, subtle white matter changes supporting HIE; KD = ketogenic diet: trialed or offered to caregiver but declined, where trialed results partially or minimally responsive; ASD = autistic spectrum disorder; Special school: either placed or likely to be needed in future (once old enough). Areas of significance emphasised in bold text.

in our region is similar to that in other international study cohorts. However, compared to the published studies, the proportion of *SCN1A*-positive DS in our cohort appeared low (41%), raising a concern about missed variants [5,6]. This proved unlikely, as the *SCN1A* coding region (26 exons) was covered at 100% capture and a good depth of coverage (> 100X on Ion Torrent and > 40X unique capture with smMIPs). Most importantly, our local NGS findings obtained with a custom panel on the Ion Torrent™PGM platform were confirmed with the published smMIPs technology [25] on the Illumina HiSeq in the USA, validating NGS in our hands for translation into the diagnostic setting. Subsequently, the diagnosis of DS was revised for 10 of the 12 variant-negative patients (seven FS+, one EOEE, one moyamoya disease and one perinatal insult), increasing our proportion of *SCN1A*-positive DS 75%, more in line with international findings. The relatively high number of

patients re-diagnosed with FS + was a likely consequence of the broad inclusion criteria of infantile-onset recurrent complex febrile seizures with normal prior development, which enhanced recruitment but also resulted in inclusion of the non-Dravet, FS + phenotypes.

Statistical analysis of the clinical demographics highlighted the DS risk score and the age at seizure onset as the most useful clinical diagnostic markers for DS. The DS risk scoring system, devised by Hattori and colleagues, compared the clinical characteristics of Japanese Dravet and non-Dravet patients with seizure onset before one year of age [29]. In our cohort, the median score in the *SCN1A*-positive group (median score 9.00, range 8.00–11.00) was significantly higher than that in the variant-negative group (median score 5.50, range 3.75–6.00). This was in agreement with Hattori and colleagues, who proposed that a child with a score of six or higher was at an increased risk of DS and should undergo *SCN1A* testing [29]. However, the utility of this scoring system may be limited in the African context, as recognition of some of its diagnostic markers e.g. hemiclonic seizures (not usually seen in the earliest presenting period of DS), requires some experience in child neurology and epilepsy. In the African setting, where many of these children are initially seen by primary healthcare workers, straightforward clinical indicators are needed to prompt early referral to specialist centres. The significantly earlier median age of onset in the *SCN1*A-positive children in our study (4 months, range 3–6 months), compared to the variant-negative group (12 months, range 8.75–13.25 months) was in line with previously published evidence showing that the onset of frequent and prolonged seizures before six months of age in an otherwise normally developing child, confers a high risk of progression to DS [31–33]. Thus the age of seizure onset presents and uncomplicated early clinical indicator and an easily implemented specialist referral criterion that could enhance early detection of DS in resource-limited settings.

The low number of *SCN1A*-DS among the indigenous black children in our cohort (only one of 10), prompted a clinical reassessment, resulting in a revised diagnosis for nine patients, the majority of whom fell into the FS + category. These children were more likely to be based in poorer socioeconomic settings, often with multiple healthcare challenges and at risk of recurrent infections, placing this group at an increased risk of recurrent FS. The results of our study therefore emphasise the importance of genetic testing not only for the variant-positive patients but also the patients in whom negative test results precipitate clinical reassessment and a revised diagnosis.

Investigators in a recent epidemiological study of DS in the United States (US) found that clinical DS in the US occurs at an incidence of 1 in 15 700 births and is more than twice as common as previously reported [34,35]. The US study also reported a higher incidence of SCN1A-associated DS (1 in 20 900 births), compared to the European estimates ranging from 1 in 22 000 to 1 in 41 000 [13,36]. Applying the US DS incidence (1 in 15 700) in SA where 969 415 live births were recorded in 2016 [37], theoretically translates into approximately 62 new DS cases in a single year. Most will not have a diagnosis at the time of publishing this study and none (or very few in private healthcare), would have had access to genetic testing. In the Western Cape region of SA, the majority of children diagnosed with clinical DS are eventually referred to the Epilepsy Clinic at the RCWMCH. At the time of recruitment, only about 30 DS/possible DS patients were referred to the Epilepsy Clinic over a period of approximately 10 years. Using the regional birth registration figure of 106 599 in 2014 [38], one might extrapolate that approximately 7 new DS patients should be referred to the clinic each year (using the US DS incidence figure). It is therefore likely that many DS patients in the Western Cape and across SA are not diagnosed or managed appropriately. Most of these children could now be clinically recognized using the age of onset and the DS risk scoring system [29], and referred for genetic testing.

Our findings are new and especially useful in the African context, where genetic epilepsy research is limited and diagnostic testing is not available. The correct diagnosis and treatment of DS and other epileptic encephalopathies may be achieved months or years after initial presentation, if at all. The DS risk score and age of onset of prolonged febrile seizures before 6 months of age present quantitative low-cost criteria to identify patients most at risk of DS and who are likely to benefit from genetic testing [29,31]. Whilst the scoring system may be more relevant to patients with established DS beyond the early febrile stage, the age at seizure onset is a simple clinical marker for early DS. In the poorest and most remote rural regions, such simple diagnostic tools can assist in the choice of treatment and alert to the need for specialist consultation and genetic testing.

As a direct result of this study, the Epilepsy Clinic at the RCWMCH now has nine patients with confirmed *SCN1A*-associated DS, and one patient with *PCDH19*-related epilepsy on its records. These findings highlighted the sometimes underestimated diagnostic precision of an informative genetic test result in a child with possible DS. Anecdotally, the appreciation of "diagnostic closure" was strongly expressed by the parents and clinicians alike, also emphasizing the role of genetic counselling. Understanding the cause of the disease in a child brought about a sense of relief, acceptance and a more focused approach to care. Whilst the management typically followed the available interventions recommended for DS, including valproate and clobazam, following the genetic confirmation parents were more committed to accessing stiripentol and trials of the ketogenic diet. The study outcomes also presented a potential focus for future research towards identifying the causes of genetic and clinical heterogeneity in the "variant-negative" patients of our cohort.

5. Conclusion

DS is, arguably, the most extensively studied epileptic encephalopathy (EE) [6] and also one of the most clinically challenging epilepsy syndromes. The intractable seizures, multiple co-morbidities and constant threat of premature mortality profoundly affect the quality of life of the children and their families. A better outcome can be achieved with appropriate intervention and more targeted therapy at an early stage of the disease, reflecting an example of precision medicine [39].

This is the first study investigating the genetic causes of DS in SA. Whilst conclusions drawn from small cohorts are generally viewed with caution, our results, as a snapshot of DS in the local population, confirm that *de novo SCN1A* variants are associated with disease in the majority of South African DS patients. Adding to the molecular findings, a significant outcome of this study was affirming the link between the DS risk score, the age at seizure onset and the presence of an *SCN1A* variant. In the poorly resourced African setting, observing these clinical signs of DS may go a long way towards embarking on the correct diagnostic course for DS and a better overall outcome. This also highlights the importance of raising awareness among healthcare practitioners of a possible genetic contribution to the seizure pathogenesis in their patients, thus beginning to bridge the significant epilepsy treatment gap in Africa.

Author contributions

AE: experiment design and analysis, article conception, drafting, collation of information, critical revision; GLC: experiment design, analysis and critical revision; RR: critical revision; SW: collation of clinical information; HCM: critical revision; JW: patient recruitment, article conception, clinical insights, critical revision.

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Conflicts of interests

The authors declare no conflicts of interests.

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