Utility of Next Generation Sequencing in Paediatric Neurological Disorders: experience from South Africa

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Article

Keywords:

Posted Date: July 5th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3086984/v1

Additional Declarations: There is no duality of interest

Version of Record: A version of this preprint was published at European Journal of Human Genetics on May 3rd, 2024. See the published version at https://doi.org/10.1038/s41431-024-01582-2.

Abstract

Next generation sequencing (NGS)-based tests have become routine first-line investigative modalities in paediatric neurology clinics in many high-income countries (HICs). Studies from these countries show that these tests are both cost-effective and reliable in diagnosing many complex childhood neurological diseases; however, NGS-based testing in low-and middle-income countries (LMICs) is limited due to cost. The primary objective of this study was to evaluate the diagnostic yield and impact of targeted gene panels in a selected paediatric cohort attending a tertiary paediatric neurology clinic in the Western Cape Province of South Africa. This retrospective study included 124 consecutive paediatric patients with neurological disease, referred for multi-gene panel testing over a 41-month period. Twenty-four different disease group-specific panels were utilized. A caregiver experience questionnaire was administered when a pathogenic variant was identified. The overall study diagnostic yield (DY) was 53% (66/124 patients). It was highest for neuromuscular disorders 64% (16/25), cerebral palsy spectrum disorders 54% (9/16) and early-onset epilepsies 44% (28/63). Testing proved inconclusive (variants of uncertain significance) in 38% (47/124). The majority of caregivers (97%) viewed NGS-based testing as a positive experience.

The diagnostic yield in this study is similar to previously reported paediatric cohorts in HICs. The high yields for neuromuscular disorders and early epileptic encephalopathies suggest that NGS-based panels may be more cost-effective as first-line testing in well-defined phenotypes. The latter finding argues for early inclusion of all children with developmental epileptic encephalopathies (DEE), as early diagnosis leads to better treatment and avoidance of unnecessary investigations.

Introduction

The rapidly expanding field of paediatric neurogenetics has transformed our understanding of mechanisms mediating neurological diseases. Precise and timeous diagnosis of these diseases are essential to be able to offer appropriate treatment and family counselling. Studies have shown that extensive non-genetic investigations of childhood neurological diseases (epileptic encephalopathies, movement disorders, neuromuscular disorders, and developmental delay, amongst others) are often costly with low diagnostic yields, resulting in diagnostic failure or delay (1).

In 2011, the World Health Organization (WHO) recommended the implementation of community genetics programmes in LMICs(2) The aim of the programmes was the reduction of congenital disorders and genetic diseases at the population level, in addition to providing genetic services, including diagnosis and counselling, for individuals and families. Implementation challenges identified by the WHO include the uneven distribution of diagnostic genetic laboratories (603 worldwide, 256 outside the USA and only 20 in LMICs) as well as cost constraints(3) Encouraging findings include that the cost of NGS-based testing is expected to decrease 30-fold within several years, whilst DNA collection via dried blood spots, buccal swabs and saliva offer an attractive solution for sample acquisition in LMICs, given the cost, ease of collection and transportation of samples. Unaffordability is often raised as an insurmountable

obstacle in LMICs. However, this notion only considers the costs of testing and not the cost of the disease burden. Paradoxically, LMICs are likely to benefit the most from NGS-based testing due to the lack of screening, higher rates of consanguinity and higher infant mortality rates (3). The WHO also reported that reconfiguration of sequence capacity to allow testing for the most relevant and potentially treatable diseases encountered in LMICs might also bring about further cost reduction (2).

Southern Africa as a region offers rich human genetic diversity, which has relevance when studying and informing diagnostic testing that is broadly applicable. Genetics data from LMICs should be included when NGS panels are configured and variants are interpreted. This study aimed to evaluate the impact of genetic testing and the diagnostic utility of targeted gene panel sequencing in a selected paediatric cohort attending a tertiary paediatric neurology clinic in the Western Cape Province of South Africa.

Materials and Methods

Participants

We retrospectively collected data from 124 consecutive paediatric patients with neurological disease aged 6 months to 17 years, referred over a 41-month period to the tertiary paediatric neurology outpatient service at Tygerberg Hospital, which is situated in the Western Cape Province of South Africa. This hospital serves a population of approximately 2.6 million people, which includes an estimated paediatric population of 1 062 911 children under the age of 13 years. Close to 2000 children are seen annually in the paediatric neurology outpatient service of the hospital. Most families reside in poor underserviced communities. A paediatric neurologist and/or developmental paediatrician and medical geneticist examined all children. Clinical data analysed included age of onset of the disease, neurological symptoms, presence of developmental delay, neurology examination findings and the results of all requested special investigations. Locally available genetic testing was preferentially offered via the National Health Laboratory Service (NHLS), however these tests are unfortunately not comprehensive. Some off these routinely available tests include Spinal Muscular Atrophy (SMA) (*SMN* homozygous deletion exon 7 only), Becker and Duchenne Muscular Dystrophy (BMD, DMD) (deletions, duplications only), *RYR1* (founder variants only), Charcot-Mary-Tooth 1A (CMT1A) (*PMP2* duplication only) and Myotonic Dystrophy (Triplet repeat, Polymerase Chain Reaction (PCR).

Sample and data collection

Following pre-test genetic counselling and informed consent, buccal swabs, assisted saliva or blood samples (3 ml) were collected from all the patients. The sampling method depended on the patient's age, clinical condition, and ability to provide a saliva sample.

Next-Generation Sequencing-based gene panels

NGS-based gene panels were outsourced (Invitae, USA). The panel selected depended on the suspected underlying neurological disorder. In total, twenty-four panels were used in the study (Supplemental Table

1), covering most of the disease groups presenting to paediatric neurology. The test results were categorized as either: positive (pathogenic/likely pathogenic variant identified), inconclusive (variant(s) of uncertain significance (VUS) identified), or negative (no variants identified). Variants of uncertain significance results were stratified into two categories:

1) Possible - “interesting candidate” (the patient's phenotype and family history is a good match but does not reach the threshold for classification as likely pathogenic; segregation of the variant in the family needed to be assessed, and/or further functional analyses conducted). For interesting candidates, testing of parents and/or other family members was requested, subject to the relevance and availability of family members.

2) Unlikely – the phenotype does not fit the genotype of the VUS. Various publicly available resources were used to compile genotype-phenotype correlations.

Qualitative questionnaire

Subsequently, six questions relating to caregiver experience were administered when a pathogenic variant was identified (Supplemental Table 2)

Statistical Analysis

Data were analysed using the statistical software SPSS version 26.0. We calculated the diagnostic yield by dividing the number of patients with a positive result over the total number of tests performed. The data obtained from the questionnaire was categorical (yes/no/some/unknown/not applicable). The variables were summarized using frequency and proportions. The study was approved by the Stellenbosch University Health Research Ethics Committee**,** HREC Reference No: S22/03/034.

Results

Patient characteristics

Overall, 124 patients underwent gene panel testing. One-hundred and twenty-seven panels were utilized: for patient 43 a broader panel was requested after the original smaller panel was negative, for patient 51 a customised panel was requested to include further genes, and for patient 86 a combined Epilepsy and Cerebral Palsy Spectrum Disorders panel was requested.. The median age of the patient at the time of gene panel analysis was 63 months (interquartile range (IQR): 12–96 months). Fifty (40%) of patients were tested by 2 years of age and 77 (62%) by 5 years of age. There was a 1.1:1 ratio of female to male patients. The median specimen turnaround time was 6 weeks (IQR: 4–8 weeks)

Pathogenic variants identified and diagnostic yield.

Table 1 illustrates the pathogenic variants identified in the study. Table 2 shows the disorder groups that were tested, the number of panels requested as well as the diagnostic yield for each panel and group.

The overall study diagnostic yield (DY) was 53% (66/124 patients). It was highest for neuromuscular disorders 64% (16/25) followed by cerebral palsy spectrum disorders 54% (9/16 patients) and epilepsies 44% (28/63 patients). High numbers of NGS-testing revealed VUS 38% (47/124 patients) complicated interpretation of NGS-derived results. The small number of patients in some phenotypic groups limited the interpretation of specific diagnostic yields.

Table 1
Number of pathogenic variants.

	Panel	Pathogenic genes per panel	Number of patients with pathogenic variants
1.	Epilepsy Panel	*SCN1A*	7
		COG5	1
		DYRK1A	1
		GABRB3	1 *
		UBE3A	1*
		STXBP1	1
		KANSL1	1
		SCN2A	1
		TPP1	1
		CDKL5	3
		KCNQ2	3
		KCNMA1	1*
		TUBB2A	1*
		GNAO1	1
		SLC6A5	1
2.	Comprehensive neuromuscular panel	*RYR1*	2
		SMN1	1
		TTN	1
		GBE1	1*
		CAPN3	1*
		STAC3	1
		DMD	1
3.	Early infantile epileptic encephalopathy panel	*CDKL5*	1
		SCN1A	1

*Indicates patients with two different variants on one panel classified as pathogenic.

	Panel	Pathogenic genes per panel	Number of patients with pathogenic variants
4.	Hereditary sensory and autonomic neuropathy panel	*SCN9A*	2
5.	Leuko-dystrophy and -encephalopathy panel	*ACADS*	1
		UGT1A1	2*
		ABCD1	1*
		PLP1	1
		ARSA	1
6.	Metachromatic and general leukoencephalopathy panel	*ARSA*	1
7.	Cerebral palsy spectrum disorders panel	*SCN2A*	1
		ADAR	2*
		BTD	2*
		SLC16A2	1
		KCNA2	1
		QDPR	1
		ATM	1
		NPHP1	1
8.	Organic acidaemias panel	*PCCA*	1
9.	Spinal muscular atrophy panel	*SMN1, SMN2copies2*	1
10.	Comprehensive myopathy panel	*STAC3*	1
		RYR1	1
11.	Rett/Angelman like variants	*IQSEC2*	1
		GABBR2	1
12.	Dystrophinopathies	*DMD*	1
13.	NF1	*NF1*	1
14.	RASopathies	*NF1*	1

*Indicates patients with two different variants on one panel classified as pathogenic.

	Panel	Pathogenic genes per panel	Number of patients with pathogenic variants
15.	Zellweger spectrum disorders panel	*HSD17B4*	1
16.	Comprehensive muscular dystrophy panel	*DMD*	2
		COL6A1	1
17.	Dystonia comprehensive panel	*KMT2B*	1
*Indicates patients with two different variants on one panel classified as pathogenic.			

Table 2
Yield per panel and neurological diseases group.

Disorder group	Panels	No. of panels requested	Yield per panel	Total yield per group
1. Neuromuscular disorders	Comprehensive neuromuscular	14	7 (50%)	16 (64%)
	Comprehensive neuropathies	1	0	
	Hereditary sensory and autonomic neuropathy	2	2 (200%)	
	Spinal muscular atrophy	1	1 (100%)	
	Comprehensive myopathy	2	2 (100%)	
	Dystrophinopathies	1	1 (100%)	
	Comprehensive muscular dystrophy	3	3 (100%)	
	Congenital Muscular dystrophy	1	0	
2. Epilepsy	Epilepsy	54	25 (46%)	28 (44%)
	Early epileptic encephalopathy	9	3 (33.3%)	
3. Movement disorders	Comprehensive Dystonia	1	1 (100%)	1 (100%)
4.Heredodegenerative disorders	Metachromatic leukodystrophy	2	1 (50%)	6 (66%)
	Leukodystrophy and Leukoencephalopathy	6	5 (83%)	
	Hereditary spastic paraplegia	1	0	
5. Neurocutaneous disorders	Neurofibromatosis 1	1	1 (100%)	1 (100%)
6. Metabolic disorders	Lysosomal storage disorders	1	0	2 (50%)
	Organic acidaemias	1	1 (100%)	
	Zellweger spectrum	1	1 (100%)	
	Glycine encephalopathy	1	0	
7. Cerebral palsy spectrum disorders	Cerebral palsy spectrum	16	9 (56%)	9 (56%)

Disorder group	Panels	No. of panels requested	Yield per panel	Total yield per group
8. Neuro-developmental disorders	Rett/Angelman like variants		2 (66%)	
	Kabuki	1	0	3 (60%)
	RASopathies	1	1 (100%)	
9. Developmental Brain malformations	Holoprosencephaly	3	0	0

Gene-phenotype matching

Table 3 shows the gene-phenotype correlation of the study population. Children with developmental epileptic encephalopathies (DDE) were excluded as they were described in a previous publication from our institution (4). For recessively inherited conditions, we noted the following: the diagnosis was confirmed in four patients (patients 62, 67, 79 and 92) as they were compound heterozygous for pathogenic variants in *RYR1*, *RYR1*, *PCCA* and *ARSA*, respectively. Phasing of the variants was confirmed after parental testing and revealed that the variants were *in trans*.

Table 3

Pathogenic mutations and clinical correlation (including two patients from the epilepsy panel not included in a previous epilepsy study)(4)

Patient	Phenotypic features	Gene	Inheritance/ zygosity	Gene resolution	Clinical relevance
10(F)	CP-like with central hypotonia and upper motor neuron signs in all limbs. Severe global developmental delay and visual impairment	*SCN2A*	AD/ heterozygous	Pathogenic	Yes
15(F)	Child with acute left hemiparesis, young stroke work-up normal except for MRI findings suggestive of leukoencephalopathy	*ACADS*	AR / heterozygous	Pathogenic but second variant not identified	Possible
17(M)	Congenital microcephaly and ventriculomegaly antenatally, developmental delay, spastic diplegia, no hyperbilirubinemia	*UGT1A1*	AR/ heterozygous	Pathogenic but second variant not identified	Unlikely
21(M)	Floppy weak infant	*RYR1*	AR/ heterozygous heterozygous heterozygous	3 *RYR1* genes. One pathogenic and two VUS Mom- one pathogenic and two VUS in *RYR1*(Dad not available)	Yes
27(F)	Intellectual disability and insensitivity to pain/Twins	*SCN9A*	AR/ homozygous	Pathogenic	Yes
28(F)	Intellectual disability and insensitivity to pain/Twins	*SCN9A*	AR/ homozygous	Pathogenic	Yes
31(M)	Neonatal onset severe weakness. Requiring respiratory support and NGT feeding	*SMN1*	AR/ Homozygous	Pathogenic	Yes
33(M)	Progressive weakness and positive Gower sign, CK > 4000	*DMD*	X-linked/ hemizygous	Pathogenic	Yes

AR- autosomal recessive, AD- Autosomal dominant, CALs- Café au lait spots, CK- Creatine kinase, *CP- Cerebral Palsy, EEG- Electroencephalogram, F- Female, M- Male, MRI- Magnetic resonance imaging, NGT- nasogastric tube, NICU-Neonatal intensive care unit, VLCFA- Very long chain fatty acids, VUS- Variant of unknown significance*

Patient	Phenotypic features	Gene	Inheritance/ zygosity	Gene resolution	Clinical relevance
34(M)	Progressive lower limb weakness and positive Gower sign, no pseudohypertrophy of calves, CK600	*COL6A1*	AD/ heterozygous	Pathogenic	Yes
38(M)	Progressive proximal weakness and positive Gower sign, high CK	*DMD*	X-linked hemizygous	Pathogenic	Yes
47(F)	Congenital myopathy with progressive musculoskeletal complications and recurrent chest infections.	*TTN*	AR/ heterozygous	No other pathogenic variant identified	Unlikely
58(F)	Mild hypotonia, cleft palate, wide spaced nipples and intermittent stridor.	*STAC3*	AR/ homozygous	Pathogenic	Yes
60(M)	Developmental delay and epilepsy then neurodevelopmental regression and progressive vision loss, MRI leukoencephalopathy	*ARSA*	AR/ homozygous	Pathogenic	Yes
62(F)	Floppy weak infant with facial diplegia	*RYR1*	AR/Compound heterozygous	Two pathogenic *RYR1* and one *RYR1* VUS	Yes
64(F)	Developmental epileptic encephalopathy with neurodevelopmental regression and defiant behaviour	*IQSEC2*	X-linked hemizygous	Pathogenic	Yes
65(M)	Normal development and then progressive lower limb weakness with positive Gower sign	*DMD*	X-linked hemizygous	Pathogenic	Yes
67(F)	Floppy weak infant, dysmorphic features, facial diplegia, micrognathia, low-set ears, prolonged oxygen support needed, feeding difficulties, multiple	*RYR1*	AR/Compound heterozygous	Two *RYR1* pathogen and one *RYR1* VUS	Yes

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Patient	Phenotypic features	Gene	Inheritance/ zygosity	Gene resolution	Clinical relevance
	admissions with respiratory distress				
68(M)	Childhood-onset dystonia, cyclical transaminitis and freckling of the skin. Bilateral striatal necrosis on MRI brain	*ADAR*	AR/ heterozygous	One *ADAR* pathogenic and one *ADAR* VUS. Mother *ADAR* pathogenic Father *ADAR* VUS Brother *ADAR* pathogenic	Yes
69(M)	Subacute onset of dense left hemiplegia. MRI features suggestive of adrenoleukodystrophy	*ABCD1* *UGT1A1*	X-linked hemizygous AR/ homozygous	All three variants are pathogenic	Yes
70(M)	Weak, low Apgar score, contractures, ventilated for 15 days in NICU.	*GBE1* *CAPN3*	AR homozygous AR heterozygous	Two pathogenic variants in *GBE1*. Carrier of *CAPN3*-related disorder	Yes Carrier
71(M)	Multiple CALs, neurofibromas with intellectual disability	*NF1*	AD heterozygous	Pathogenic	Yes
72(M)	Pulmonary stenosis Multiple CALs	*NF1*	AD heterozygous	Pathogenic	Yes
73(M)	Developmental delay, intellectual disability. Hypomyelination on MRI	*PLP1*	X-linked hemizygous	Pathogenic	Yes

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Patient	Phenotypic features	Gene	Inheritance/ zygosity	Gene resolution	Clinical relevance
75(M)	Neonatal seizures, requiring NGT feeding, Polymicrogyria on MRI, VLCFA indicative of peroxisomal disorder	*HSD17B4*	AR/ heterozygous	Dad HSD17B4 pathogenic Mom is negative Possible other pathogenic variant in *HSD17B4* not detected	Possible
79(F)	Previously well infant presented with infantile encephalopathy and raised ammonia levels	*PCCA*	AR/Compound heterozygous	One pathogenic and one likely pathogenic variant	Yes
88(M)	Central hypotonia with peripherally increased tone and reflexes. Global developmental delay. MRI demyelination	*SLC16A2*	X-Linked hemizygous	Pathogenic	Yes
89(F)	Developmental delay, minimal speech, autistic-like features, microcephaly, unusual hand movements. No significant family history	*GABBR2*	AD/ heterozygous	Pathogenic	Yes
90(M)	5 year old boy presents with 3 year history of spastic diplegia of unknown cause.	*BTD*	AR/ heterozygous	No other pathogenic variant identified	(Carrier)
91(M)	Infantile onset febrile seizures, also focal seizures tonic clinic and generalised tonic seizures. Initial EEG normal	*SCN1A*	AD/ heterozygous	Pathogenic	Yes
92(M)	4-and-a-half year old with 2 episodes of ataxia, resolved, developed dystonia and spastic diplegia in 2021, now with regressing milestones.	*ARSA*	Compound heterozygous	Two pathogenic variants in *ADAR*	Yes

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Patient	Phenotypic features	Gene	Inheritance/ zygosity	Gene resolution	Clinical relevance
93(M)	Seizures, frontal cortical dysplasia, developmental delay and progressive spastic paraplegia	*KCNA2*	AD/ heterozygous	Pathogenic	Yes
94(F)	CP-like presentation but normal Birth history and two other siblings with similar presentation	*QDPR*	AR/ homozygous	Pathogenic	Yes
96(F)	Profound bilateral Sensory-neural hearing loss and global developmental delay	*ATM*	?AD/ ?AR/ heterozygous	No other pathogenic variants identified	(carrier)
97(F)	Previously normal development presenting with progressive dystonia	*KMT2B*	AD/ heterozygous	Pathogenic	Yes
98(F)	Delayed walking and spastic paraplegia with normal Brain and spinal MRI	*NPHP1*	AR/ heterozygous	Incidental	(Carrier)
107(F)	Floppy weak infant	*STAC3*	AR/ homozygous	Pathogenic	Yes
110(M)	Previously normal development, now proximal weakness and clumsiness when walking. Hypertrophy of the claves.	*DMD*	X-linked hemizygous	Pathogenic	Yes
113(M)	Floppy weak infant, no dysmorphic feature	*SMN1*	AR/ homozygous	Pathogenic	Yes
119(F)	Paroxysmal involuntary movements and dystonia with normal intellect	*BTD* *ADAR*	AR/ heterozygous AR/ heterozygous	Incidental One *ADAR* pathogenic and one *ADAR* VUS	No Possible
122(F)	Abrupt onset Childhood onset focal motor seizures with generalisation, up to 30 times per day. EEG confirmed semiology.	*NPRL3*	AD heterozygous	Pathogenic	Yes

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Patient	Phenotypic features	Gene	Inheritance/ zygosity	Gene resolution	Clinical relevance
	Normal MRI. Normal development				
123(F)	Baby with exaggerated startle and then tonic posturing	*SLC6A5*	AR/ heterozygous	One pathogenic and one VUS in *SLC6A5*	Yes

AR- autosomal recessive, AD- Autosomal dominant, CALs- Café au lait spots, CK- Creatine kinase, *CP- Cerebral Palsy, EEG- Electroencephalogram, F- Female, M- Male, MRI- Magnetic resonance imaging, NGT- nasogastric tube, NICU-Neonatal intensive care unit, VLCFA- Very long chain fatty acids, VUS- Variant of unknown significance*

In patients 58 and 60, one pathogenic/ likely pathogenic homozygous variant was found that causes autosomal recessive disease. For some patients, only one pathogenic/ likely pathogenic heterozygous variant was identified in a gene known to cause disease in an autosomal-recessive manner (patients 15 and 75). The second variant in that gene was not identified, thus the patients were only confirmed to be heterozygous carriers and a confirmatory diagnosis could not be made despite a high clinical suspicion. In some cases (patients 68, 119 and 123), the second variant identified in the same gene was classified as a VUS, making the diagnosis more likely, however without reclassification of the VUS a definitive diagnosis could not be made, although clinically the phenotype would fit. In a further 2 patients (69 and 70), another pathogenic heterozygous variant in a different recessive gene was found. Those patients were thus carriers of pathogenic variants in two distinct genes.

The last group (patients 17, 47, 90, 96 and 98), had pathogenic variants identified in genes which were not deemed clinically significant, for example patient 96 was found to be a carrier of a pathogenic heterozygous variant in *ATM*, related to increased susceptibility to autosomal-dominant breast cancer which was not related to the current phenotype; however the gene was included because in the homozygous/compound heterozygous state, variants in *ATM* cause ataxia telangiectasia.

Utility to parents

Table 4 illustrates the caregiver responses to positive (pathogenic) test results. Twenty-eight parents (97%) felt that knowing the result brought closure for the family and 28 (97%), indicated that should prenatal testing be available that they would use it in future pregnancies. In 14 (48%) of the cases, treatment was adjusted to some extent once the pathogenic variant was known. Fourteen parents (48%) felt that it assisted in helping them take better care of their child.

Table 4
Care-giver questionnaire answers.

Did the result help explain the reason for your child illness?	**Results of participants**
Yes	25 (86%)
No	3 (10%)
Some/little	0
Unknown	1 (3%)
Not applicable	0
Were any changes made to the treatment/ medication after the diagnosis?	
Yes	14 (48%)
No	11 (37%)
Some/little	3 (10%)
Unknown	1 (3%)
Not applicable	0
Did the result help you to take better care for your child?	
Yes	14 (48%)
No	13 (44%)
Some/little	2 (7%)
Unknown	0
Not applicable	0
Did the results help you plan for future pregnancies?	
Yes	8 (28%)
No	5 (17%)
Some/little	1 (3%)
Unknown	3 (10%)
Not applicable	12 (41%)
Should prenatal testing become available, would you make use of it?	
Yes	28 (97%)

Did the result help explain the reason for your child illness?	Results of participants
No	0
Some/little	0
Unknown	0
Not applicable	1 (3%)
Did knowing the result bring you and your family any closure?	
Yes	28 (97%)
No	0
Some/little	1 (3%)
Unknown	0
Not applicable	0

Discussion

This study aimed to evaluate the diagnostic yield and the impact of targeted gene panel sequencing in a selected cohort attending a LMIC tertiary paediatric neurology outpatient service. The overall study diagnostic yield (DY) was 53% (66/124 patients). It was highest for neuromuscular disorders 64% (16/25) followed by cerebral palsy spectrum disorders 54% (9/16) and epilepsies 44% (28/63).

To date, few studies have investigated the diagnostic yield of molecular testing in paediatric neurological disorders. The overall rate of molecular diagnosis in previous studies range from 20 to 60%. However, the results of previous studies are not generalizable, due to heterogeneous study designs, sample size, inclusion criteria, and specific diagnostic techniques. To the best of our knowledge, this is the first study that specifically investigated the diagnostic yield of commercially available gene panels in a large cohort attending a tertiary paediatric neurology outpatient service in a LMIC. The overall diagnostic yield in this study is similar to previously reported paediatric cohorts in HICs (See Supplementary Table 3)

Clinical interpretation of genetic test results is increasingly complicated by variants of uncertain significance (VUS) that have an unknown impact on health. High volumes of returned VUS (47/124 patients) in the study similarly complicated interpretation of results. This is true across disease category and across panels, as our population is understudied and underrepresented in global databases (5). Reclassification can clarify a variant's clinical significance and it is increasingly facilitated by the availability of updated information about human genetic diversity, especially among underrepresented populations. This furthermore highlights the importance of studies, which include LMICs and diverse populations.

The most common genes identified in the study were *SCN1A* (n = 8), *CDKL5* (n = 4), *DMD* (n = 4), *KCNQ2* (n = 3), *RYR1* (n = 3). These genes have been well described in other international paediatric studies. *SCN1A, CDKLA5* and *KCNQ2* were also among the top five genes with the highest yield using NGS in studies focusing on paediatric epilepsy in a study by Mei *et al.* (6). A recent study from the South Africa using a panel of 71 DEE-associated genes identified Pathogenic/Likely pathogenic candidate single nucleotide variant or short indels in 12% of cases (28/234 patients) (7). Similar to our study, *SCN1A* proved the most prevalent gene in this category (n = 13). Of interest was the failure to identify any *KCNQ2* cases despite the gene's inclusion in the testing panel. The three *KCNQ2* cases identified in our study highlight the importance of including this gene in any DEE or Epilepsy panel, as it is relevant and potentially allows clinicians to offer precision therapy (sodium blockers).

Spinal muscular atrophy is a commonly encountered neuromuscular disease in our region. Studies have shown that the birth incidence of SMA in black South Africans is higher than one in 3574 (8). Local testing for *SMN1* does not include a determination of *SMN2* copy numbers. The latter may be employed to correlate with the disease phenotype, predict disease evolution, and stratify patients that are eligible for gene therapy. The Invitae SMA panel used in this study offered the additional advantage of identifying the *SMN2* copy numbers, facilitating easier entry into clinical trials for these patients.

With regards to the three patients where additional panels were requested: Patient 43 clinically had features suggestive of Rett syndrome but a pathogenic variant was only found once a re-requisition was made to the extensive Epilepsy panel. Patient 51 was diagnosed on the main panel (Epilepsy panel) and the additional panel was not helpful. Patient 86's results still yielded VUS even though the panel was expanded to more than 600 genes.

Interesting cases

Unexplained cause of muscle weakness and hypotonia

A female infant presented with hypotonia, muscle weakness, recurrent chest infections and progressive scoliosis presumed to be related to a congenital myopathy. Investigations including creatine kinase (CK) were normal. Muscle biopsy was deferred due to high risk of anaesthetic complications. Genetic testing via the comprehensive neuromuscular NGS panel identified one pathogenic variant in *TTN* (OMIM #188840), which is associated with autosomal-dominant dilated cardiomyopathy (OMIM #604145) as well as a group of disorders affecting skeletal muscles, including autosomal-dominant tibial muscular dystrophy and autosomal-recessive limb-girdle muscular dystrophy type 2J (LGMD2J: OMIM #608807), autosomal-recessive centronuclear myopathy (CNM; OMIM # 611705) and autosomal-dominant hereditary myopathy with early respiratory failure (HMERF; OMIM # 603689). This patient is a carrier for a variant in a domain of TTN known to be associated with autosomal recessive *TTN*-related conditions. The second variant in TTN was not identified on the panel and the clinical significance of his variant remains unknown A definitive diagnosis has thus not been made. Further testing in the form of genome sequencing or transcriptomics may uncover the underlying cause of her myopathy.

ADAR-related bilateral striatal necrosis with cyclical transaminitis

A teenage boy presented with a longstanding history of acquired dystonia and recurrent episodes of transaminitis associated with lethargy and vomiting. Clinical examination revealed hyperpigmented macules arranged in reticulated patterns in the face and the dorsal aspects of the extremities of the patient, as well as his mother and brother. Metabolic investigations proved unremarkable, whilst magnetic resonance imaging (MRI) of the brain demonstrated bilateral high signal intensity involving the striatum. The Cerebral Palsy Spectrum Disorders panel revealed that the boy had two variants in RNA-specific adenosine deaminase (*ADAR) namely ADAR* c.2128_2131dup classified as pathogenic and *ADAR* c.577C > G, classified as a VUS. On familial testing, the mother and brother had the pathogenic *ADAR* c.2128_2131dup variant only. The father had the *ADAR* c.577C > G VUS only, thus confirming that the variants were *in trans* in the patient. *ADAR* is associated with autosomal-dominant dyschromatosis symmetrica hereditaria (DSH; OMIM #127400) and autosomal-recessive Aicardi Goutières syndrome (AGS; OMIM #615010). It thus confirmed that the hyperpigmented rash could be attributed to dyschromatosis symmetrica hereditaria (9). *ADAR* is also an essential molecule for liver homeostasis as it inhibits the pro-inflammatory effects of interferon. Genetic testing therefore allowed molecular insights and may in future assist with more targeted anti-interferon therapies.

Adrenoleukodystrophy

A 5-year old child presented with a left hemiplegia and neurodevelopmental regression. Investigations including MRI brain and very long chain fatty acids suggested a diagnosis of adrenoleukodystrophy. Genetic testing (Invitae leukodystrophy panel) revealed pathogenic variants in 2 genes: *ABCD1* (hemizygous) and *UGT1A1* (homozygous). *ABCD1* (OMIM #300100) on the X chromosome provides instructions for producing the adrenoleukodystrophy protein and *UGT1A1* (OMIM #191740) provides instructions for making enzymes called UDP-glucuronosyltransferases. Both have been implicated in genetic white matter disorders.

The phenotype of this patient strongly suggested a diagnosis of X-linked adrenoleukodystrophy: a metabolic disorder caused by impaired peroxisomal beta oxidation that leads to the accumulation of saturated very long chain fatty acids in numerous tissues throughout the body. The majority of males with X-linked adrenoleukodystrophy develop adrenocortical insufficiency in childhood, followed by progressive myelopathy and peripheral neuropathy in adulthood (10)The *UGT1A1* gene identified is associated with autosomal-recessive hyperbilirubinemia (11)This finding was deemed of no clinical significance as the boy never presented with jaundice. Importantly, the mother also tested positive for the *ABCD1* variant, and was a heterozygous carrier of the *UGT1A1* variant. Genetic counselling was of utmost importance here, to explain X-linked inheritance and the 50% chance of recurrence in future male offspring. Patients with X-linked adrenoleukodystrophy benefit from haematopoietic stem cell transplants if they are diagnosed and transplanted before they become symptomatic. While it was already too late for this patient to be considered for a transplant, he had a younger brother, who was

asymptomatic and in whom a genetic diagnosis may have facilitated early treatment. The younger brother subsequently tested negative for the pathogenic variant in *ABCD1*.

A glycogen storage disorder

A male neonate was born at 36 weeks' gestation via Caesarean-section for pathological cardiotocography (CTG). The mother had polyhydramnios. The baby was noted to be hypotonic with poor respiratory effort even on continuous positive airway pressure and was intubated and ventilated in the neonatal intensive care unit. The baby had minor dysmorphic features including low-set ears and a high-arched palate. No tongue fasciculations were noted. The baby's power remained 1/5 in the limbs with marked global hypotonia. Cranial ultrasound revealed ventriculomegaly but no other structural abnormalities. Cardiac echocardiography showed a normal structured heart with poor ventricular contractility. Unfortunately, the baby demised after 15 days. NGS results revealed two pathogenic variants: Glycogen branching enzyme (*GBE1*) (homozygous) and calpain-3 encoding gene (*CAPN3)* (heterozygous).

GBE1 (OMIM # 607839) is associated with glycogen storage disorder type 4 (GSD IV). Diminished enzyme activity results in a build-up of structurally abnormal glycogen in affected tissues. GSD IV has a highly variable phenotype. Affected systems can include musculoskeletal, cardiac, neurological, and hepatic. The neuromuscular form of GSD IV varies greatly in onset (perinatal, congenital, juvenile, or adult) and severity. This variant explained the patient's phenotype. This patient was also a carrier for a pathogenic heterozygous variant in *CAPN3* (OMIM #114240) related conditions. This result is important for the family and has future reproductive implications. This case therefore demonstrates the importance of NGS for future family planning.

When it is not cerebral palsy

A 4-year old female was referred to the paediatric neurology outpatients service as she and two of her siblings were labelled as having cerebral palsy. The family was visiting the Western Cape Province and presented to Tygerberg Hospital when the patient and one of her siblings developed pneumonia. The treating clinician was concerned that they might have a cerebral palsy mimic as there was no past birth or medical history or radiological findings to correlate with the clinical findings of severe global developmental disability, seizures and upper motor signs. A cerebral palsy spectrum panel was performed and quinoid dihydropteridine reductase (*QDPR)* (homozygous) pathogenic variant was found. This is a treatable condition using tetrahydrobiopterin (BH4*)* (12)This result could have a transformative impact on this family and their relatives and newborns can be tested immediately and started on BH4 as soon as possible after birth, thereby optimising their clinical and neurological outcomes. Patient 94 unfortunately passed away shortly after her return to the Eastern Cape.

A variant with possible adult-onset disease

A 2-year old female was referred to the paediatric neurology outpatients service via the Audiology Department where she was followed-up for profound bilateral sensory-neural hearing loss requiring

cochlear implants. She also presented with acquired microcephaly, global developmental delay and marked dystonia. MRI brain was normal and the Cerebral Palsy Spectrum Disorders panel was requested. One pathogenic variant was identified in *ATM* Serine/Threonine Kinase (*ATM*; OMIM *#*607585)*)* (heterozygous*).* The autosomal dominant form of *ATM (*OMIM #114480) is associated with predisposing to breast cancer and autosomal-recessive inheritance is associated with ataxia-telangiectasia (AT; OMIM #208900). A second disease-causing variant was not identified and clinically, she did not have features of AT. Therefore, the clinical phenotype cannot be explained at this time. However, the patient may be at increased risk for adult-onset breast and ovarian cancers. This is a secondary finding, because of broad testing. We would normally not intentionally test minors for adult-onset diseases, but in this case biallelic pathogenic variants in *ATM* also result in a childhood-onset disorder (AT; OMIM #208900). The family has received genetic counselling to explain the result.

New-onset refractory status epilepticus (NORSE)

A previously well, developmentally age-appropriate teenager presented with NORSE. MRI brain, metabolic, infectious and autoimmune investigations all proved normal. Electroencephalogram (EEG) video telemetry confirmed left frontal focal-onset seizures with secondary generalisation. Initially, the seizures proved refractory to sodium valproate, levetiracetam, lamotrigine and phenytoin. Fortunately, immunomodulation with corticosteroids and cyclophosphamide brought about marked reduction in the seizure frequency. The Epilepsy panel revealed a heterozygous pathogenic variant in Nitrogen Permease Regulator-like3 (*NPRL3;* OMIM *#* 600928). The latter is an important component of the GATOR1 complex and its mutations can promote the activity of the mTOR signalling pathway, thereby causing epilepsy. Sleep related hyper motor epilepsy and frontal lobe epilepsy are the most common clinical presentations, always with a high rate of drug resistance. Identifying the pathogenic variant allowed for a better understanding of the clinical phenotype of *NPRL3* related epilepsies. Moreover, the underlying molecular mechanism suggests that mTOR inhibitors such as rapamycin and sodium channel blockers (carbamazepine) may offer potential targeted treatment for drug-resistant cases.

Baby with an excessive startle response

A 3-month old baby with presumed epilepsy was found to have an exaggerated startle response. The Epilepsy panel revealed one pathogenic variant as well as one VUS in Solute Carrier Family 6 Member 5*(SLC6A5*: OMIM # 604159*).* Family testing was performed to assist in phasing the variants, and the mother was found to be a carrier of the pathogenic variant in *SLC6A5*. The father was not available for testing. However, as the results fit the clinical picture of the patient, we are treating her as having Hyperekplexia (OMIM # 614618), and she is currently doing well on a benzodiazepine and behavioural adjustments e.g., avoiding load noise. VUS resolution may be possible with additional information in future.

Utility to the parents

Parents of patients with positive results were given the opportunity to partake in this study by voluntarily answering a six-question questionnaire (Supplementary Table 2). The majority of parents felt that

knowing the result brought closure and that if prenatal testing should be available, they would make use of it in future pregnancies. In almost half of the cases, treatment was adjusted to some extent once the pathogenic variant was known. Treatment changes included adjusting anti-seizure medications eg stopping sodium channel blockers like Lamotrigine in patients with *SCN1A* or adding sodium channel blockers like carbamazepine in patients with *KCNQ2* pathogenic variants (4). The questionnaire was given to parents only and not the treating physicians therefore full details of treatment changes were not covered by this study.

Strengths and limitations

The study was unique to a specific population of patients from a single centre. VUS resolution was not performed on all patients, as either one (especially the father) or both biological parents were not available to provide samples. As this was a gene panel testing approach, the analysis was limited to the genes in the panel and the diagnostic assays used. For the genes included in the panel, certain types of variants may be missed (intronic, structural, some deletions/duplications). In addition, cost limitations prohibited more detailed genetic testing (for example, exome or genome for patients with a negative result) or further functional analysis (for example, RNA sequencing or *in-vitro* ion channel analysis for patients with VUSs in ion-channel genes).

Despite these limitations, our study provides insights into the diagnostic yield of NGS in a resource-constrained, previously non-investigated, LMIC setting with high levels of genetic diversity. Testing allowed clinicians to optimise genetic counselling, patient care and prognostication when pathogenic variants were identified. Caregivers were able to receive closure and make plans for their families.

Conclusion

The study enforces that NGS testing is achievable in resource-constrained settings. The high diagnostic yield in this study suggests that it is feasible to recommend NGS as a first-tier testing approach for children with neurological disorders.

Declarations

Declaration of competing interests

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Acknowledgements

We thank all the families involved in this study and the clinicians who care for them. This study was undertaken as part of an MPhil thesis (MvN) supervised by RS, RvT and SM. SM is supported by the

South African Medical Research Council through its Division of Research Capacity Development under the Early Investigators Programme from funding received from the South African National Treasury. The content hereof is the sole responsibility of the authors and do not necessarily represent the official views of the SAMRC.

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