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ORIGINAL ARTICLE

THE UTILITY OF WHOLE EXOME SEQUENCING IN DIAGNOSING PEDIATRIC NEUROLOGICAL DISORDERS

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ABSTRACT

Pediatric neurological disorders have a wide spectrum of clinical presentations and can be challenging to diagnose. Whole exome sequencing (WES) is increasingly becoming an integral diagnostic tool in medicine. It is cost-effective and has high diagnostic yield, especially in consanguineous populations. This study aims to review WES results and its value in diagnosing neurological disorders. A retrospective chart review was performed for WES results between the period of January 2018 to November 2019. Whole exome sequencing was requested for children with unexplained neurological signs and symptoms such as epilepsy, developmental delay, visual impairment, spasticity, hypotonia and magnetic resonance imaging (MRI) brain changes. It was conducted for children in a pediatric neurology clinic of a tertiary center at Jeddah, Saudi Arabia. Twenty-six children with undiagnosed neurological conditions were identified and underwent WES diagnosis. Nineteen patients (73.0%) of the cohort were diagnosed with pathogenic variants, likely pathogenic variants or variants of unknown significance (VUS). Consanguinity was positive in 18 families of the cohort (69.0%). Seven patients showed homozygous mutations. Five patients had heterozygous mutations. There were six patients with VUS and six patients had negative WES results. Whole exome sequencing showed a high diagnostic rate in this group of children with variable neurological disorders.

Keywords: Consanguinity; Developmental delay; Neurological disorders; Saudi Arabia; Whole exome sequencing (WES).

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INTRODUCTION

Childhood neurological disorders are a vast group of heterogenous conditions with a myriad of clinical presentations. Reaching a diagnosis can be challenging, time-consuming and costly. Sometimes, multiple imaging, laboratory investigations and ancillary procedures are exhausted trying to reach a final diagnosis [1]. Families also suffer from the dilemma of multiple investigations. Many neurological conditions in children present with variable degrees of presentations as well as different stages of progression, thus, multiple metabolic and genetic investigations are requested. Recently, whole exome sequencing (WES) has become an important diagnostic tool for many presumed genetic or idiopathic neurological conditions [2]. Whole exome sequencing is becoming rapidly available, cost-effective and can be a shortcut to the diagnosis. Reaching a diagnosis in a child with a neurological disorder, counseling parents and preventing recurrences of similar conditions in his/her family is the essence of pediatric neurology practice. Nowadays, phenotype-based genetic testing and panels are slowly being substituted by WES [3]. The depth of screening using WES and variant coverage as well as identification of novel and pathogenic variants is rapidly improving. The increasing rate of WES sensitivity, expanding genetic databases, shorter turn-around time and decreasing prices of WES are appealing and promising [4].

In the Middle East region and Africa, consanguinity is common. In Saudi Arabia, 52.0-67.0% of marriages are from the same family and tribe [5,6]. This cultural practice can be associated with a higher percentage of neurogenetic and metabolic conditions [7]. The diagnostic yield of WES can range from 20.0 to 70.0% with a higher yield in consanguineous populations [8-12]. Few studies in the Middle East reviewed the outcomes of WES in children with neurological disorders in a population with

high consanguinity rate such as Saudi Arabia [13-16]. Such characteristics could increase the yield of WES. In this cohort, the clinical characteristics and WES results of children with variable neurological disorders in Saudi Arabia are reviewed.

MATERIAL AND METHODS

A retrospective chart review for 26 children with undiagnosed neurological conditions was performed from January 2018 to November 2019. These neurological disorders ranged between developmental delay, hypotonia, epilepsy, loss of vision, ataxia, white matter changes, intellectual delay, encephalopathy and metabolic abnormalities. This review was conducted for children attending the pediatric neurology clinic at King Abdulaziz University, Jeddah, Saudi Arabia. Medical history, patients' demographics, history of consanguinity (second cousin or closer), clinical examination and neuroimaging were reviewed and documented. All patients underwent proband-only WES. Further segregation analyses were performed for parents planning future pregnancies. Whole exome sequencing was performed in an accredited laboratories, Centogene in Rostock, Germany and Blueprint laboratories in Helsinki, Finland. Variant classifications into five classes were based on American College of Medical Genetics and Genomics (ACMG) guidelines [17] (Table 1). All patients signed a consent form for WES. The study was approved by the King Abdulaziz University Research Ethics Committee.

RESULTS

There were 19 males and seven females included in the study. Age of presentation ranged from 1 to 12 years (mean 4.8 years). Nineteen patients had positive WES results (73.0%), five patients with variants of unknown significance (VUS) that matches the clinical picture, one patient with VUS that does not explain the observed clinical characteristics. The remaining six patients were negative. The average age in the WES-positive group was 4.9 years and the average age in the WES-negative group was 4.5 years. Consanguinity was positive in 69.0% of the cohort, most of them were in the WES-positive group (Table 2).

The WES-positive cohort results were classified into pathogenic, likely pathogenic variants and VUS. In the homozygous and heterozygous groups, pathogenic and likely pathogenic mutations were found. These mutations, when paired with the phenotype, confirms the clinical picture (Tables 3 and 4). The VUS group was mainly clinically upgraded to clinically significant variants as those patients have matched genotype and phenotype. No benign or likely benign variants were detected in this cohort.

Table 1. American College of Medical Genetics and Genomics (ACMG) classification of variants [17].

Class 1	Pathogenic
Class 2	Likely pathogenic
Class 3	Variant of uncertain significance (VUS)
Class 4	Likely benign
Class 5	Benign

Table 2. Cohort demographics: WES positive and negative.

	Positive ***n*** **(%)**	**Negative** ***n*** **(%)**
Males	14	5
Females	6	1
Total	20 (77.0)[a]	6 (23.0)
Age (mean)	4.9 years	4.5 years
Consanguinity	14 (53.0)	4 (15.0)

[a] One of the 20 patients had positive WES results but does not explain the phenotype (patient 15 in Table 3).

Eight patients had homozygous mutations, six patients had heterozygous mutations and six patients were carrying VUS. All patients had variable neurological presentations such as: epilepsy, intellectual delay, motor delay, regression of milestones, hypotonia, visual abnormalities, ataxia and MRI brain changes. Similar family history in another sibling or relative was reported in seven patients with positive WES result.

One patient had *ITGA7* classified as VUS that does not explain the phenotype. He was developmentally normal then developed regression of milestones and a middle cerebral artery stroke after meningoencephalitis. An *ITGA7* mutation was reported to be associated with congenital muscular dystrophy, neonatal hypotonia, proximal atrophy and scoliosis ,which were not apparent in this patient before his regression.

Variants of unknown significance with consistent phenotypes were seen in five patients (Table 3). The *MLC1* mutation in patient #16 was consistent with megalencephalic leukoencephalopathy with subcortical cysts type 1. The patient developed ataxia and convulsions. Brain MRI also showed expected white matter changes and temporal cysts. Patient #17 had a *SLC6A3* mutation compatible with infantile Parkinsonism-dystonia type 1. He presented with orolingual, upper and lower limbs dystonia and was initially diagnosed as cerebral palsy. Patient #18 was diagnosed with intractable infantile spasms and failed to respond to multiple antiseizure medications for several months. He also developed regression of milestones. A *PNPO* mutation

Table 3. Homozyogous, heterozygous and variants of uncertain significance VUS groups.

#	Sex-Age	Family History	Consanguinity	Clinical Characteristics	Onset	Genes	Variant	Diagnoses
Homozygous Mutations								
1	F-6	yes	yes	motor delay; hypotonia; scoliosis; respiratory difficulties in neonatal period; normal congnitions, areflexia	1 year	*P1EZO2*	pathogenic: c.273_279del, p.(Pro92Thrfs*18)	piezo-type mechanosensitive ion channel component; OMIM: 613629
2	M-1	no	yes	severe hypotonia; reduced tendon reflexes; motor/speech delay; cerebellar atrophy; cerebellar cyst; elevated serum CPK	birth	*FKRP*	likely pathogenic: c.204del, p.(Ser69Profs*60)	MDDGA5 (congenital with brain/eye anomalies), type A5 (MDDGA5); OMIM: 613153
3	M-2	no	yes	vision loss; nystagmus; severe retinal dysfunction	2 months	*RPGRIP1*	pathogenic: c.1107del, p.(Glu370Asnfs*5)	Leber congenital amaurosis type 6, OMIM: 613826
4	M-7	no	yes	intractable epilepsy; global developmental delay; poor vision	3 years	*TPP1*	pathogenic: c.616C>T, p.(Arg206Cys)	neuronal ceroid lipofuscinosis type 2, OMIM: 204500
5	M-8	no	yes	poor hearing; encephalopathy; MRI: white matter changes	4 years	*BTD*	pathogenic: c.1618C>T, p.(Arg540Cys)	biotinidase deficiency
6	M-6	yes	yes	ataxia; delayed motor milestones; mild intellectual delay; MRI: cerebellar atrophy	1 year	*SPTBN2*	likely pathogenic: c.6258_6261delGAGA, p.(Lys2088Glyfs*228)	infantile-onset spinocerebellar ataxia type 5
7	F-8	yes	yes	ataxia; delayed motor milestones; mild intellectual delay; MRI: cerebellar atrophy	1 year	*SPTBN2*	likely pathogenic: c.6258_6261delGAGA, p.(Lys2088Glyfs*228)	infantile-onset spinocerebellar ataxia type 5
8	M-9	yes	yes	ataxia; oculomotor apraxia; telangiectasia; MRI: cerebellar atrophy	3 years	*ATM*	likely pathogenic: c.9066del, p.(Gly3023Alafs*10)	ataxia telangiectasis
Heterozygous Mutations								
9	F-7	no	no	delayed language/motor development; intellectual disability; hypotonia; generalized seizures; infantile spasms; visual impairment; normal MRI; normal metabolic profile	1 year	*NTRK2*	pathogenic: c.1301A>G, p.(Tyr434Cys)	early infantile epilectic encephalopathy type 58, OMIM: 617830
10	M-3	yes	no	acute necrotizing encephalopathy; generalized seizures; spasticity; coma and death; brain MRI: symmetric thalamic hyperintense lesions	3 years	*RANBP2*	pathogenic: c.1754C>T, p.(Thr585Met)	acute infection-induced encephalopathy-type 3, OMIM: 608033
11	M-4	no	no	developmental delay; neonatal hypotonia; autistic-like behavior; epilepsy	1 year	*SHANK3*	likely pathogenic: c.2313+1G>A	Phelan-McDermid syndrome, OMIM: 606232
12	M-9	no	no	ataxia; ADHD; delayed speech/ language development; motor delay; hypotonia; normal EEG and brain MRI	2 years	*KAT6A*	likely pathogenic: c.1483-1G>A	mental retardation type 32, OMIM: 616268
13	M-1	no	no	intractable neonatal seizures; normal brain MRI	1 month	*PACS2*	pathogenic: c.625G>A, p.(Glu209Lys)	early infantile epileptic encephalopathy type 66, OMIM: 618067
14	F-4	no	yes	intractable focal seizures; normal brain MRI	3 months	*SCN1A*	likely pathogenic: c.1377G>C, p.(Gln459His)	early infantile epileptic encephalopathy type 6 (Dravet syndrom), OMIM: 607208
Variant(s) of Uncertain Significance								
15	M-6	no	yes	MCA stroke; dystonia; spasticity; regression of milestones; delayed language/ motor development; focal seizures and abnormal brain myelination on MRI	4 years	*ITGA7* AR	c.1601C>T, p.(Ala534Val)	congenital muscular dystrophy/hypotonia, OMIM: 613204

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16	F-5	no	yes	ataxia; frequent falls; macrocephaly, epilepsy and ADHD; MRI: megalencephalic leukoencephalopathy with subcortical cysts	2 years	*MLC1* AR	c.275C>A, p.(Pro92His)	megalencephalic leukoencephalopathy with subcortical cysts type 1, OMIM: 604004
17	M-3	no	yes	delayed speech/language development; dyskinesia; dystonia; infantile onset of the disease; paroxysmal dystonia; MRI: brain atrophy	2 years	*SLC6A3* AR	c.851G>A, p.(Gly284Glu)	infantile Parkinsonism dystonia type 1, DTDS, PMID: 21777827
18	M-1	yes	yes	intractable infantile spasms	3 months	*PNPO* AR	c.256T>C, p.(Cys86Arg)	PNPO, OMIM: 603287
19	F-6	yes	yes	ataxia and oculomotor apraxia; brain MRI: molar tooth sign	2 years	*CC2D2A* AR	c.916_927del, p.(Pro306_Leu309del)	Joubert syndrome type 9, OMIM: 612285
20	M-2	no	yes	fair hair; global developmental delay; hearing impairment; infantile onset of the disease; motor delay; muscular hypotonia; visual impairment; focal epilepsy; MRI: brain atrophy	3 months	*SPATA5* AR and *TIMMDC1*[a]	c.1058A>T, p.(Asp353Val) and c.230T>C, p.Ile77Thr)	EHLMRS, OMIM: 616577 mitochondrial complex I deficiency, OMIM: 618251

#: patient number; F: female; M: male; OMIM: Online Mendelian Inheritance in Man; CPK: creatine phosphokinase; MDDGA5: muscular dystrophy-dystroglycanopathy type A5; MRI: magnetic resonance imaging; ADHD: attention deficit hyperactivity disorder; EEG: electroencephalogram; MCA: middle cerebral artery; DTDS: dopamine transporter deficiency syndrome (DTDS); PMID: PubMed reference number; AR: autosomal recessive; PNPO: pyridoxamine 5'-phosphate oxidase; EHLMRS: epilepsy, hearing loss and mental retardation syndrome.

[a] Autosomal mitochondrial.

Table 4. Whole exome sequencing negative group.

#	Sex-Age	Family History	Consanguinity	Clinical Characteristics	Onset
1	F-1	no	yes	epilepsy and developmental delay; brain MRI: normal	4 months
2	M-7	no	yes	epilepsy and developmental delay; brain MRI: normal	2 years
3	M-2	no	no	intractable epilepsy; poor vision and global developmental delay; brain MRI: normal	1 year
4	M-12	no	yes	global developmental delay; brain MRI: basal ganglia enhancement; metabolic work-up: negative	6 years
5	M-4	yes	yes	intractable focal epilepsy and ADHD	1 year
6	M-2	no	yes	microcephaly; motor delay; brain MRI: white matter changes	6 months

MRI: magnetic resonance imaging; ADHD: attention deficit hyperactivity disorder.

was reported once. He was put on pyridoxal 5 phosphate cofactor therapy. No more seizures were reported by the parents and he is now developmentally up to age after 1 year of follow-up. Targeted analysis of both parents showed heterozygous mutations in both (Figure 1). Patient #19 was found to have ataxia and oculomotor apraxia. Brain MRI showed classic molar tooth sign. The *CC2D2A* mutation is associated with Joubert syndrome. Patient #20 had infantile onset of developmental delay, hearing impairment, hypotonia, visual impairment, focal epilepsy, high serum lactic acid and brain MRI showed brain atrophy. Mutations in *SPATA5* and *TIMMDC1* were reported to cause epilepsy, hearing loss, and mental retardation syndrome and mitochondrial complex I deficiency, respectively.

DISCUSSION

The introduction of WES in medicine has changed the way of physician's approach to patients. The number of newly diagnosed neurogenetic conditions and mutations are increasing.

Multiple studies worldwide reviewed WES findings, however, few in the Middle East region. In Saudi Arabia, the Arabic ethnic background is the main population structure. Consanguinity is common in marriages. Thus, more metabolic and neurogenetic conditions are prevalent in our region.

In this study, 19 patients (73.0%) out of 26 patients had genetically and phenotypically consistent findings. In the WES-positive group, consanguinity was present in

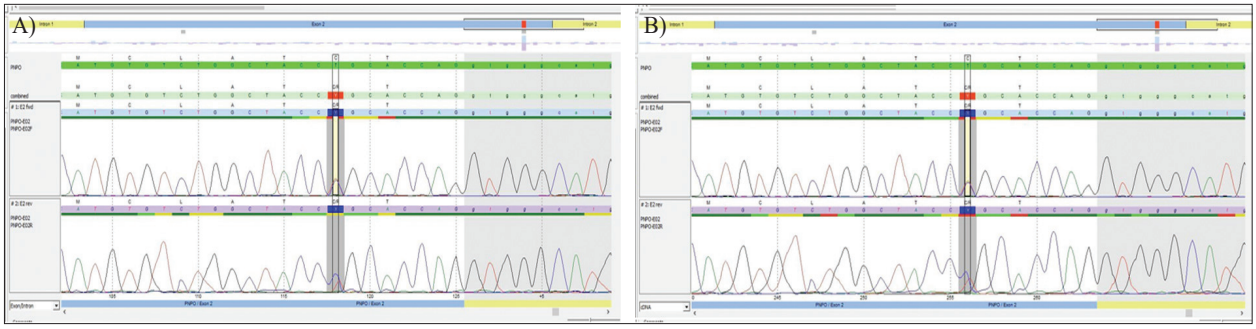

Figure 1. Parents of a child with an inherited *PNPO* gene mutation. They are cousins and both are heterozygous. A) Mother; B) father.

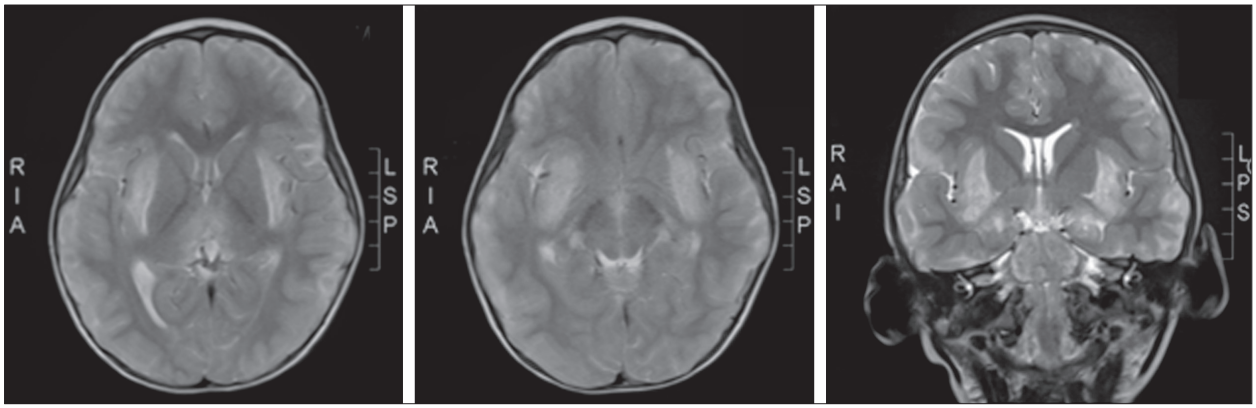

Figure 2. Brain MRI in a child with acute necrotizing encephalopathy and a positive *RANBP2* gene mutation. Brain MRI showing increased T2 hyperintensity of basal ganglia (A, B, and C).

53.0% of the families whereas in the WES-negative it was 15.0%. Compared to other studies in the region [18,19] 43.0-49.0% of WES results showed clinically significant results in Saudi Arabia. Other studies from the United Arab Emirates and Qatar, reached 54.0-68.0% genetically confirmed diagnoses [14,15]. Larger studies including 2000-3000 WES samples from different ethnic regions, showed a lower diagnostic rate, around 25.0% from the USA [11], Care4Rare Canada [20] and Finding of Rare Disease Genes (FORGE) [21]. Consanguinity was positive in 18 families of the cohort (69.0%). In other studies, a similar rate of 66.0-75.0% of consanguinity was reported [12,14]. The most common features of WES results in the Middle East are a high percentage of consanguinity and a higher positivity rate of WES results [22,23].

Whole exome sequencing has a direct impact on management [24]. For example, patient number #5 (Table 3) had a positive *BTD* mutation classified as pathogenic. He was 7 years old when diagnosed with biotinidase deficiency. His symptoms were noticed by his parents since he was 3 years old. He had hearing problems, ataxia, convulsions and intermittent encephalopathy. He was in coma and ventilatory-dependent when WES was requested. Once started on biotin supplements, he gradually started to improve. Currently, he is off the ventilator. He is redeveloping motor gains with physical therapy. He has not experienced any more convulsions and his hearing has improved.

Another patient in the cohort has a pathogenic *RANBP2* gene mutation. He presented with acute necrotizing encephalopathy (ANE) (Figure 2). A few months after being diagnosed, his sister was also diagnosed with clinical ANE. Unfortunately, both siblings died despite aggressive immunotherapy.

In conclusion, WES is an integral diagnostic tool in a pediatric neurology clinic. It is of great importance to unravel the diagnostic odyssey of many neurological and neurogenetic conditions. Family counseling, prevention of recurrence and treatment depends on proper genetic diagnoses. Pricing of WES is still a challenge at many centers and countries [25,26]. Sometimes, WES results can also take more than 2-3 months that can delay WES-focused medical care.

The high yield of WES results in this study, though a small number of patients, is compatible with similar studies. This study promotes performing WES in childhood neurological disorders, especially when these is a similar family history and positive consanguinity.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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