ORIGINAL ARTICLE

The Results of Whole Exome Sequencing Performed On Previously Undiagnosed Pediatric Neurology Patients

How to Cite This Article: Ahmadnia N, Beiraghi Toosi M, Ghayour Karimiani E, Ashrafzadeh F, Faraji Rad M. The Results of Whole Exome Sequencing Performed On Previously Undiagnosed Pediatric Neurology Patients. Iran J Child Neurol. Spring 2021; 15(2): 17-31

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Received: 20-Jul-2019
Accepted: 20- Jan-2020

Abstract

Objective

Whole exome sequencing (WES) is a new molecular diagnostic test, used in pediatric medicine, especially pediatric neurology. The diagnostic yield of WES is higher than conventional methods. Therefore, this study aimed to assess the diagnostic yield of WES in a pediatric neurology clinic and to report positive results.

Materials & Methods

This retrospective study was performed on patients, presenting to the pediatric neurology clinic of Ghaem Hospital in Mashhad, Iran, between March 2015 and March 2017, with various neurological disabilities and unrevealing workup before WES. The patients' clinical features and molecular diagnoses based on the WES results were reported in this study.

Results

The overall diagnostic yield of WES was 82.71% (67/81 patients). Two patients were excluded for the lack of data. Sixty-five patients with pathogenic or possibly pathogenic variants exhibited various abnormalities, including intellectual disability/developmental delay (n=44), seizure (n=27), developmental regression (n=11), myopathy (n=9), microcephaly (n=8), neuropathy (n=2), autism spectrum disorder (n=2), and neuromuscular disease (n=2). Overall, 93.84% of the patients were born to consanguineous parents. Also, 62 patients had an autosomal recessive disorder, and three patients had an autosomal dominant disorder.

Conclusion

The present findings indicating the high diagnostic yield of WES,

besides the important role of this test in determining the etiology of non-specific and atypical presentations of genetic disorders, support the use of WES in pediatric neurology practice.

Keywords: Whole exome sequencing; Diagnostic yield; Pediatric neurology

DOI:10.22037/ijcn.v15i2.26401

Introduction

The causes of neurodevelopmental disorders are not identified in many patients (1). Accurate genetic diagnosis can improve the patient's health by identifying, starting, or changing the existing treatments (2). Current diagnostic approaches, including clinical assessments, laboratory tests, chromosomal microarrays, single gene sequencing, and gene panel sequencing, are effective in 46% of patients in pediatric clinics (3). Also, these approaches are diagnostic in 25% of patients with neurodevelopmental disorders (2).

Whole exome sequencing (WES) is a new molecular diagnostic test, with a higher diagnostic yield than conventional methods (2). The diagnostic yield of WES is variable in heterogeneous groups of patients (2, 4). In previous studies evaluating heterogeneous groups of pediatric neurology patients, the diagnostic yield of WES was estimated at 49.1%, 41%, and 19%, respectively (5-7). Therefore, the present study aimed to assess the diagnostic yield of WES in our pediatric neurology clinic and to report positive results between March 2015 and March 2017.

Materials & Methods

In this retrospective study, we evaluated the medical records of patients, who presented to the pediatric neurology clinic of Ghaem Hospital in Mashhad, Iran between March 2015 and March 2017 and underwent WES. WES was ordered by pediatric neurologists when genetic causes were suspected based on their clinical judgment. We extracted and analyzed the patients' data, including age, sex, disease phenotype, family history, affected siblings, parental consanguinity, and gene variants. However, we excluded patients with insufficient medical records.

All families completed a written informed consent form before WES. This study was approved by the Ethics Committee of Mashhad University of Medical Sciences (code: IR.MUMS.fm.REC.1396.390).

Results

WES was performed on DNA of 81 patients, presenting to the pediatric neurology clinic of Ghaem Hospital in Mashhad, Iran between March 2015 and March 2017. An unknown genetic disorder was suspected in all patients before WES. Diagnostic evaluations, such as clinical assessments, biochemical and metabolic tests, imaging tests, biopsy, and genetic tests (i.e., karyotyping, chromosomal microarray, and single gene sequencing), had been carried out prior to WES for the majority of patients.

Genomic DNA was extracted from the peripheral blood samples using standard methods and sent

to laboratories for WES. The result of WES was approved by the pediatric neurologist who had visited the patient. A proven or hypothesized molecular effect related to the patient's phenotype was required in the genetic variant; also, the variant should follow the Mendelian inheritance model, consistent with the status of each affected family member. All results were confirmed via Sanger sequencing.

The overall diagnostic yield of WES was estimated at 82.71% (67/81 patients); therefore, 67 patients had pathogenic or possibly pathogenic variants. Two patients were excluded for the lack of data. Full clinical and molecular data of all 65 patients are presented in Table 1. Of 65 eligible patients, 39 (60%) were male, and 26 (40%) were female, with the mean age of 8.125.15± years.

Sixty-five patients with pathogenic or possibly pathogenic variants exhibited various abnormalities, including intellectual disability/ developmental delay (n=44), seizure (n=27), developmental regression (n=11), myopathy (n=9), microcephaly (n=8), neuropathy (n=2), autism spectrum disorder (n=2), and neuromuscular diseases (n=2). Moreover, of 65 eligible patients, 61 (93.84%) were born to consanguineous parents: first cousins, 48 (78.69%); second cousins, 6 (9.83%); and double cousins, 1 (1.64%). Forty-three (66.2%) out of 65 eligible patients had an affected sibling with a similar phenotype, although its severity varied.

Of 65 patients with a genetic diagnosis, 62 (95.39%) had an autosomal recessive disorder, and 3 (4.61%) had an autosomal dominant disorder. The variant types in 65 patients with a genetic diagnosis were missense (n=34), nonsense (n=12), frameshift (n=11), splice site (n=5), deletion (n=2), and insertion (n=1).

Table 1. Molecular and Clinical Features of Patients with a Genetic Diagnosis

	Age*	Sex	Phenotype	Affected Siblings	Parental Consanguinity	Genes And Variants/ Inheritance And Genotype	Associated Disorder Or Evidence Of Pathogenicity [OMIM]
1	15	F	Joubert syndrome	+	+ DC	TMEM67, c.725A>G, p.Asn242Ser/ AR HMZ	Joubert syndrome 6, [610688]
2	0.5	F	Lissencephaly posterior dominant type, cerebral hypoplasia, horizontal nystagmus and strabismus	-	+ FC	APC2, uc002lss.1:exon13:c.438_439del: p.s146fs* / AR HMZ	Sotos syndrome 3, [617169]
3	4	M	DD, drug-resistant epilepsy, FTT	+	+ FC	GAD2, c.C187A, p.63T/ AR HMZ	Neurodevelopmental disease [138275]
4	2	M	DD, microcephaly	+	+ SC	ASPM, c.9697C>T, (p.Arg3233Ter)/ AR HMZ	Microcephaly 5, primary, [608716]
5	4	F	Microcephaly, DD, spastic quadriplegia, Aqueductal stenosis, ventriculomegaly, FTT, bilat. Club foot	-	+ FC	MFSD2A, .2:exon11:c.C1010T:p. P337L/ AR HMZ	Microcephaly 15, primary, [616486]
6	13	F	ID, Epilepsy, dandy walker malformation, Corpus callosum Agenesis, XY in karyotype and no uterus	+	+ SC	TOE1:NM_025077:exon8:c.A1496G:p. H499R/ AR HMZ	Pontocerebellar hypoplasia, type 7, [614969]
7	9	M	ID, epilepsy, developmental regression since 5 y/o, optic nerve atrophy and cherry red spots around macula	+	+ FC	FLRT1, c.931C>T, p.Arg311Cys/ AR HMZ	Spastic paraplegia 68 (8)
8	12	F	L-2-hydroxyglutaric aciduria	+	+ FC	L2HGDH (NM_024884.2): c.[584A>G];[584A>G], p.[Tyr195Cys];[Tyr195Cys]/ AR HMZ	L-2-hydroxyglutaric aciduria, [236792]

9	3.5	M	Epilepsy started since 2y/o, Myo Myo syndrome stage III in brain MRA	-	+	GNB4 c.665dupt p.Thr233HisF fs*24/ AD HTZ	Charcot-Marie-Tooth disease, dominant intermediate F, [615185]
10	8	F	Progressive imbalance, talking difficulties, and spastic quadriplegia from 2 years ago, temporal disc paleness in eye examination, normal brain MRI	-	+ FC	FA2H, c.G772A, p.G258S, exon5/ AR HMZ	Spastic paraplegia 35, autosomal recessive, [612319]
11	12	F	ID, epilepsy, myopathy, marphanoid feature, scoliosis	+	+ FC	HERC1, NM_003922:exon38:c.7846+1G>A/ AR HMZ	Macrocephaly, dysmorphic facies, and psychomotor retardation, [617011]
12	15	M	Myopathy	+	+ FC	SYNE1, NM_033071:exon145:c. C25954T:p.R8652X/ AD HTZ	Emery-Dreifuss muscular dystrophy 4, autosomal dominant, [612998]
13	7	M	Macrocephaly, ID, leukodystrophy	+	+ FC	Del exon 2 of gene MLC1/ AR HMZ	Megalencephalic leukoencephalopathy with subcortical cysts, [604004]
14	1.5	M	GDD, Infantile spasms, seizure, developmental regression since 6m/o, cerebellar and cerebral atrophy in MRI	-	+ FC	HEXA, NM_000520:exon3:c.C409T:p. R137X/ AR HMZ	Tay-Sachs disease, [272800]
15	8	M	Microcephaly	+	+ FC	ASPM, c.2650-2651insg, p.(Lys884Argfs*15)/ AR HMZ	Microcephaly 5, primary, autosomal recessive, [608716]
16	2.5	M	GDD, congenital glaucoma, developmental regression since 1 y/o, leukodystrophy in brain MRI	-	+ FC	CYP1B1:NM_000104:exon2:c. G182A:p.G61E/ AR HMZ	Glaucoma 3A, primary open angle, congenital, juvenile, or adult onset, [231300]

17	3.5	M	GDD, Developmental regression since 3 y/o, Megalencephalic leukoencephalopathy with subcortical cysts	+	+ FC	HEPACAM:NM_152722:exon2:c.G100A:p.V34M/ AR HMZ	Megalencephalic leukoencephalopathy with subcortical cysts 2A, [613925]
18	4	M	DD, HMSN, anemia, elevated CPK	+	+ FC	MTMR2, c.1164 G>A, p.W388*/ AR HMZ	Charcot-Marie-Tooth disease, type 4B1, [601382]
19	17	M	Irritable myopathy	+	+	(GMPPB):c.859C>T (p.Arg287Trp) / AR HMZ	Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 14, [615352]
20	3	F	Neuronal ceroid lipofuscinosis, cerebellar atrophy in brain MRI	+	+ FC	TPP1:NM_000391:exon3:c.177_180del :p.e59fs/ AR HMZ	Spinocerebellar ataxia, autosomal recessive 7, [609270]
21	12	M	ID, Epilepsy, elevated CPK, mildly abnormal EEG	+	+ FC	COQ4:NM_016035:exon6:c.T611C:p.I204T/ AR HMZ	Coenzyme Q10 deficiency, primary, 7, [616276]
22	12	F	Imbalance and Ataxia, GDD, FTT	+	+ FC	SPG20, NM_015087, p.Ala442Pro/ AR HMZ	Troyer syndrome, [275900]
23	0.5	F	Epilepsy, normal brain MRI, her sister had ID	+	+ FC	HSPG2, c.11830G>A, p.Ala3944Thr/ AR HMZ	Schwartz-Jampel syndrome, type 1, [255800]
24	3	M	Charcot-Marie-Tooth disease	-	+ FC	GDAP1 (NM_018972.2): c.[154G>T];[154G>T], p.[(Glu52*)];[(Glu52*)]/ AR HMZ	Charcot-Marie-Tooth disease, [606598]

25	2	F	Hypotonia, DD, facial dysmorphism, loss of white matter, and thinning of the corpus callosum, respiratory distress, recurrent respiratory infections, Hepatosplenomegaly, Optic nerve atrophy, Hypothyroidism	-	+ FC	PPP1r21, c.1607dupt p.(Leu536Phefs*7)/ AR HMZ	Neurodevelopmental syndrome with symptoms of mild endosomal-lysosomal dysfunction (9)
26	9	M	ID, spastic quadriplegia, dystrophy of retinal cone cells, FTT, an elevated level of Arg and Ala in metabolic studies, hypospadias, bilateral hydrocele	-	+ FC	Deletion of the third exon of c12orf65/ AR HMZ	Spastic paraplegia 55, autosomal recessive, [615035]
27	6	M	ID, imbalance, episodic ataxia, developmental regression since 1.5 y/o, expired in 6 y/o	+	+ FC	ADPRHL2, (c.530C>T (p.Ser177Leu))/ AR HMZ	Neurodegeneration, childhood-onset, stress-induced, with variable ataxia and seizures, [617180]
28	12	F	Myopathy, brain MRI: vermis atrophy and mild lateral ventriculomegaly	+	+ SC	EXOSC3, {NM_016042.3} p.Asp132Ala/ AR HMZ	Pontocerebellar hypoplasia, type 1B, [614678]
29	8	M	Microcephaly, ID, Epilepsy, normal brain MRI	+	-	FDXR c.A791G p.D264G/ AR HMZ	Auditory neuropathy and optic atrophy, [617717]
30	5	M	DD, Epilepsy	+	+	AP3B2 {NM_004644.4} p.Arg67Ter/ AR HMZ	Epileptic encephalopathy, early infantile, [617276]
31	18	M	ID, Epilepsy	+	+ FC	ALDH5A1:NM_001080:exon9:c. T1397A:p.L466X / AR HMZ	Succinic semialdehyde dehydrogenase deficiency, [271980]

32	2	F	DD, drug-resistant epilepsy, Conductive hearing loss, developmental regression since 1 y/o	-	+ FC	PIGL p.Met244Leu/ AR HMZ	CHIME syndrome, [280000]
33	10	M	ID, drug-resistant epilepsy, floppy baby	+	+	ALDH5A1:NM_001080:exon9:c. T1397A:p.L466X / AR HMZ	Succinic semialdehyde dehydrogenase deficiency, [271980]
34	0.7	F	Microcephaly, GDD, drug-resistant epilepsy	- Affected cousin+	+ FC	SLC25A22, NM_001191060, exon7, c.458dela, p.q153fs / AR HMZ	Epileptic encephalopathy, early infantile, 3, [609304]
35	12	M	Progressive proximal weakness, vitiligo, DM I, muscle biopsy: muscular dystrophy with peripherally located large mitochondria	+	+ FC	CHKB, c.259T>C, p.Leu87Pro/ AR HMZ	Megaconial Congenital Muscular Dystrophy (10)
36	0.4	M	Myopathy, normal brain MRI, muscle weakness started since 2 months old	-	+ FC	TK2, c.388C>T, p.Arg130Trp/ AR HMZ	Mitochondrial DNA depletion syndrome 2 (myopathic type), [609560]
37	12	M	Myopathy, floppy baby since infancy, recurrent rhabdomyolysis	+	+ FC	ITGA7 RS760407686 NM_002206.2:c.2779C>T R [CGG] > W [TGG]/ AR HMZ	Muscular dystrophy, congenital, due to ITGA7 deficiency, [613204]
38	11	M	Distal sensorimotor peripheral polyneuropathy, axonal type, inability to walk from 2 years ago	-	+ FC	SBF2, (c.1395+1G>A)/ AR HMZ splicing mutation	Charcot-Marie-Tooth disease, type 4B2, [604563]
39	15	F	Epilepsy and ataxia since 7 y/o	+	+ FC	POLG, c.911T>G, p.Leu304Arg/ AR HMZ	Mitochondrial recessive ataxia syndrome, [607459]

40	9	M	ID, epilepsy	+	+ FC	PLXNB1, chr3, 48422209, A>T , splice region variant; intro variant; NMD transcript variant/ AR HMZ	Developmental disability (11)
41	6	F	ASD	-	-	PLXNB1, chr3, 48422209, A>T , splice region variant; intro variant; NMD transcript variant/ AR HMZ	Developmental disability (11)
42	18	M	ID, epilepsy started from 7 months, congenital disorder of glycolysation	+	-	ALG1, NM_019109:exon11:c. C1076T:p.S359L/ AR HMZ	Congenital disorder of glycosylation, type Ik, [608540]
43	12	M	ID, behavioral disorder, Microcephaly, Spasticity, no seizure occurred since 7 y/o	+	+ FC	AP4B1 NM_001253852:exon1:c.52_53del:p. c18fs/ AR HMZ	Spastic paraplegia 47, autosomal recessive, [614066]
44	11	F	Fahr's syndrome	-	+ FC	PDGFRB, c.2705C>T, c.2705C>T, p.Thr902Ile/ AD HTZ	Basal ganglia calcification, idiopathic, 4, [615007]
45	2.5	F	DD, epilepsy, movement disorder, normal brain MRI	-	+ FC	UNC80 c.4963C to T p.Arg1655Cys / AR HMZ	Hypotonia, infantile, with psychomotor retardation and characteristic facies 2, [616801]
46	5.5	M	DD, behavioral problems, amblyopia	+	+ FC	TMEM67 c.A725G p.Asn242ser/ AR HMZ	Joubert syndrome 6, [609884]
47	18	M	ID, epilepsy	+	+ SC	MRPS35:NM_001190864:exon3:c. G281A:p.G94D / AR HMZ	mitochondrial multisystem disorder [611995] intellectual disability (12)
48	15	F	ID, polydactyly, Bardet-Biedl syndrome	+	+ FC	BBS1, NM_018848.3, c.110A>G, p.(Tyr37Cys)-- c.882A>C, p.(Gln294His)/ AR HMZ	Bardet-Biedl syndrome 1, [209900]

49	2	F	GDD, congenital heart disease, hepatomegaly, congenital cataract	+	+ FC	HMBS, NM_001258209, c.C550T, p.R184W, rs118204109/ AR HMZ	Acute Intermittent Porphyria, [176000]
50	7	F	ID, Microcephaly, congenital cataract	+	+ FC	RAB3GAP2 NM_012414:exon21:c.2227dupt:p. w743fs/ AR HMZ	Warburg micro syndrome 2, [614225]
51	6	F	ID, Microcephaly, seizure and developmental regression since 1 y/o	+	+ FC	DPM3:NM_018973:exon1:c.A221G:p. Y74C / AR HMZ	Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 15, [612937]
52	10	M	ID, epilepsy, spasticity, developmental regression, brain atrophy in MRI	-	+ FC	NGLY1 {NM_001145293}:exon9:c. C1351T:p.R451X / AR HMZ	Congenital disorder of deglycosylation, [615273]
53	3.5	F	DD, dystonia, abnormal gait, developmental regression since 1.5 y/o	+	-	PANK2:NM_153638:exon2:c.734dupt :p.l245fs/ AR HMZ	Neurodegeneration with brain iron accumulation 1, [234200]
54	17	M	ID, drug resistance epilepsy, osteomalacia, mild atrophy of cerebellar vermis in brain MRI	+	+ FC	CACNA1A:NM_001127221:exon36:c. C5482G:p.H1828D / AR HMZ	Epileptic encephalopathy, early infantile, 42, [617106]
55	11	M	ID, ASD, drug-resistant epilepsy, FTT	+	+ FC	CHKB, c.844 dup, p.Cys282Leufs*/ AR HMZ	Muscular dystrophy, congenital, megaconial type, [602541]
56	5	M	DD, epilepsy, decreased visual acuity, ventriculomegaly, cortical cerebral atrophy, and cerebral dysgenesis	+	+ FC	USH2A, c.8713C/G p.His2905Asp/ AR HMZ	Usher syndrome, type 2A, [276901]
57	13	M	ID, epilepsy, FTT, normal brain MRI	+	+	TUSC3 {NM_006765}:exon2:c. C163T:p.Q55X/ AR HMZ	Mental retardation, autosomal recessive 7, [611093]

58	11	M	ID, obesity, congenital cataract, anemia, little sized testis	-	+ SC	ALMS1, c.3859_3860instat, {NM_015120.4}, Ser1286_ Gln1287insLeu*/ AR HMZ	Alstrom syndrome, [203800]
59	10	M	Neuromuscular junction disorder	-	+ SC	COLQ, c.444G>A, p.Trp148*/ AR HMZ	Myasthenic syndrome, congenital, 5, [603034]
60	13	F	Neuromuscular junction disorder	+	+ FC	CHRNE:NM_000080:exon12:c.1327del g:p.e443fs / AR HMZ	Congenital myasthenic syndrome, [100725]
61	9	F	ID, developmental regression, congenital blindness, strabismus, epilepsy started at 3 y/o	+	+ FC	LRP5 NM_02335 EX6 c.1042C>T p.R348W/ AR HMZ	Osteoporosis-pseudoglioma syndrome, [259770]
62	1	M	Muscular dystrophy, developmental regression since 2 mo/old, DD, resistant epilepsy, elevated CPK	+	+ FC	SGCA, c.718T>A, p.Gly91Ser/ AR HMZ	Muscular dystrophy, limb-girdle, autosomal recessive 3, [608099]
63	5	M	Megalencephaly, GDD	-	+ FC	MOCS1:NM_005943:exon6:c.G777C:p. K259N / AR HMZ	Molybdenum cofactor deficiency A, [252150]
64	1.5	M	Obesity, polydactyly, DD	-	+ FC	BBS12, c.954C>A, p.(Cys318*)/ AR HMZ	Bardet-Biedl syndrome 12, [615989]
65	10	F	Muscular dystrophy	-	+	CAPN3, c.2380+2T>G, rs761935462/ AR HMZ	Muscular dystrophy, limb-girdle, autosomal recessive 1, [253600]

*Age at referring to Genetic Clinics

M: male, F: female, ID: Intellectual Disability, DD: Developmental Delay, GDD: Global Developmental Delay, FTT: Failure To Thrive, ASD: Autism Spectrum Disorder, CPK: Creatine Phosphokinase, MRA: Magnetic Resonance Angiography, DMI: Diabetes Mellitus type 1, MRI: Magnetic Resonance Imaging, EEG: Electroencephalography, HMSN: Hereditary Motor and Sensory Polyneuropathy, FC: First Cousin, SC: Second Cousin, DC: Double Cousin

Discussion

The diagnostic yield of WES in the present study was remarkably high for 81 previously undiagnosed pediatric neurology patients, with a success rate of 82.71%. In heterogeneous pediatric neurology cohorts, the diagnostic yield of WES varies substantially. In this regard, a study on 78 children with undiagnosed neurological diseases showed that the diagnostic yield of WES was 41% (6). Moreover, in a neurogenetic clinic, the diagnostic yield of WES was 49% among 57 patients with various undiagnosed neurological diseases (5). Also, in a WES study of 118 patients with neurodevelopmental disorders, for whom conventional genetic tests were undiagnostic, 8% of cases had a mutation in known genes causing diseases, and 19% had a mutation in novel genes possibly causing diseases (7).

In a previous study evaluating 47 genetic centers, the success rate of WES was estimated at 22.8% (13). The diagnostic yield of WES was 25% in a study on 250 probands, who were referred from genetic, pediatric, and neurology clinics (14). Moreover, in a study on patients with neuromuscular diseases, associated with the parents' consanguineous marriage, the diagnostic yield of WES was 73.3% (3). The high diagnostic yield of WES in the present study, where 93.4% of the patients were born to consanguineous parents, could be attributed to our patient population, as WES was performed for those with highly suspected genetic factors.

As shown in Table 1, the same variant of TMEM67 gene was found in two patients (case 1 and case 46). The clinical and imaging findings suggested Joubert syndrome in case 1, whereas in case 46, developmental delay, behavioral disorder, and amblyopia were found, which are not the usual diagnostic signs of this disease. These findings indicate that WES can be utilized for identifying the unusual manifestations of rare diseases.

Eight different mutations have been found in Plexin B1 (PLXNB1) gene, five of which are considered pathogenic, and one is considered to be possibly pathogenic. However, there is not sufficient evidence to identify the phenotypes of these mutations (15). In the present study, we found the same mutation in two related patients with the central nervous system (CNS) involvement; however, their phenotypes were different (case 40 and case 41 in Table 1).

A mutation in the abnormal spindle-like microcephaly associated (ASPM) gene is the most common cause of genetic microcephaly. More than 100 mutations in this gene have been identified so far (16). We found two different variants of ASPM gene in two patients. The c.9697C>T (p.Arg3233Ter) variant was identified as a disease-causing variant (17), whereas the c.2650_2651insG (p.Lys884Argfs*15) variant was a novel mutation in our study.

In the present study, case 25 was one of the four patients, who had the same clinical manifestations and different mutations in the PPP1R21 gene. This patient had been evaluated for developmental delay since the age of eight months, and a thin cerebral cortex, especially in the frontotemporal regions, large lateral ventricles, thinning of the corpus callosum, and atrophy/hypoplasia of the optic nerve were detected in the brain MRI. The function of protein, translated from the PPP1R21 gene, was not definitely identified. However, based on previous studies, its deletion causes a specific neurodevelopmental syndrome with symptoms of mild endosomal/lysosomal dysfunction (9).

In routine clinical practice, indications for WES are not as complicated as the interpretation of its

results. Determination of the patient's phenotype is the first step in requesting WES. If the phenotype is compatible with a known syndrome, the next step is ordering conventional targeted genetic tests for evaluations. Based on the American College of Medical Genetics and Genomics (ACMG), WES should be performed for the following patients: (1) patients with a suspected genetic disorder, whose phenotype is not compatible with the suspected syndromes; (2) patients with clinical manifestations which have various genetic causes; and (3) patients without an acceptable cause for the phenotype (18). In this study, WES was requested after the routine diagnostic tests yielded unsuccessful results, while according to recent studies, it is more cost-effective if clinicians request WES for patients with a suspected genetic disorder in the primary visits (2, 9, 15, 19).

Despite the high diagnostic yield of WES, this test has several limitations. WES cannot identify large insertions and deletions, structural chromosomal rearrangements, trinucleotide repeats, epigenetic modifications, or mutations in non-exon regulatory regions. There are also technical limitations in sequencing mitochondrial DNA (5, 6, 20). Also, uncertainty about the function of possible genetic findings is another limitation of WES, which can lead to the misidentification of genes and misdiagnosis (5, 6). On the other hand, the neurodevelopmental phenotype of a patient is affected by many factors, such as complicated interactions between various genes and environmental factors. Due to the unavailability of WES in Iran, in the present study, the extracted DNAs were sent abroad for sequencing; therefore, establishing the final diagnosis can be time-consuming.

In conclusion

The diagnostic rate of WES was remarkably high in our pediatric neurology clinic, with a success rate of 82.71%. Considering the high diagnostic yield of WES, its application in prenatal care can expand the genetic knowledgebase; therefore, clinicians should consider requesting WES in their routine practice.

Acknowledgement

We appreciated all patients who participated in this study, and would like to thank of all technicians in genetic lab for doing tests and had very cooperation with children during sampling.

Author's Contribution

Negin AHMADNIA: Carried out the research and wrote the article.

Mehran BEIRAGHI TOOSI: Literature review and referred patients

Ehsan GHAYOUR KARIMIANI: Conceptualized the study, did the collection of data and reviewed articles.

Mohammad FARAJIRAD: Helped in literature review, and approved the final manuscript as submitted.

Farah ASHRAFZADEH: Conceptualized the study, reviewed articles and writing article

All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest

This study was carried out with no specific grant or funding. This article has not been previously published and/or presented as a poster or an oral presentation in any other national or international

meeting. All authors declare that there is no conflict of interest.

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