Osmangazi Tıp Dergisi
Osmangazi Journal of Medicine 2023;

Research Article / Araştırma Makalesi

Genetic Variants in Rare Diseases Identified by WES Analysis

Nadir Hastalıklarda Tüm Ekzom Dizileme Analizi ile Saptanan Genetik Varyantlar

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Abstract

Next-generation sequencing tests have become a part of the diagnostic process in most fields of medicine. Especially with whole-exome sequencing (WES) studies, the rate of diagnosis has increased in rare hereditary diseases. In this study, we aimed to present the results together with the clinical findings of 65 cases whose diseases are suspected to be of genetic origin. Between 2016 and 2019, patients who underwent WES testing in Bursa Yüksek İhtisas Training and Research Hospital Medical Genetics Unit were retrospectively screened and included in the study with their analysis results and clinical findings. In 27 of the 65 cases (41.5 %) included in the study, 30 significant variants were found in relation to their clinical findings. Twenty of these variants (66.7 %) have not been previously reported in literature. Rare diseases encountered in patients within a wide age range, from the fetus to 66 years of age, are presented along with their clinical findings and WES results. Thus, this study contributes to the mutation spectrum of hereditary diseases.
Keywords: Next generation sequencing, Novel, rare disease, Whole-exome sequencing

Özet

Yeni nesil dizileme testleri, tıbbın çoğu alanında tanı sürecinin bir parçası haline gelmiştir. Özellikle tüm ekzom dizileme (WES) çalışmaları ile nadir kalıtsal hastalıklarda tanı oranı artmıştır. Bu çalışmada, hastalıklarının genetik kökenli olduğundan şüphelenilen 65 olgunun sonuçlarını klinik bulguları ile birlikte sunmayı amaçladık. Bursa Yüksek İhtisas Eğitim ve Araştırma Hastanesi Tıbbi Genetik Birimi'nde, 2016-2019 yılları arasında WES testi ile değerlendirilen hastalar retrospektif olarak taranarak analiz sonuçları ve klinik bulguları ile birlikte çalışmaya dahil edildi. Çalışmaya dahil edilen 65 olgunun 27'sinde (% 41.5) klinik bulgularıyla ilişkili 30 anlamlı varyant bulundu. Bu varyantların 20'si (% 66.7) daha önce literatürde bildirilmemişti. Fetustan 66 yaşına kadar geniş bir yaş aralığındaki hastalarda görülen nadir hastalıklar klinik bulguları ve WES sonuçları ile birlikte sunulmuştur. Sonuç olarak bu çalışma ile kalıtsal hastalıkların mutasyon spektrumuna katkıda bulunulmuştur.
Anahtar Kelimeler: Yeni nesil dizileme, Yeni varyant, Nadir hastalık, Tüm ekzom dizileme

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Received 10.05.2022 Accepted 05.08.2022 Online published 25.08.2022

Gorukmez O, Genetic Variants in Rare Diseases Identified by WES Analysis , Osmangazi Journal of Medicine, 2023;45(1): 22-27
Doi: 10.20515/otd.1115008

1. Introduction

Over the past few decades, with the advent of next-generation sequencing (NGS) technologies, we have witnessed significant advances in molecular biology and genetics. With access to extensive genetic information, there have been important advances in diagnosis and treatment in many fields related to medicine. However, linking rare variations to a complex phenotype continues to be difficult (Jalkh et al., 2019). Indeed, determining the cause of a disease that is presumed to be of genetic origin requires a multidisciplinary approach that begins with the identification of phenotypic findings with a detailed family history. In some cases, functional studies are needed to establish a genotype-phenotype relationship (Soden et al., 2014). Approximately 80–85 % of the mutations known to cause Mendelian diseases are located in the coding and splicing regions of a gene. The whole-exome sequencing (WES) method, which scans the coding sequence including the splicing sites representing 1–1.5 % of the human genome, is a practical and cost-effective method (Dixon-Salazar et al., 2012). For this purpose, we aimed to retrospectively evaluate the results of WES analysis in 65 patients who were followed up for a long time and/or could not be diagnosed by routine methods (Karyotype, clinical microarray, capillary electrophoresis).

2. Materials and Methods

This study was approved by the local ethics committee (Bursa Yüksek İhtisas Training and Research Hospital) and complied with the principles of the Declaration of Helsinki. Patients who were evaluated at our medical genetics unit and underwent WES study from January 2016 to January 2019 were included in this study. WES applications were performed using the SureSelect XT Library Prep Kit (Agilent, Santa Clara, CA, USA) on the Novaseq platform (Illumina, San Diego, CA, USA). All bioinformatics analyses, variant filtering, and interpretation were performed on the Sophia DDM™ platform (Sophia Genetics, Saint Sulpice, Switzerland). All variants (Single nucleotide variants and small InDels) significant in terms of genotype-phenotype compatibility were confirmed by sequencing on a capillary electrophoresis device (Applied Biosystems™ 3500, Thermofisher, USA), and segregation analyses were performed (Supplementary material). Whether the variants were reported in the literature was checked with the Human Gene Mutation Database (HGMD; https://www.hgmd.cf.ac.uk). Classification of changes not reported in the literature was made according to American College of Medical Genetics and Genomics (ACMG) criteria (Richards et al, 2015).

3. Results

Patients who were evaluated at the Medical Genetics Unit of Bursa Yüksek İhtisas Training and Research Hospital and were suspected to have a genetic origin were assessed retrospectively. As the last step, the results of the patients who underwent diagnostic WES test (no pathology detected in routine cytogenetic, molecular cytogenetic, and molecular tests according to their preliminary diagnosis) were evaluated in the light of clinical findings, and significant changes were listed (Table 1). No study was conducted to research candidate genes in undiagnosed patients, and all detected variants were on previously identified genes. The ages of the 65 patients included in the study varied widely, and most of them were in the pediatric group. A total of 57 patients (one fetus and five newborns) were under 18 years of age. Sex distributions were close to each other (female: 33, male: 32). In terms of clinical features, most of the probands (29/65) were analyzed for neurological symptoms. Others applied to our unit for different complaints related to gastrointestinal (8), multiple (6), metabolic (4), skeleton (4), muscular (3), mitochondrial (3), immune (3), endocrine (2), cardiovascular (1), respiratory (1), and genitourinary (1) systems. There was consanguinity between the parents of 38 individuals, and 16 of them had a positive family history. Three of the remaining 27 individuals had a positive family history. Thirty different variants, mostly homozygous, explaining the clinical features, were detected in 27 patients.

Table 1. Demographic/clinical characteristics and identified variants in the study patients.

ID	Sex	Age[10,11]	Clinical Findings (Pre-diagnosis)	Gene (Transcript)	cDNA (Protein)	Type	Zygosity	R/N[1]	ACMG[2]	gnomAD[3]	Inherited from	OMIM[4]	C[5]	Family History	IP[6]
3	F[7]	8m	Cholestasis	*ABCB11* (NM_003742)	c.2448+1G>A	Splicing	Homozygous	N	P[12]	0	parents	Cholestasis, progressive familial intrahepatic 2 (MIM: 601847)	Yes	No	AR[16]
4	F	28y	Spastic gait, urinary incontinence, keratoconus	*SPG11* NM_001160227	c.200_203delCTTT (p.Ser67Ter)	Nonsense	Homozygous	N	P	2:216.866	parents	Spastic paraplegia 11, autosomal recessive (MIM: 604360)	Yes	Yes	AR
5	M[8]	15y 7m	Mental retardation, seizures, ataxia	*GAMT* NM_138924	c.327G>A (p.Lys109=)	Synonymous	Homozygous	Stöckler S, et al. (1996) Am J Hum Genet, volume:58, issue:5			parents	Cerebral creatine deficiency syndrome 2 (MIM: 612736)	Yes	Yes	AR
7	M	7y 5m	Progressive liver failure	*HSD3B7* (NM_025193)	c.45_46delAG (p.Gly17LeufsTer26)	Frameshift	Homozygous	Molho-Pessach V, et al. (2012) Hepatology, volume:55, issue:4			parents	Bile acid synthesis defect, congenital, 1 (MIM: 607765)	Yes	Yes	AR
11	F	12y 10m	Progressive encephalopathy, sensorineural hearing loss	*SUCLA2* (NM_003850)	c.751G>A (p.Asp251Asn)	Missense	Homozygous	Jaberi E, et al. (2013) J Hum Genet, volume:58, issue:8			parents	Mitochondrial DNA depletion syndrome 5 (MIM: 612073)	Yes	No	AR
12	F	5y	Microcephaly, progressive vision loss, hypotonia, irritability, cerebral atrophy	*PPT1* (NM_000310)	c.538dupC (p.Leu180ProfsTer9)	Frameshift	Homozygous	Kousi M, et al. (2012) Hum Mutat, volume:33, issue:1			parents	Ceroid lipofuscinosis, neuronal, 1 (MIM: 256730)	Yes	No	AR
16	M	6y 9m	Intellectual disability	*SHROOM4* (NM_020717)	c.3012C>A (p.Cys1004Ter)	Nonsense	Hemizygous	N	P	0	mother	Stocco dos Santos X-linked mental retardation syndrome (MIM: 300434)	No	No	XL[17]
17	F	11m	Hypotonia, hydrocephalus, lack of psychomotor development, corneal opacity, febrile convulsion, increased serum creatine kinase	*POMGNT2* (NM_032806)	c.791T>G (p.Ile264Ser)	Missense	Homozygous	N	UCS[14]	0	parents	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies, type A, 8) (MIM: 614830)	Yes	No	AR
19	M	3y 1m	Severe delayed psychomotor development, dysmorphism, operated for cleft palate	*PGAP3* (NM_033419)	c.496-39_498del (?)	Splice junction loss	Homozygous	N	P	0	parents	Hyperphosphatasia with mental retardation syndrome 4 (MIM: 615716)	Yes	No	AR
20	M	11y 7m	Feeding difficulties, rigidity, hypertonicity	*SLC6A3* (NM_001044)	c.1234T>G (p.Phe412Val)	Missense	Homozygous	N	UCS	0	parents	Parkinsonism-dystonia, infantile, 1 (MIM: 613135)	Yes	No	AR
22	F	11y 5m	Generalized hypotonia, muscle weakness, ptosis	*CHAT* (NM_001142934)	c.761T>C (p.Ile254Thr)	Missense	Homozygous	Kraner S, et al. (2003) Arc Neurol, volume:60, issue:5			parents	Myasthenic syndrome, congenital, 6, presynaptic (MIM: 254210)	Yes	Yes	AR
23	M	NB[9]	Hyperammonemia	*PCCA* (NM_000282)	c.1746+4A>G (?)	Splicing	Homozygous	N	UCS	0	parents	Propionic acidemia (MIM: 606054)	Yes	No	AR
25	M	9m	Cholestasis	*SCYL1* (NM_001048218)	c.460G>T (p.Glu154Ter)	Nonsense	Heterozygous	N	P	0	mother	Spinocerebellar ataxia, autosomal recessive 21 (MIM: 616719)	No	No	AR
					c.1577C>A (p.Ala526Asp)	Missense	Heterozygous	N	UCS	0	father				

28	F	1y 9m	Developmental and psychomotor delay	*GPR56* (NM_001145770)	c.898C>T (p.Gln300Ter)	Nonsense	Homozygous	N	P	0	parents	Polymicrogyria, bilateral frontoparietal (MIM: 606854)	Yes	No	AR
29	M	1y 5m	Febrile convulsion, oculomotor apraxia, polydactyly, syndactyly, global developmental delay, molar tooth sign (Joubert Syndrome)	*CPLANE1* (NM_023073)	c.3821G>T (p.Arg1274Ile)	Missense	Homozygous	N	UCS	0	parents	Joubert syndrome 17 (MIM: 614615)	Yes	No	AR
30	F	9y	Intellectual disability	*SON* (NM_001291411)	c.4895_4898delTAAC (p.Thr1633LeufsTer9)	Frameshift	Heterozygous	N	P	0	de novo	ZTTK[19] syndrome (MIM: 617140)	No	No	AD[15]
32	F	6m	Neonatal hypotonia	*AP4M1* (NM_004722)	c.1012C>T (p.Arg338Ter)	Nonsense	Homozygous	Tuysuz B, et al. (2014) Am J Med Genet A, volume:164A, issue:7			parents	Spastic paraplegia 50, autosomal recessive (MIM: 612950)	Yes	No	AR
34	M	39y	Paraparesis, ataxia	*GJB1* (NM_001097642)	c.271G>A (p.Val91Met)	Missense	Hemizygous	Bone LJ, et al. (1995) Neurology, volume:45, issue:10			mother	Charcot-Marie-Tooth neuropathy, X-linked dominant, 1 (MIM: 302800)	No	Yes	XLD[18]
35	F	4y 5m	Dystonia, loss of ambulation, basal ganglia abnormalities	*MECR* (NM_001024732)	c.772C>T (p.Arg258Trp)	Missense	Heterozygous	Heimer G, et al. (2016) Am J Hum Genet, volume:99, issue:6			father	Dystonia, childhood-onset, with optic atrophy and basal ganglia abnormalities (MIM: 617282)	No	No	AR
					c.1009C>T (p.Arg337Ter)	Nonsense	Heterozygous	N	P	3:250.898	mother				
39	M	39y	Proximal muscle weakness, difficulty walking, wheelchair dependent	*DYSF* (NM_001130976)	c.1622G>C (p.Arg541Pro)	Missense	Homozygous	N	LP[13]	0	parents	Muscular dystrophy, limb-girdle, autosomal recessive 2 (MIM: 253601)	Yes	Yes	AR
40	F	39y	Muscle weakness, wheelchair dependent	*TCAP* (NM_003673)	c.75G>A (p.Trp25Ter)	Nonsense	Homozygous	Chamova T, et al. (2018) Neuromuscul Disord, volume:28, issue:8			parents	Muscular dystrophy, limb-girdle, autosomal recessive 7 (MIM: 601954)	Yes	Yes	AR
42	F	26y	Growth retardation, Alopecia, Gonadal dysfunction,	*ANTXR1* (NM_018153)	c.152+1G>T (?)	Splicing	Homozygous	N	P	0	parents	GAPO syndrome (MIM: 230740)	Yes	No	AR
43	M	6y	Dextrocardia, situs inversus, recurrent respiratory infections	*CCDC151* (NM_145045)	c.703_704insACCTA (p.Ala235AspfsTer5)	Frameshift	Homozygous	N	P	0	parents	Ciliary dyskinesia, primary, 30 (MIM: 616037)	Yes	No	AR
44	F	3m	Protein losing enteropathy	*PLVAP* (NM_031310)	c.339dupT (p.Ala114CysfsTer9)	Frameshift	Homozygous	N	P	0	parents	Diarrhea 10, protein-losing enteropathy type (MIM: 618183)	Yes	No	AR
46	F	NB	Short stature, midface hypoplasia, anal atresia	*HSPA9* (NM_004434)	c.376C>T (p.Arg126Trp)	Missense	Homozygous	Royer-Bertrand B, et al. (2015) Sci Rep, volume:5 article:17154			parents	Even Plus Syndrome (MIM: 616854)	Yes	Yes	AR
55	M	1m	Hypotonia, increased serum creatine kinase, cerebellar atrophy, patent foramen ovale, epilepsy, feeding difficulties	*GMPPB* (NM_013334)	c.316G>C (p.Val106Leu)	Missense	Heterozygous	N	UCS	0	father	Muscular dystrophy-dystroglycanopathy, type A, 14 (MIM: 615350)	No	Yes	AR
					c.1162T>G (p.Ter388Gly)	Stop loss	Heterozygous	N	UCS	0	mother				
56	F	14y 6m	Aplasia cutis congenita of the scalp, dysmorphism, psychomotor retardation (Adams Oliver Syndrome)	*DOCK6* (NM_020812)	c.1963G>A (p.Gly655Ser)	Missense	Homozygous	N	UCS	0	parents	Adams-Oliver syndrome 2 (MIM: 614219)	Yes	Yes	AR

1R/N: reported/novel; 2ACMG: American College of Medical Genetics and Genomics; 3gnomAD, Genome Aggregation Database; 4OMIM, Online Mendelian Inheritance in Man; 5C, consanguinity; 6IP, inheritance pattern; 7F, female; 8M, male; 9NB, newborn; 10y, year; 11m, month; 12P, pathogenic; 13LP, likely pathogenic; 14UCS, uncertain significance; 15AD, autosomal dominant; 16AR, autosomal recessive; 17XL, X-linked; 18XLD, X-linked dominant; 19ZTTK, Zhu-Tokita-Takenouchi-Kim.
Note: The classification of novel variants according to the ACMG and their frequencies in gnomAD are given. Reference publications have been listed for the reported variants.

4. Discussion

In the current study, WES results of patients who were consulted at our center from different units are presented. In 27 of 65 probands, we identified 30 different variations in known disease genes in the Online Mendelian Inheritance in Man (OMIM; https://www.omim.org/) database. Although it varies between studies, the diagnostic rate of WES is approximately 25–30 % (Bhatia et al., 2021). In our study group, the diagnostic rate was 41.5 %. Although we predicted their diagnosis, some patients were tested with WES since their genetic etiologies are quite heterogeneous. Because for these patients, the study of candidate genes sequentially or as a panel would increase the cost and cause a waste of time. Therefore, we think that our diagnosis rate is higher than reported in other studies.

In a study of 213 cases, the rate of novel variants was reported to be 69.5 % (Nair et al., 2018). In another study including 200 patients, this rate was found to be 66.9 % (Jalkh et al., 2019). Twenty of the detected variants have not previously been reported in the literature. Our rate of novel variants was 66.7 % and compatible with the literature. This study has shown us that the variants detected at the time of diagnosis in NGS-based applications such as WES which gives us a broad perspective compared to other techniques are likely to be novel.

Most of our cases showed a recessive inheritance pattern. In societies where consanguineous marriages are common, it is known that the frequency of homozygosity is high (Monies et al., 2017). In the light of this information, 24 of the diseases detected in our 27 probands were autosomal recessive, and 21 of them were homozygous for the variants identified in associated recessive disease genes.

WES is an appropriate NGS test for individuals or families whose etiology is thought to have a possible monogenic disorder (Ormondroyd et al., 2017). In our study, carried out in parallel with this aim, very rare diseases were diagnosed. In fact, the syndromes associated with genetic disorders detected in cases 44 and 35 have only been reported as case studies in the literature. Therefore, these cases have been previously contributed to the literature (Gorukmez et al., 2019; Gorukmez et al., 2019). In addition to these patients, we found pathogenic variants in the *SHROOM4* and *SON* genes, which are associated with Stocco dos Santos X-linked mental retardation syndrome and Zhu-Tokita-Takenouchi-Kim syndrome, respectively, which are extremely rare in the literature. Although the diseases observed in 26 individuals evaluated in our study were induced by Mendelian inheritance, a 9-year-old female patient carrying a pathogenic variant of the *SON* gene was sporadic, and this was the only case of a *de novo* pathogenic variant. Another rare occurrence that causes symptoms in independent systems is *SCYL1* gene mutation. Biallelic mutations in the *SCYL1* gene, under the title of spinocerebellar ataxia in OMIM, also cause hepatic problems and skeletal anomalies. This gene is also listed among the familial causes of intrahepatic cholestasis in current publications (Li et al., 2021). Compound heterozygous changes in the *SCYL1* gene were found in a 9-month-old male patient with cholestatic liver disease findings. It should be noted that in defects of this gene whose molecular pathogenesis is unclear, extrahepatic findings will emerge at a later period; therefore, our patient will need to be followed up for a long time (McNiven et al., 2021).

An interesting situation was that individuals 39 and 40 were married. Despite their kinship, both were wheelchair-dependent because of pathologies in different genes. Their situation became clear through WES analysis and family studies.

In this study, based on the variants detected in patients, we had the opportunity to diagnose other family members who were not evaluated earlier and showed similar characteristics to their

probands. This shows the importance of family screening, along with segregation analysis. Thus, while clarifying our definition, we simultaneously identified other sick individuals. This was also an important element in detailed genetic counseling.

5. Conclusion

Finding the cause of the disease using tests that provide high-level data on the human genome, such as WES, can be expressed as "looking for a needle in a haystack" which is frequently used worldwide. Clinical and laboratory findings, segregation and family studies, multidisciplinary approaches, and functional studies are the most important approaches to facilitate this process (Manolio et al., 2013; Bowdin et al., 2015). In particular, rare syndromes and the presentation of data related to them will shed light on future studies. Hence, in this study, we presented variants of 27 different genes that cause rare diseases.

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Ethics

Ethics Committee Approval: The study was approved by Bursa Yüksek İhtisas Training and Research Hospital Ethical Committee (Number: 2011-KAEK-25 2019/08-01, Date: 07.08.2019).

Informed Consent: The authors declared that it was not considered necessary to get consent from the patients because the study was a retrospective data analysis.

Authorship Contributions: Surgical and Medical Practices: ÖG. Concept: ÖG. Design: ÖG. Data Collection or Processing: ÖG. Analysis or Interpretation: ÖG.Literature Search: ÖG. Writing: ÖG.

Copyright Transfer Form: Copyright Transfer Form was signed by all authors.

Peer-review: Internally peer-reviewed.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure:. I thank all our patients and their relatives included in the study for their compliance with routine examinations.