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ARTICLE

Secondary findings in 622 Turkish clinical exome sequencing data

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Abstract

CES (Clinical Exome Sequencing) is a method that we use to diagnose rare diseases with nonspesific clinical features. Besides primary indication for testing genetic information may be detected about diseases which have not yet emerged. ACMG guidelines recommend to report pathogenic variations in medically actionable 59 genes. In this study we evaluated CES data of 622 cases which were tested for various indications. According to ACMG recommendations 59 genes were screened for reportable variations. The detected variations were reviewed using distinct databases and ACMG variation classification guidelines. Among 622 cases 13 (2.1%) had reportable variations including oncogenetic, cardiogenetic disorders, and malignant hyperthermia susceptibility-related genes. In 15 cases (2.4%) heterozygous pathogenic and likely pathogenic variations were detected in genes showing autosomal recessive inheritance. Ten novel variations causing truncated protein or splicing defect were reported. We detected 11 variations having conflicting interpretations in databases and 30 novel variations, predicted as likely pathogenic via insilico analysis tools which further evaluations are needed. As to our knowledge this is the first study investigating secondary findings in Turkish population. To extract the information that may lead to prevent severe morbidities and mortalities from big data is a valuable and lifesaving effort. Results of this study will contrbute to existing knowledge about secondary findings in exome sequencing and will be a pioneer for studies in Turkish population.

Background

Advances in molecular genetic methods allow us to obtain high input data about human genome and diseases. It became easier and faster to screen the diseases having high genetic heterogenity or nonspecific findings to diagnose using these high input methods such as whole genome sequencing, whole exome sequencing (WES), and clinical exome sequencing (CES). Also these methods may disclose variations in life-threatening and/or preventable disease causing genes unrelated to primary disease that causes the patient to undergo genetic testing. Genetic conditions may concern all family members therefore to report the variants of proband which of most are underage may bring out ethical dilemmas.

In 2013 American College of Medical Genetics and Genomics Working Group published recommendations on incidental findings in clinical exome and genome sequencing. According to this report it is recommended to report for most genes, variants only known cause of the related disease (Known Pathogenic: KP) or variants that are previously unreported but are of the type that is expected to cause the disorder (Expected Pathogenic: EP), as defined by prior ACMG guidelines regardless of the age of the patients. In this publication variations in 56 genes associated with 24 conditions causing severe morbidity or mortality risk if not recognized were recommended [1]. In 2016 name of this findings revised as "secondary findings" and updated the gene list that one gene removed (*MYLK*) and four (*BMPR1A*, *SMAD4*, *ATP7B*, *OTC*) were added [2]. Majority of this list consists of malignacies and cardiac disorders

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such as arrhythmia and cardiomyopathy which may result in sudden death, related genes [3].

Following ACMG recommendations several studies have been published reporting secondary findings in distinct ethnicities. Evaluation of 56 ACMG genes in Phase I of 1000 Genomes dataset revealed the frequency of secondary findings as 1% (12/1092) in 1092 unrelated individuals. In this study majority of the identified medically actionable findings were in individuals from the European (5/379), American (4/181) ancestry groups, with fewer findings in Asian (2/286) and African (1/246) ancestry groups [4]. In another study including 954 East Asians SFs were detected 2.5% of cases [5]. Amendola et al. reported SF frequency 0.7% and 0.5% in 4300 European and 2203 African-ancestry participants respectively [6]. One of the biggest reported data from 50.726 WESs revelaed the frequency of pathogenic or likely pathogenic variations that meet criteria for clinical action as 3.5% [7].

The variant spectrum of the genes and variant frequencies may vary according to ancestry. In this study we aimed to determine the prevalence of SFs in CES datas in Turkey population. This is the first report of SFs in Turkey population therefore it will contribute to database and could be used as a guide for clinical genetic testing practitioners.

Materials and methods

Patients

CES data of 622 patients with diagnosis of distinct disorders including neurodevelopmental delay, epileptic encephalopathy, nonsyndromic deafness, retinal disorders, metabolic and endocrinologic disorders, skeletal disorders between 2017 and 2020 were evaluated retrospectively in the study. All patients or guardians of patients were informed face to face and written informed consents were obtained. The study was approved by the Ethics Committee of Marmara University.

Molecular study

From every patient 2 ml of peripheral blood samples were collected into EDTA tubes and stored at −20 °C. Genomic DNA was isolated from peripheral blood leukocytes using QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocols. All coding exons and exon-intron boundaries of 4493 genes were amplified using Clinical Exome Solution v2 kit (SOPHiA Genetics,Boston USA). Prepared library was sequenced on the Illumina NextSeq platform (Illumina Inc., San Diego, CA, USA).

Data analysis and variant classification

The data were analyzed using Sophia DDM data analysis software (SOPHiA Genetics, Boston USA). Coverage was 99.4% at a minimum depth of 20 reads in 59 ACMG recomended genes. In order to call variants sequencing data was aligned to human reference genome, hg19. All variations in exons and exon-intron boundaries having allele frequency (MAF) under 0.01 in GnomAD and affecting protein sequence with a minimum read depth of 20 were evaluated [8]. Numbers and cathegorization of variations are shown in Fig. 1. The Human Gene Mutation Database Professional (HGMD, 2020) and ClinVar databases were screened for the KP variants [9, 10]. The variants explaining

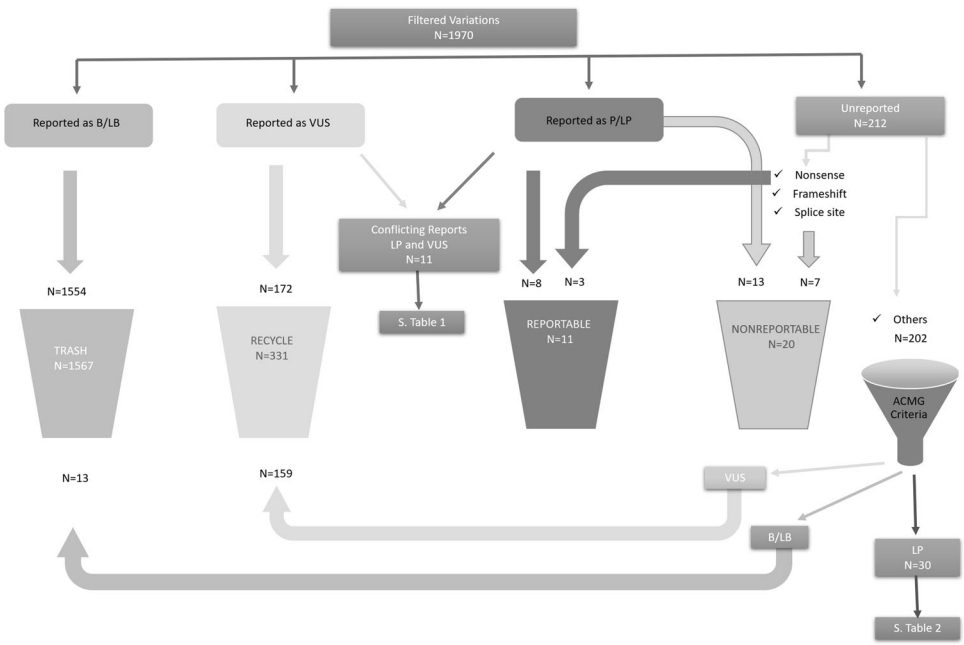


Fig. 1 Cathegorization of detected 1970 vairants in 622 Cases. B benign, LB likely benign, VUS variant of uncertain significance, LP likely pathogenic, P pathogenic, S. Table Supplement Table

the clinical features that caused the patient to be referred to us were excluded. Previously unreported variations that are predicted to result in the introduction of a stop codon or a missense mutation of the normal stop codon; mutate the initiation codon (ATG); alter the sequence at a splice junction, particularly the invariant AG/GT nucleotides; or delete one or more nucleotides or exons in such a manner as to lead to a shift in the mRNA reading frame are listed as EP according to ACMG recommendations [11]. VarSome was used to interprete novel nontruncating variations which is based on ACMG guidelines [12, 13].

Results

A total of 1970 protein altering or splicing variants were detected in 622 patients. Twenty-one distinct variations detected in 22 (3.5%) cases which were reported as pathogenic (P)/likely pathogenic (LP) in ClinVar and/or DM (Disease Causing Mutation) in HGMD were categorized as KP variations. Ten variations causing nonfunctional protein (nonsense, frameshift, and splice-site variations) were categorized as EP variations were detected in 12 (1.9%) cases. The number of the cases carrying KP and EP variants and related conditions were summarized in Table 1 and Fig. 2.

In 22 patients, 21 distinct KP variants which were reported previously in ClinVar and/or HGMD as causative variants of associated diseases were detected in 10 genes. Most common variants were in *ATP7B* (7 distinct variants in 7 patients) and *MUTYH* (6 distinct variants in 7 patients) genes. The other genes detected KP variants were *BRCA1, BRCA2, MSH6, PTEN, SDHD, PKP2, KCNQ1*, and *RYR1* (Table 2). Most of the variants were unique in this cohort, except *MUTYH c.842C>T* (p.Pro281Leu) and *RYR1* c.7268T>A (p.Met2423Lys) variations which were detected in two cases. *MSH6* c.2764C>T (p.Arg922*) and *MUTYH* c.1145G>A (p. Gly382Asp) variations were detected in the same case, all the other cases had only one variation in an actionable gene.

We detected 10 novel variations causing truncated protein or effecting splicing which were described as EP in 9 distinct genes in 12 (1.9%) patients. Variations were detected in *MUTYH, RET, KCNH2, SMAD3, RYR2, MYH7`, GLA, APOB*, and *CACNA1S* genes which of seven were frameshift, two were nonsense and three were splice site variants. In EP variants only *SMAD3* c.72delG (p. Arg25Glyfs*47) and *RET* c.2393–2 A>C variant was detected in two unrelated cases, all the others were unique (Table 3).

Among 34 cases carrying KP and EP variants 7 were heterozygous for an OD malignancy prone disorder, and 9 were heterozygous for a cardiogenetic disorder (Fig. 3).

All KP variants in the 59 actionable genes are recommended to report (In case of presence of biallelic variants for *ATP7B* and *MUTYH* genes) however there are exceptions for EP variants [2]. According to the list of these genes among 622 cases 13 (2,1%) had 11 reportable variants in 11 genes including *BRCA1, BRCA2, MSH6, PTEN, SDHD, TSC1, TSC2, SMAD3, PKP2, KCNQ1, KCNH2*, and *RYR1* associated with autosomal dominant conditions, one case had a variation in GLA gene (X-linked inheritance), and 15 (2.4%) cases were carriers of two autosomal recessive conditions Wilson Disease (WD) and *MUTYH*-Associated Polyposis (MAP). All variations were detected in heterozygous state. Among 34 cases having KP and EP variants in ACMG recommended genes, 7 (20%) cases had EP variants in *RET, MYH7, RYR2, APOB, CACNA1S* genes which were not recommended to report.

In 18 (2.8%) cases we detected 11 variants which have conflicting interpretations in databases reported as LP, P, or variant of uncertain significance (VUS) in distinct studies, however predicted as LP according to ACMG criteria (Supplementary Table 1). Eleven of these patients had variations in genes related with autosomal dominant cardiogenetic disorders. It may be confusing to decide these variations to report or not, because of incomplete penetrance and variable expressivity in autosomal dominant conditions and there is no clear recommendation about these variants in ACMG report [2].

Among 1970 variations 30 novel missense variations were predicted as LP according to ACMG criteria. These candidate variations were listed Supplementary Table 2 and planned to be evaluated after a clear segregation analysis and clinical assesment.

Discussion

In this study we evaluated CES data of 622 unrelated cases for 59 ACMG recommended genes and detected KP or EP variants in 34 (5.4%) of the cases. Among 34 cases 15 were heterozygous variation carriers for autosomal recessive conditions and ACMG recommends the variations in these genes should be reported only if they are biallelic. According to ACMG recommendations reportable variants were present in 13 of 622 which accounts 2.1% of all cases in this study. Tang et al. detected SFs in 2.5% of 954 East Asians' WESs [5]. Earlier in 1303 Korean WES study, 2.46% carrier frequency is reported in 56 ACMG recommended genes [14]. In another study including 4300 European and 2203 African-ancestry participants, SFs were detected in 0.7% and 0.5% of the participants respectively [6]. Our result seems to be similar with East Asians and SF frequency in Turkish population is higher than Europeans and Africans. However our cohort is smaller than the

Table 1 Number of cases having variations in 59 ACMG recommended genes and related conditions

Related condition	Heridty pattern	Gene	No. of cases carrying KP variations	No. of cases carrying EP variations
Hereditary breast and ovarian cancer	AD	*BRCA1*	**1**	**0**
		BRCA2	**1**	**0**
Lynch syndrome	AD	*MSH6*[a]	**1**	**0**
MYH-associated polyposis	AR	*MUTYH*[a]	7	1
Multiple endocrine neoplasia type 2 Familial medullary thyroid cancer	AD	*RET*	**0**	2
PTEN hamartoma tumor syndrome	AD	*PTEN*	**1**	**0**
Hereditary paragangliomapheochromocytoma syndrome	AD	*SDHD*	**1**	
Marfan syndrome, Loeys-Dietz syndromes, and familial thoracic aortic aneurysms and dissections	AD	*SMAD3*	**0**	**2**
Hypertrophic cardiomyopathy, dilated cardiomyopathy	AD	*MYH7*	**0**	1
	XL	*GLA*	**0**	**1**
Catecholaminergic polymorphic ventricular tachycardia	AD	*RYR2*	**0**	2
Arrhythmogenic right ventricular cardiomyopathy	AD	*PKP2*	**1**	**0**
Romano-Ward long-QT syndrome types 1, 2, and 3, Brugada syndrome	AD	*KCNQ1*	**1**	**0**
	AD	*KCNH2*	**0**	**1**
Familial hypercholesterolemia	AD	*APOB*	**0**	1
Wilson disease	AR	*ATP7B*	7	0
Malignant hyperthermia susceptibility	AD	*RYR1*	**2**	0
		CACNA1S	**0**	1
Total			22	12

AD autosomal dominant, *AR* autosomal recessive, *XL* X-linked

Bold values indicate the variations should be reported according to ACMG recommendations

A recurrent MUTYH variant was detected in two case

[a]The case carrying *MSH6* mutation also carries a *MUTYH* mutation

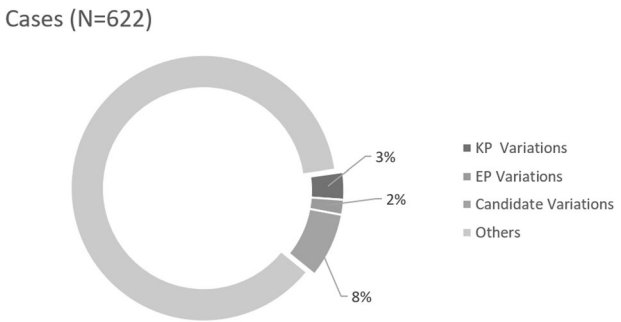


Fig. 2 Cases evaluated for secondary findings. KP known pathogenic, EP expected pathogenic

European and African cohort, more studies and large cohorts are needed for a more accurate comparision and also distinct ratios may be a result of the differences in filtering criteria and variant interpretation. In addition, the fact that these cohorts are not homogenous in terms of factors that may change the frequency of variants such as ethnicity and sequencing indications the assesment requires a more complex approach. There may be an increase in these ratios as the pathogenicity of novel variants is clarified with additional studies in next years.

Among 13 cases having reportable variants, 5 (38.4%) were carrying a variation in genes causing cancer prone disorders, which may be preventable by lifestyle changes, proper screening programmes and prophylactic surgeries. *BRCA1* and *BRCA2* genes are the most common genes associated with hereditary cancer syndromes and cause Hereditary Breast Ovary Cancer (HBOC) Syndrome. Estimated prevalance of pathogenic variants in *BRCA1* and *BRCA2* genes are 0.07% and 0.14% respectively in general population [15]. In this study we detected a nonsense variant (c.3607C>T, p.Arg1203*) in *BRCA1* and a frameshift variant (c.2957dupA, p.Asn986Lysfs*2) in *BRCA2* genes in two of 622 cases, indicating pathogenic variant prevelance as 0.16% for each gene. Both variations were previously reported in HBOC

Table 2 KP variants in 59 ACMG recommended genes

Gene	Transcript	Nucleotide change	Protein change	dbSNP ID	MAF (GnomAD)	ClinVar	HGMD	Carriers
ATP7B	NM_000053	c.1934T>G	p.Met645Arg	rs121907998	0.0005169	P/LP	CM970138	1
		c.2293G>A	p.Asp765Asn	rs28942075	–	P	CM950110	1
		c.2332C>G	p.Arg778Gly	rs137853284	0.000008018	LP,P	CM950112	1
		c.2804C>T	p.Thr935Met	rs750019452	0.0001885	P/LP	CM004606	1
		c.2817G>T	p.Trp939Cys	rs1057517310	–	P	CM119350	1
		c.2906G>A	p.Arg969Gln	rs121907996	0.00003608	P	CM950116	1
		c.3809A>G	p.Asn1270Ser	rs121907990	0.0001	P	CM930060	1
MUTYH	NM_001048171	c.503G>A	p.Arg168His	rs143353451	0.00001193	P	CM042753	1
		c.692G>A	p.Arg231His	rs140342925	0.00008390	LP,P	CM053997	1
		c.817delG	p.Ala273Profs*32	rs761468459	0.000003986	P/LP	CD066380	1
		c.842C>T	p.Pro281Leu	rs374950566	0.00001195	P/LP	CM064129	2
		c.1145G>A	p.Gly382Asp	rs36053993	0.003027	P/LP	CM020287	1
		c.1395_1397delGGA	p.Glu466del	rs587778541	0.00009544	P	CD031064	1
MSH6	NM_000179	c.2764C>T	p.Arg922*	rs587779246	0.000003985	P	CM117497	1
PTEN	NM_000314	c.1003C>T	p.Arg335*	rs121909231	–	P	CM971278	1
BRCA1	NM_007294	c.3607C>T	p.Arg1203*	rs62625308	0.00001195	P	CM940176	1
BRCA2	NM_000059	c.2957dupA	p.Asn986Lysfs*2	rs80359365	–	P	CI063656	1
PKP2	NM_001005242	c.2357 + 1G>A		rs111517471	0.00002784	P	–	1
KCNQ1	NM_000218	c.1097 G>A	p.Arg366Gln	rs199473410	0.000003980	P	CM002330	1
SDHD	NM_003002	c.147dupA	p.His50Thrfs*19	rs876659130	–	P	CI032161	1
RYR1	NM_000540	c.7268T>A	p.Met2423Lys	rs118192174	0.00001989	P	CM057506	2

P pathogenic, *LP* likely pathogenic

cases [9]. Lynch Syndrome (LS) is another wellknown hereditary cancer predisposition syndrome related with distinct types of cancer especially colorectal and endometrial cancer. Mismatch repair gene (*MLH1, PMS2, MSH2*, and *MSH6*) defects are responsible for LS. The estimated frequency of LS is between 1:2000 and 1:660 in general population [16]. Although *MSH2* is the most common affected gene in LS, in this study no case was found to carry pathogenic variant in *MSH2* however we detected a previously reported pathogenic variant (c.2764C>T; p.Arg922*) in *MSH6* gene in 1 of 622 cases. Due to small number of cases, this can not be considered as a population data for Turkey.

Six out of 13 (46.1%) cases were carrying a reportable variant in genes affecting cardiovascular system including *SMAD3, GLA, PKP2, KCNQ1*, and *KCNH2*. These genes are related to sudden cardiac death due to causing aortic aneurysms and dissections, arrhytmias and cardiomyopathies. This information suggests that approximately half of these patients are at risk of sudden cardiac death where urgent examination is needed. Jalkh et al. reported these situations among 18 variations, 6 (33%) and 3 (20%) in genes causing oncogenetic and cardiogenetic disorders respectively [17]. Interestingly we detected a novel frameshift variation (c.72delG; p.Arg25Glyfs*47) in exon 1 of *SMAD3* gene in two cases, Haplo-insufficiency and loss-of-function variations in *SMAD3* gene result in Loeys-Dietz syndrome (LDS, OMIM ID #609192) characterized by hypertelorism, bifid uvula/cleft palate and aortic/arterial aneurysms and tortuosity. Among all LDS cases about two third of cases are the consequence of de novo mutations and have more severe phenotype, whereas the other one third are familial. The prevalence of LDS is unknown however *SMAD3* gene defects are responsible ~5–10% of LDS cases [18]. As to our knowledge there is no molecular data about LDS cases in Turkish population. Therefore we can speculate that this variation may be frequent in Turkish population however more studies are needed to make a definitive interpretation.

In two cases we detected *RYR1* variants associated with malignant hyperthermia susceptibility (MHS), which is a lifethreating condition in case of exposure to anesthetic triggering agents [19]. These two unrelated cases had the same missense variant (c.7268T>A; p.Met2423Lys) which of MAF is reported as 0.00001989 in GnomAD, however our data suggests an allele frequency of 0.003215 (~160-fold higher) [8]. The incidence of malignant hyperthermia (MH) is reported as 1:15.000 and 1:50.000 in children and adults respectively. *RYR1* and *CACNA1S* gene defects are associated with MHS [20]. As to our knowledge there is no data about the pathogenic variant frequency of *RYR1* gene in Turkish population. In our cohort pathogenic variant

Table 3 EP Variants in 59 ACMG recommended genes

Gene	Transcript	Nucleotide change	Protein change	Coding consequence	MAF (GnomAD)	ACMG	Carriers	Reportable
APOB	NM_000384	c.13096G>T	p.Glu4366*	Nonsense	–	PVS1, PM2, BP4	1	No
KCNH2	NM_000238	c.3400C>T	p.Arg1134*	Nonsense	0.000005894	PVS1, PM2, PP3	1	Yes
SMAD3	NM_001145103	c.72delG	p.Arg25Glyfs*47	Frameshift	–	PVS1, PM2, PP3	2	Yes
RYR2	NM_001035	c.9257delA	p.Gln3086Argfs*36	Frameshift	–	PVS1, PM2, PP3	1	No
		c.14217dupT	p.Ala4740Cysfs*56	Frameshift	0.000008587	PVS1, PM2, PP3	1	No
MYH7	NM_000257	c.816_817del	P.Arg272Serfs*20	Frameshift	–	PVS1, PM2, PP3	1	No
GLA	NM_000169	c.158delA	p.Asn53Thrfs*68	Frameshift	–	PVS1, PM2, PP3	1	Yes
RET	NM_020630	c.2393-2A>C		Splicing	–	PVS1, PM2, PP3	2	No
MUTYH	NM_001048171	c.462 + 1G>T		Splicing	–	PVS1, PM2, PP3	1	No
CACNA1S	NM_000069	c.2550 + 1G>C		Splicing	–	PVS1, PM2, PP3	1	No

PVS1 pathogenic very strong 1, *PM2* pathogenic moderate 1, *BP4* benign supporting 4, *PP3* pathogenic supporting 3

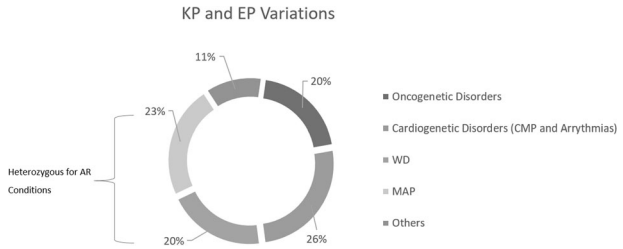


Fig. 3 Distribution of detected KP and EP variations and related disorders. AR autosomal recessive, CMP cardiomyopathy, WD Wilson Disease, MAP MUTYH-associated polyposis

frequency is calculated as 0.3% higher than expected worldwide population frequency however close to the report of Amendola et al. in which 7 of 6503 (0.1%) participants had P and LP variants in *RYR1* gene [6]. Jalkh et al. reported three individuals carrying pathogenic *RYR1* variants in 280 Lebanese which accounts 1% of participants [17]. Another study including 954 East Asians had reported no variants in *RYR1* gene [5].

MUTYH gene encodes a component of mismatch repair system and causes autosomal reccessive *MUTYH*-Asspciated Polyposis (MAP). Monoallelic *MUTYH* mutations are also associated with malignancies such as colorectal and breast cancer [21]. In this study we detected heterozygous pathogenic *MUTYH* variants in eight cases which accounts 1.2% of this cohort. Although there is no data about *MUTYH* variant frequency in Turkish population, similar with our cohort, Win et al. had reported the estimated carrier frequency of pathogenic *MUTYH* variation as 1:45 [22]. Among eight pathogenic variation seven were recurrent and we detected a novel variation (c.462 + 1G>T) disturbing splice site. The c.842C>T (p.Pro281Leu) variation was detected in two cases, which is one of two reported variations in a Turkish adenomatous polyposis cohort including eight distinct families, it is reported in our previous study (unpublished data).

In seven patients we detected heterozygous pathogenic variations in *ATP7B* gene. WD carrier frequency is calculated as 1% in this cohort. This result was compatible with previous studies which reported WD carrier frequency as 1:90 in Mediterranean populations [23]. All variations in *ATP7B* were perviously reported missense variations.

The variations in *MUTYH* and *ATP7B* were not recommended to report in case of presenting in heterozygous state. However in the populations where consanguineous marriages are common, such as Turkish population, it may be appropriate to evaluate them in terms of prenatal and preimplantation genetic diagnosis possibilities for the next generations.

Although in this study the CES data of 622 patients evaluated in a single center in Istanbul hosting people from various regions of Turkey, it is hard to claim that these results reflect whole Turkish population.

Conclusion

Exome sequencing is an advanced molecular technique provides geneticists an advantage in diagnosing rare diseases with nonspecific clinical features and genetically heterogenous diseases in a short time. On the other hand while analysing exome data molecular geneticists come across variations which are causing or predisposing life-threating and/or preventable disorders concerning the other family members. Deciding to report them is difficult, as they may cause ethical and psychosocial problems. Revised ACMG recommedations, reported in 2016, are guiding us about reportable variations, suggesting to report KP and EP variations 59 genes. However every population differs from others with sociodemographic characteristics besides variation spectrum so revision and expansion of this list seems inevitable. As our knowledge there is no study published evaluating SFs in Turkish population, therefore this study evaluating 622 CES data of Turkish population may be a pioneering study.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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