Carrier Rates of Phenylketonuria, Biotinidase Deficiency and Cystic Fibrosis in Turkey

Türkiye'de Fenilketonüri, Biotinidaz Eksikliği ve Kistik Fibrozis Taşıyıcı Oranları

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ABSTRACT

Objective: It is known that the number of diseases associated with autosomal recessive inheritance pattern is increasing in our country where consanguineous marriages (including within the same village/region) are high. In this study, we aimed to evaluate the carrier rate of Phenylketonuria, Biotinidase Deficiency and Cystic Fibrosis by evaluating the next generation sequencing (NGS) data collected.
Methods: The data of 279 cases who underwent NGS between March 2021 and April 2022 for different clinical indications were investigated retrospectively. Cases with pathogenic variants in *PAH*, *BTD*, and *CFTR* genes were figured out as carriers.
Results: In this study, pathogenic variants were found in the *PAH* gene in 9, *BTD* gene in 23 and *CFTR* genes in 14 of a total of 279 individuals. Additionally, 2 people had pathogenic variants in both the *BTD* and the *PAH* genes. The carrier rates for phenylketonuria, biotinidase deficiency and cystic fibrosis were 3.2%, 8.2%, and 5%, respectively.
Conclusion: These results suggest that carrier rate of Phenylketonuria, Biotinidase Deficiency, and Cystic fibrosis may be significantly high in our country. Carrier screening is very important in diseases with high carrier rates. When both couples are known to be carriers, prenatal or pre-implantation genetic diagnosis testing options can be offered.

Keywords: Phenylketonuria, Biotinidase Deficiency, Cystic Fibrosis, Next-Generation Sequencing, carrier rate, consanguineous marriages

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ÖZET

Amaç: Akraba evliliklerinin (aynı köy/bölge içi de dahil) yüksek olduğu ülkemizde otozomal resesif kalıtım paterni ile ilişkili hastalıkların sayısında artışın olduğu bilinmektedir. Bu çalışma ile toplanan yeni nesil dizileme (NGS) verilerini değerlendirilerek Fenilketonüri, Biotinidaz Eksikliği ve Kistik Fibrozis taşıyıcı sıklığını değerlendirmeyi amaçladık.
Yöntem: Farklı klinik endikasyonlarla 2021 Mart ve 2022 Nisan ayları arasında NGS yapılan 279 olgunun dataları retrospektif olarak değerlendirildi. *PAH*, *BTD* ve *CFTR* genlerinde patojenik varyanta sahip vakalar taşıyıcı olarak belirlendi.
Bulgular: Bu çalışmaya dahil edilen 279 hastanın 9'unda *PAH* geninde, 23'inde *BTD* geninde ve 14'ünde *CFTR* geninde patojenik varyant saptandı. Ayrıca, 2 hastada hem *BTD* geninde hem de *PAH* geninde patojenik varyant vardı. Fenilketonüri, Biotinidaz Eksikliği ve Kistik Fibrozis hastalıkları için taşıyıcılık oranları sırasıyla %3.2, %8.2 ve %5 olarak saptandı.
Sonuç: Bu sonuçlar, ülkemizde Fenilketonüri, Biotinidaz eksikliği ve Kistik Fibrozis taşıyıcılığının önemli ölçüde yüksek olabileceğini düşündürmektedir. Taşıyıcılık oranlarının yüksek olduğu hastalıklarda taşıyıcılık taraması oldukça önemlidir. Her iki eşinde taşıyıcı olduğu bilindiğinde prenatal veya pre-implantasyon genetik tanı testi sunulabilir.

Anahtar Sözcükler: Fenilketonüri, Biotinidaz Eksikliği, Kistik Fibrozis, Yeni Nesil Dizileme, taşıyıcı oranı, akraba evliliği

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INTRODUCTION

Phenylketonuria (PKU; OMIM# 261600) is the most common congenital metabolic disorder caused by the deficiency of phenylalanine hydroxylase or its cofactor tetrahydrobiopterin (BH4).

Mutations in the *phenylalanine hydroxylase* gene (*PAH;* OMIM* 612349) are associated with PKU. Phenylalanine hydroxylase is a liver enzyme that catalyzes the hydroxylation of the amino acid phenylalanine to tyrosine (1). The accumulation of phenylalanine in the body can lead to developmental delay/intellectual disability, abnormal movements, and seizures (2). Early diagnosis is crucial to prevent this accumulation from causing irreversible damage and to ensure that patients live a healthy life. For all of these reasons, the Newborn Screening Program for Phenylketonuria was initiated in the United States in 1962 (3). In Turkey, Newborn Screening Programme started in 1987 with the Phenylketonuria Screening Program (4). The prevalence of PKU is approximately 1/15000, with ethnic and regional variations (5). The incidence in Turkey is approximately 1/3500-1/4000 newborns (6).

Biotinidase deficiency (BTD deficiency; OMIM# 253260) is a disease mainly associated with neurologic, dermatologic, and ocular findings (7). It was caused by biallelic pathogenic variants in the *biotinidase* (*BTD*; OMIM* 609019) gene. BTD deficiency is observed in approximately 1/60000 newborns (8).

Cystic fibrosis (CF; OMIM# 219700) is a multisystem disease that causes abnormal ion transport, affecting mainly the respiratory epithelium, exocrine pancreas, intestine, hepatobiliary system and exocrine sweat glands (9). It was caused by mutations in the *cystic fibrosis transmembrane conductance regulator* gene (*CFTR*; OMIM* 602421) (10). Although the estimated incidence of CF is 1/3000, it shows regional and ethnic differences (11).

PKU, BTD deficiency, and CF are diseases with autosomal recessive inheritance pattern. The PKU Screening Program has been performed compulsorily all over Turkey since 2006 (12). In October 2008, biotinidase deficiency and in January 2015, cystic fibrosis were added to the Newborn Screening Program in Turkey. The screening is based on guthrie card and biochemical analysis. However, carrier frequencies in Turkey have not yet been clarified by genetic methods in large series for these diseases.

Developments in next-generation DNA sequencing (NGS) technologies allow rapid and highly accurate sequencing of not only a specific region of the genome, but also the whole genome. NGS technologies mainly Clinical Exome Sequencing (CES) and Whole Exome Sequencing (WES) have increased the carrier detection rate in diseases with autosomal recessive inheritance patterns (13,14).

It is known that there is an increase in the number of diseases associated with autosomal recessive inheritance pattern in our country where consanguineous marriages are high (%24) (15). If both couples are carriers, genetic counseling for prenatal diagnosis is crucial. In this study, we determined the carrier rates of PKU, BTD deficiency, and CF by retrospectively evaluating the genetic data of patients who underwent CES and/or WES.

METHODS

Patients

The data of 279 patients who underwent next-generation sequencing (CES or WES) for different clinical indications in the Medical Genetics Department of Balıkesir Atatürk City Hospital between March 2021 and April 2022 were collected. NGS data from 279 cases were retrospectively screened for variants in each of the *PAH*, *BTD,* and *CFTR* genes. Cases with pathogenic variants in the *PAH*, *BTD,* and *CFTR* genes are described as carriers. The median age for all carriers was 16.1 years. The female and male ratio was 1.2 (24/20).

The exclusion criteria in the study are as follows: Cases with mutations detected in *PAH*, *BTD,* and *CFTR* gene by single gene sequencing.

Data collection and DNA extraction

Informed consent was provided from all patients before collection of blood sample. DNA extraction was performed from these blood samples. The obtained blood sample was 2 ml of venous blood collected in an EDTA tube. We extracted DNA from 200 µl peripheral blood samples using the High Pure PCR Template Preparation Kit (Roche, Pleasanton, CA 94588 USA) protocol.

Clinical exome sequencing (CES)/ Whole-exome sequencing (WES)

The obtained genomic DNA was enriched using two capture kits used for CES: Twist CES kit (South San Francisco, USA) and KAPA HyperCap DS CES Target Enrichment Probes (Roche, Germany). These were sequenced with the MGI-DNBSEQ-G400 (China). Additionally, WES was performed using the TruSeq Exome Enrichment kit from Illumina following the manufacturer's protocol. It was sequenced using a HiSeq2500 Sequencer (Illumina).

Data analysis

GenomizeSeq (Version 6.13.1) software was used for analysis with an average read depth of 20X and coverage of 94.17%. Exon-intron junction boundaries (±10 bç) were included in the analysis. The Human Genome Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php), Franklin (https://franklin.genoox.com/clinical-db/home), VarSome (https://varsome.com/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), and Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/) novel variants in the databases were checked. The pathogenicity score of new variants was interpreted using in silico variant prediction programs Mutation Taster, CADD (Combined Annotation Dependent Depletion). The GRCh37 assembly genome build name was used. The data obtained were analyzed according to the American College of Medical Genetics and Genomics (ACMG) criteria (16).

Ethical Publication Statement

This study was conducted at Balıkesir Atatürk City Hospital. Ethical approval was obtained from the institutional Ethics Committee, dated 06/04/2023 and numbered 2023/1/5. The study was evaluated as a research file and it was decided that it was scientifically and ethically appropriate. All the participants of the study gave their informed consent.

RESULTS

In our study investigating the carrier rates of PKU, BTD deficiency and CF diseases, a total of 44 carrier individuals were identified. Of these individuals, 9 were carriers of PKU, 23 were carriers of BTD deficiency, and 14 were carriers of CF.

Seven different *PAH* gene variants were observed in 9 unrelated PKU carriers (Table 1). All of these variants were of the missense type. We detected the same variant, *PAH* gene: c.1208C>T: p.Ala403Val, in three different carriers.

In 23 cases, 5 different *BTD* gene variants were identified. Nineteen (82.6%) of the BTD deficiency carriers had the same NM_001370658.1: c.1270G>C: p.Asp424His variant in the *BTD* gene.

In the CF group, the most common variant (n=7 individuals, 50%) was c.3154T>G: p.Phe1052Val, while the second common variant (n=5 individuals, 35.7%) was c.1521_1523del: p.Phe508del. All these individuals also had the 7T variant. In case 43, we detected dual carriage with the *PAH* gene: c.782G>T: p.Arg261Leu variant and the *BTD* gene: c.1270G>C: p.Asp424His variant. In addition, we detected 2nd dual carriage in case 44 with *PAH* gene: c.782G>T: p.Arg261Leu variant and *BTD* gene: c.1270G>C: p.Asp424His variant.

Table 1. Pathogenic variants in the *PAH*, *BTD* and *CFTR* genes

Gene Ref. Seq. ID	Case	Age	Gender	Variant Type	Location	Nucleotide variation	Amino acid variation	ACMG Clasification	Associated phenotype
PAH (NM_000277.3)	1	5 y	M	missense	Exon 2	c.143T>C	p.Leu48Ser	Pathogenic	PKU carrier
	2	10 y	F	missense	Exon 6	c.688G>A	p.Val230Ile	Pathogenic	
	3	6 y	F	missense	Exon 12	c.1208C>T	p.Ala403Val	Pathogenic	
	4	16 y	F	missense	Exon 12	c.1208C>T	p.Ala403Val	Pathogenic	
	5	3 y	M	missense	Exon 12	c.1208C>T	p.Ala403Val	Pathogenic	
	6	65 y	M	missense	Exon 12	c.1218A>G	p.Ile406Met	Pathogenic	
	7	8 y	F	missense	Exon 12	c.1243G>A	p.Asp415Asn	Pathogenic	
	8	5 y	F	frameshift	Exon 2	c.38_44delGCGGCTGinsTCC	p.Cys13PhefsTer36	Pathogenic	
	9	2 y	F	missense	Exon 4	c.410G>A	p.Arg137His	Pathogenic	
	10	46 y	F	missense	Exon 4	c.476G>A	p.Arg159His	Pathogenic	
	11	2 y	F	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	12	12 y	M	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	13	41 y	F	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	14	9 y	F	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
BTD (NM_001370658.1)	15	10 y	F	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	BTD deficiency carrier
	16	2 y	F	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	17	2 y	M	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	18	5 y	M	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	19	8 y	F	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	20	11 y	F	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	21	9 y	M	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	22	4 y	M	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	

Table 1. continued

	23	38 y	F	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	24	19 y	F	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	25	4 y	F	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	26	4 y	M	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	27	3 y	M	missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	
	28	7 y	M	missense	Exon 4	c.1309G>A	p.Val437Met	Pathogenic	
	29	6 y	M	missense	Exon 4	c.328G>A	p.Asp110Asn	Pathogenic	
	30	54 y	F	in frame	Exon 11	c.1521_1523 del	p.Phe508del	Pathogenic	
	31	4 y	M	in frame	Exon 11	c.1521_1523 del	p.Phe508del	Pathogenic	
	32	5 y	M	in frame	Exon 11	c.1521_1523 del	p.Phe508del	Pathogenic	
	33	7 y	M	in frame	Exon 11	c.1521_1523 del	p.Phe508del	Pathogenic	
	34	50 y	F	in frame	Exon 11	c.1521_1523 del	p.Phe508del	Pathogenic	
	35	18 y	M	missense	Exon 20	c.3154T>G	p.Phe1052Val	Pathogenic	
CFTR (NM_000492.4)	36	20 y	M	missense	Exon 20	c.3154T>G	p.Phe1052Val	Pathogenic	CF carrier
	37	14 y	F	missense	Exon 20	c.3154T>G	p.Phe1052Val	Pathogenic	
	38	17 y	F	missense	Exon 20	c.3154T>G	p.Phe1052Val	Pathogenic	
	39	46 y	F	missense	Exon 20	c.3154T>G	p.Phe1052Val	Pathogenic	
	40	3 y	M	missense	Exon 20	c.3154T>G	p.Phe1052Val	Pathogenic	
	41	15 y	M	missense	Exon 20	c.3154T>G	p.Phe1052Val	Pathogenic	
	42	56 y	F	missense	Exon 21	c.3454G>C	p.Asp1152His	Pathogenic	
PAH (NM_000277.3	43	16 y	F	missense	Exon 7	c.782G>T	p.Arg261Leu	Pathogenic	PKU carrier
BTD (NM_001370658.1)				missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	BTD deficiency carrier
PAH (NM_000277.3	44	11 y	M	missense	Exon 7	c.842C>T	p.Pro281Leu	Pathogenic	PKU carrier
BTD (NM_001370658.1)				missense	Exon 4	c.1270G>C	p.Asp424His	Pathogenic	BTD deficiency carrier

y:years, M:male, F: female

DISCUSSION

Newborn Screening Programs are part of public health programs within the scope of preventive health services worldwide. Newborn Screening Programs for various diseases are also carried out in our country. These programs aimed an early diagnosis and possible treatment of common diseases. In this study, NGS data collected from 279 cases were retrospectively evaluated to determine the carrier rate of PKU, BTD deficiency and CF. We detected 9 variants in the *PAH* gene, 23 variants in the *BTD* gene, and 14 variants in the *CFTR* gene. Variants in both the *PAH* and *BTD* genes were found in two individuals.

In 2022, Toktaş et al. investigated the incidence of PKU and BTD deficiency in Diyarbakır, where consanguineous marriages are high, and found the incidences to be 1:7878 and 1:2359, respectively. In this study, they reported the carrier frequency as 4% for PKU only. (17). The PKU carrier rate in our study was similar to this study with 3.2%, whereas our BTD deficiency carrier rate was 8.2%. In the study by Aytaç et al. in Adana province, PKU was observed in 1/20000 newborns in 2010, while it was observed in 1/21000 newborns in 2011. BTD deficiency was observed in one in 8,120 and 14,261 births in 2010 and 2011, respectively. However, there is no information about carrier rates (18). In the study of Akova et al. in which they reported their 10-year experience in Sivas, the lowest and highest incidence rates were 1:8375-1:657 for PKU, and 1:6815-1:1861 for BTD deficiency, respectively. In this study, no data were found about carriers (19). It has been shown in previous studies that variants of BTD gene, p.R157H, p.D444H, p.C13Ffs and p.T532M are common in Turkey (15, 20-22). The p.D444H amino acid variant was observed most frequently (38.7%) in the study by Seker Yilmaz et al. and we found the carrier frequency of this variant to be the highest with a rate of 82.6% (n=19 individuals) (21).

The CF incidence in our country is thought to be approximately 1/3000, but there are not enough studies (11). In the study of Rueda-Nieto et al. in 2022, they found Phe508del (58.3%) as the most common variant in their study with 192 CF patients in Spain (23). The p.Phe508del variant, which is seen in approximately 85% of individuals with CF, is also the most frequently reported variant in the USA (24). Although CF has a heterogeneous mutation spectrum in Europe, the p.Phe508del variant is the most common (25, 26). Hacettepe University Department of Pediatric Chest Diseases and Department of Medical Biology evaluated 2458 patients between 1990 and 2015. In this study, the p.Phe508del variant was observed at a rate of 13.79%, while the delta variant was found at a rate of 0.04% (27). In our study, unlike all these studies, p.Phe1052Val was the most common (%50), and the p.Phe508del variant was second most common. There is not enough information in the literature about the p.Phe1052Val variant (28). This ratio should be supported by further studies. We suggest that panels used to screen for *CFTR* gene mutations also include the most common variant, p.Phe1052Val.

In conclusion, we obtained carrier rates of 3.2%, 8.2% and 5% using CES or WES in PKU, BTD deficiency and CF diseases included in the Newborn Screening Program, respectively. The most common variants detected in our province were p.Ala403Val (33.3%) in *PAH* gene, p.Asp424His (82.6%) in *BTD* gene, and p.Phe1052Val (50%) in *CFTR* gene. To date, most studies have been aimed at elucidating the genetic etiology of patients. These results, although showing regional and ethnic differences, suggest that the carrier rate of these diseases is high in Turkey. Carrier screening allows prospective parents to learn about the likelihood of having a sick child. Adding these diseases to premarital screening will provide at-risk couples with genetic counseling and information about prenatal or preimplantation genetic diagnosis.

Our study include several limitations. More cases will help to evaluate the true carrier frequency of the population and identify regional differences. No segregation studies have been conducted in the families of carrier individuals. CNV analysis was not performed in this study.

Conflict of interest

No conflict of interest was declared by the authors.

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