GMJ 2024;35:173-178

DOI: http://dx.doi.org/10.12996/gmj.2023.3795

Wilson Disease in a Turkish Population: Molecular Insights into an Old Disease with Reported and Novel Variants

Bir Türk Popülasyonunda Wilson Hastalığı: Bildirilen ve Yeni Varyantlarıyla Eski Bir Hastalığa Moleküler Bakış

Abdullatif Bakır[1,2], Vehap Topçu[3,4], Büşranur Çavdarlı[3,4]

[1]Ankara Training and Research Hospital, Clinic of Medical Genetics, Ankara, Türkiye
[2]University of Health Sciences Türkiye, Dr. Sami Ulus Maternity and Children's Training and Research Hospital, Clinic of Medical Genetics, Ankara, Türkiye
[3]Ankara Numune Training and Research Hospital, Clinic of Medical Genetics, Ankara, Türkiye
[4]Ankara City Hospital, Clinic of Medical Genetics, Ankara, Türkiye

ABSTRACT

Objective: Wilson's disease (WD) is a rare autosomal recessive genetic liver disorder with hepatic, neurological, or psychiatric manifestations between 1st to 5th decades. WD is caused by homozygous or compound heterozygous pathogenic variants in the *ATP7B* gene. In this study, we aimed to contribute to the *ATP7B* gene variant spectrum in a Turkish population of WD patients.

Methods: We investigated 49 patients from 46 families to determine the underlying molecular etiology of WD. DNA samples were extracted from peripheral blood. The molecular genetic diagnosis was performed using the next-generation sequencing method.

Results: Molecular genetic analysis revealed 26 different variants, two of which were novel c.1707+4A>G (IVS4+4A>G) and p.M497K in 34 patients from 31 different families. p.M769Hfs*26 was the variant with the highest allele frequency at 11.3%, followed by the p.H1069Q variant (8%). The classification of the variants according to the molecular mechanism was as follows: missense 61.5%, splice site and frameshift 15.4%, and non-sense 0.08%.

Conclusion: In this study, we aimed to contribute the variant spectrum of the *ATP7B* gene in the Turkish population and the genetic profile of WD with the obtained data.

Keywords: Wilson disease, *ATP7B* gene, variants

ÖZ

Amaç: Wilson hastalığı (WH), 1. ve 5. dekatlar arasında hepatik, nörolojik veya psikiyatrik belirtiler gösteren, otozomal resesif geçişli, nadir görülen bir genetik karaciğer hastalığıdır. WH, *ATP7B* genindeki homozigot veya bileşik heterozigot patojenik varyantlardan kaynaklanır. Bu çalışmada, WH hastalarının Türk popülasyonunda *ATP7B* gen varyant spektrumuna katkıda bulunmayı amaçladık.

Yöntemler: WH'nin altında yatan moleküler etiyolojiyi belirlemek için 46 aileden 49 hastayı inceledik. DNA örnekleri periferik kandan ekstrakte edildi. Moleküler genetik tanı, yeni nesil dizileme yöntemi kullanılarak gerçekleştirildi.

Bulgular: Moleküler genetik analizde, 31 farklı aileden 34 hastada ikisi yeni olmak üzere c.1707+4A>G (IVS4+4A>G) ve M497K) 26 farklı varyant ortaya çıkardık. p.M769Hfs*26 %11,3 ile en yüksek alel frekansına sahip varyan idi ve onu p.H1069Q varyantı (%8) takip ediyordu. Varyantların moleküler mekanizmaya göre sınıflandırılması şu şekildeydi; yanlış anlamlı %61,5, splays ve çerçeve kayması %15,4, anlamsız %0,08.

Sonuç: Bu çalışmada elde edilen verilerle *ATP7B* geninin Türk popülasyonundaki varyant spektrumuna ve WH'nin genetik profiline katkıda bulunmayı amaçladık.

Anahtar Sözcükler: Wilson hastalığı, *ATP7B* geni, varyantlar

Address for Correspondence/Yazışma Adresi: Abdullatif Bakır MD, University of Health Sciences Türkiye, Dr. Sami Ulus Maternity and Children's Training and Research Hospital, Clinic of Medical Genetics, Ankara, Türkiye
E-mail / E-posta: latif.225@gmail.com
ORCID ID: orcid.org/0000-0002-3931-4168

Received/Geliş Tarihi: 16.01.2023
Accepted/Kabul Tarihi: 02.08.2023

INTRODUCTION

Wilson's disease [(WD), MIM#277900] is a rare autosomal recessive inherited metabolic disease of copper metabolism (1). It manifests as hepatic, neurological, or psychiatric disorders, or a combination thereof, in individuals aged three to fifty years. The disease is diagnosed on the basis of typical clinical and laboratory findings, such as low ceruloplasmin concentration and high urinary copper excretion (2). Early symptoms of WD are usually observed between the ages of 5 and 35, but in some cases, the disease can manifest in both infancy and old age (3,4). In most cases, clinical manifestations are usually latent and may not be noticeable until progressive liver failure and/or neurological dysfunction (5). Although the Kayser-Fleischer ring is a diagnostic ophthalmologic feature, it can be seen in a limited number of patients with WD (6). Biochemical indicators for early diagnosis may not be helpful for WD, particularly in children (7).

The prevalence of WD is considered to be 1/30,000 to 1/100,000 live births and a carrier frequency of 1 in 90 (8). According to some studies on the European population, the disease is observed much more commonly than previous estimates, with an estimated prevalence of 1:10,000 to 1:7,000 (9,10). This discrepancy probably originates from difficulties in the clinical diagnosis of WD, the presence of latent forms, and limitations of diagnostic methods.

WD is typically inherited in an autosomal recessive manner and is caused by either homozygous or compound heterozygous pathogenic variants in the *ATP7B* gene, which encodes a copper-transporting ATPase. The P-type copper-transporting ATPase ensures that copper is incorporated into ceruloplasmin and excreted into the bile via the apical membrane of hepatocytes. *ATP7B* dysfunction causes hepatocyte damage due to the failure of hepatic excretion of copper into the bile (11). Free copper enters the bloodstream and causes damage to many organs and tissues, predominantly in the liver, brain, cornea, and kidney. With an early diagnosis and treatment of WD, liver cirrhosis and other complications can be prevented before they cause disability or death (12).

The *ATP7B* gene (MIM#606882) is located on chromosome 13q14.3 and is approximately 80 kb in size, consisting of 21 exons, 20 introns, and 4.3 kb open reading frames (13). It encodes a protein called copper-transporting ATPase 2 that contains 1464 amino acids. Copper binding sites in the NH2-terminal domain, the ATP-binding domain that converts the energy of ATP (containing the nucleotide binding and phosphorylation subdomains), and the six transmembrane proteins are the domains of this protein. In WD patients, most pathogenic variants are missense.

Although the *ATP7B* gene is frequently studied worldwide, there is a lack of data from Türkiye. In addition, the spectrum of *ATP7B* variants differs significantly between populations. This study contributes to the variant distribution of the *ATP7B* gene in the Turkish population.

MATERIALS AND METHODS

All procedures were performed following the tenets of the Declaration of Helsinki. Informed consent was obtained from the participants' parents for molecular genetic analysis and the publication of patient data before their enrollment in the study. This study was approved by the Ankara City Hospital Ethics Committee (approval number: EI/878/2020, date: 02.07.2020).

Patients

Between 2016 and 2019, 49 index cases from 46 different families were referred to our clinic for molecular diagnosis of WD.

The clinical diagnosis process included physical examination, anamnesis, complete blood count, urine analysis, biochemical analysis of blood (alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, CE), parameters of copper metabolism ceruloplasmin, and 24-h urine copper excretion). The patients were evaluated as WD patients according to clinical findings (presence of Kayser-Fleischer ring, neurological abnormalities, abnormal liver function) and laboratory findings; (low serum ceruloplasmin, increased urinary copper excretion). The exclusion criteria were chronic viral hepatitis, autoimmune hepatitis, and other metabolic liver diseases.

Genetic Analyses

Genetic analyses were performed for diagnosis using a parental informed consent form. DNA was extracted from peripheral blood using a QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All exons of the *eATP7B* (NM_000053.4) gene all exons and exon-intron boundaries were sequenced using in-house designed primers on the MiSeq system (Illumina, San Diego, CA, USA) with next-generation sequencing according to the manufacturer's instructions.

In Silico Bioinformatics Analyses

The potential functional effects of novel missense variants were predicted using Alamut® Visual 2.4 Software (SIFT, Polyphen-2, and Variant Taster). Information about the location of AA and predicted transmembrane domains was obtained from UniProt (http://www.uniprot.org/). The classification of variants was made according to the ACMG 2015 criteria (14).

RESULTS

Between 2016 and 2019, 49 patients from 46 families were referred to our clinic for molecular genetic diagnosis of WD. In this study, we confirmed the molecular diagnosis of WD in 34 of 49 patients (34/49, 69%) (Figure 1). The average age of patients was 16.6. Twenty-two of the patients were between 6 and 18 years old, 5 of them were between 18-30, and the remaining 3 were over 30 years old. The age information of 4 patients was not available (Figure 2). In the molecular genetic analyses positive group, the number of male patients was 22, and the number of female patients was 12, which indicates male dominance (65%) (Figure 3).

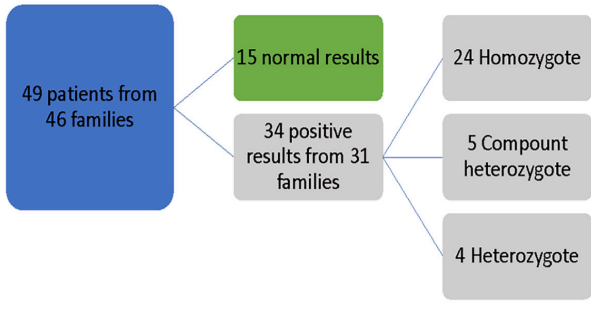

Figure 1. Classification of patients according to the results of molecular genetic analyses.

In this series, we found 26 unique genetic variants, two of which were novel. In terms of variant effect, missense type was the most common (61.5%, 16/26). The splice site and frameshift frequency were the same at 15.4% (4/26), and non-sense was 0.08% (2/26). While 24 of the 34 patients were homozygous, 5 were compound heterozygous and 4 were heterozygous states. All compound heterozygous results were confirmed by parental molecular genetic analyses. In addition, all variants are classified as pathogenic or likely pathogenic according to the ACMG 2015 classification criteria (Table 1).

Variants were relatively frequent in the exon 8 region. The distribution of the variants tended to be balanced when exon 8 was excluded (Figure 4). p.M769Hfs*26 and p.H1069Q were the most common variants with allele frequencies of 7/62 (11.3%) and 5/62 (8%), respectively. Patients 31 and 32 were siblings and both had c.1285+5G>T (IVS2+5G>T) alterations, which were also shared by unrelated patient 22. Moreover, p.R778G and p.L1327V were also shared variants by two unrelated families. Patients 7, 8 (c.1707+4>G or IVS4+4>G) and 24, 25 (Y1384*) were affected siblings from two different families, respectively. p.M297K and c.1707+4>G were the only 2 novel variants in the study, and both were classified as likely pathogenic according to ACMG criteria.

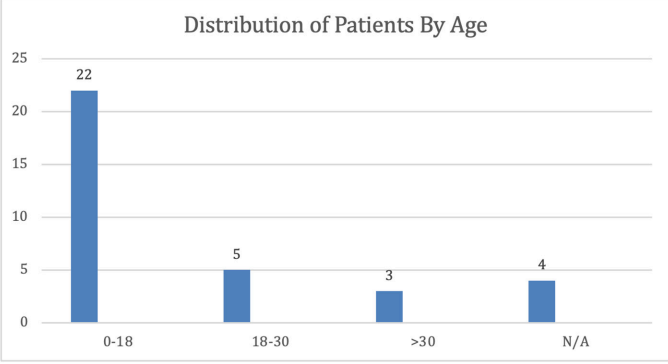

Figure 2. Distribution of admitted patients by age group All patients were divided into three groups: 0-18, 18-30, and over 30. The age information of 4 patients was not available.

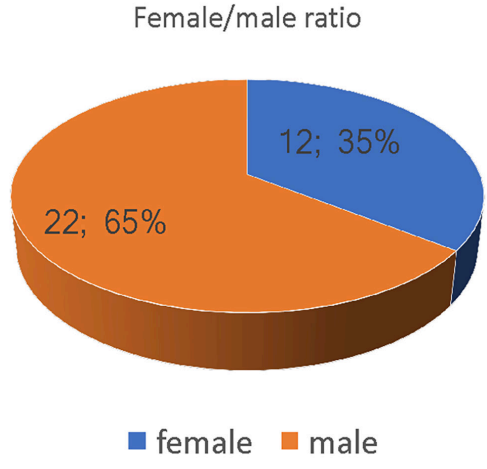

Figure 3. Female/male ratio of the patients.

DISCUSSION

To the best of our knowledge, this is the largest study conducted in Türkiye. A total of 26 unique variants were detected in 34 index cases from 31 different families with a 67% (31/46) diagnostic rate, which is much lower than that reported in previous studies reporting variants in approximately 80% of all clinically confirmed WD (15). The highest mutation detection rates reported in the literature are 98% and 97.35% in cohorts from the United Kingdom and Vietnam, respectively (9,16). Higher variant detection rates may be related to complementary techniques implemented in DNA analysis (such as MLPA) to detect large deletions/duplications, the varying prevalence of WD in different populations, or specific patient selection criteria used in clinical diagnosis. Similar previous cohorts from Türkiye reported variant detection rates of approximately 65% (30/46) and 71% (23/32) using Sanger sequencing, which is comparable to the present study (17,18). Considering the number of patients included in our study and other studies from Türkiye, the differences could be interpreted as insignificant. In our study, the age of the patients evaluated ranged 6 to 39 years at the time of molecular diagnosis. The age range of the evaluated patients was highly variable, consistent with other studies in the literature.

As a typical feature of WD, the common variant responsible for the disease differs among populations. In Europe, Mediterranean countries, and North America, the most common variant is p.H1069Q with a prevalence varying from 10% to 40%, whereas in Asian populations, R778L is more prevalent, with a frequency of 20% to 44% (19). Based on data from Eastern, Central, and Northern Europe, p.H1069Q is suggested to be the most frequent variant with an allele frequency of 30% to 70% in molecularly confirmed WD patients (20). However, in this study, p.M769Hfs*26 was the most frequent variant with an allele frequency of 11.3% (7/62), followed by p.H1069Q with a frequency of %8 (5/62). p.H1069Q was previously reported as the most common variant in other studies from Türkiye with a 17.39% and 15.625% allele frequency (17,18). The number of patients in our cohort was insufficient to infer common WD allele in the Turkish community. A larger group of patients with molecularly identified WD might reveal a higher allele frequency in favor of the H1069Q variant. Nevertheless, we specify p.M769Hfs*26 as one of the frequent alleles in Turkish patients with WD. The p.M769Hfs*26 variant is mentioned in the list of major causative mutations in some other European countries (21). Our study is the first to suggest that p.M769Hfs*26 is also a common allele in the Turkish WD population. The other recurrent variants detected in different families included c.1285+5G>T, p.R778G, and p.L1327V.

According to previous studies, missense variants were observed as a more common molecular mechanism compared with non-sense and frameshift effects (50% vs. 16.7%) in WD (22). Similar rates were found in our study (missense type 61.5%, splice site, and frameshift %15.4) except for non-sense variants, which we found only twice with a 0.08% (2/26) prevalence. WD appears to have high allelic heterogeneity between different populations. The distributions of variants within the gene were evaluated, and the most common variants were seen in exon 8, which we thought could be the hot-

Table 1. The entire list of index cases and all identified variants

Patient	Age (year)	Gender	Variants*	Zygosity	Classification†	Variant ID**
1	29	F	c.2131G>A (p. G711R)	Het	P	CM992817
2	13	M	c.3007G>A (p. A1003T) /c.3207C>A (H1069Q)	Comp Het	P	CM980180/CM930059
3	39	M	c.3402delC (A1135Qfs*13)	Hom	P	CM930912/rs137853281
4	13	F	c.1369C>T p. Q457*	Hom	P	CM992591
5	18	M	c.3979C>G (p. L1327V)	Hom	P	CM993113
6	19	M	c.3979C>G (p. L1327V)	Hom	P	CM993113
7	17	F	IVS4+4>G (c.1707+4A>G)	Hom	LP	NOVEL
8	6	M	IVS4+4A>G (c.1707+4A>G)	Hom	LP	NOVEL
9	N/A	M	c.2304_2305insC (p. M769Hfs*26)	Hom	P	CI951903
10	15	M	c.3506T>C (p. M1169T)	Hom	P	CM992826
11	6	M	c.3207C>A (p. H1069Q)	Hom	P	CM012441
12	6	M	c.2141_2142delTC (p. P714Lfs*40)	Het	P	NOVEL
13	27	F	c.2332C>G (p. R778G)	Hom	P	CM950112/ rs137853284
14	28	M	IVS13-1G>A (c.3061-1G>A)	Hom	P	CS961487
15	11	F	c.122A>G (p. N41S)	Het	P	CM040679).
16	36	M	c.1490T>A p. M497K	Hom	LP	NOVEL
17	18	F	c.2304_2305insC (p. M769Hfs*26)	Het	P	CI951903
18	10	M	c.2332C>G (p. R778G)	Hom	P	CSM950112
19	8	F	c.1639delC (p. G547Rfs*22)	Hom	P	CD982472
20	16	M	c.2304_2305insC (p. M769Hfs*26)	Hom	P	CI951903
21	11	M	c.2383C>T p. L795F/c.2519C>T p. P840L	Comp Het	P	CM970141/CM980172
22	17	F	IVS2+5G>T(c.1285+5G>T) /c.2957C>T p. S986F/ c.3451C>T p. R1151C	Comp Het	P	CS982084/CM107540/CM076004
23	9	M	c.2621C>T (p. A874V)	Hom	P	CM980173
24	9	F	c.2141T>G p. Y1384*	Hom	P	rs1431633756
25	18	M	c.2141T>G p. Y1384*	Hom	P	rs1431633756
26	11	F	IVS2+2dupT (c.1707+2dupT)	Hom	P	CI992786
27	11	M	c.1639delC (p. Q547Rfs*22)	Hom	P	CD982472
28	37	M	c.2906G>A p. R969Q	Hom	P	rs121907996
29	19	F	c.2557C>T p. R919W	Hom	P	CM980176
30	N/A	M	c.2621C>T (p. Ala874Val) /c.3809A>G (p. A1270S)	Comp Het	P	CM980173/930060
31	N/A	F	IVS2+5G>T (c.1285+5G>T) / IVS13-1G>A (c.3061 1G>A)	Comp Het	P	CS982084/ CS961487
32	15	M	IVS2+5G>T(c.1285+5G>T) /IVS13-1G>A c.3061-1G>A	Comp Het	P	CS982084/CS961487
33	6	M	c.3207C>A (p. H1069Q)	Hom	P	CM012441???
34	N/A	M	c.2304_2305insC (p. M769Hfs*26)	Hom	P	rs137853287

N/A: Not available, F: Female, M: Male, P: Pathogenic, LP: Likely pathogenic, †ACMG 2015, *_ATPTB_ (NM_000053.4), **Human Gene Mutation Database (HGMD®), and dbSNP.

spot region (Figure 4). Previous studies have reported different exons for potential hot spot regions. For example, while Coffey et al. (9) reported exons 8, 14, and 18 as hot spot regions for the United Kingdom population, Huong et al. (16) pointed to exon 2 as a hot spot region for Vietnamese populations. Also, Balashova et al. (22) considered exons 7, 8, and 17as hot spots for the Russian Federation. This confirms the thesis that "hotspots" of the *ATP7B* gene vary significantly by geographic region. Because relatively frequent variants were observed in exon 8 (4/26, 15.4%), this region was thought to be a potential hot spot region for the *ATP7B* gene within the Turkish population. However, this is a low rate to provide an advantage in early molecular genetic diagnosis, and variants in

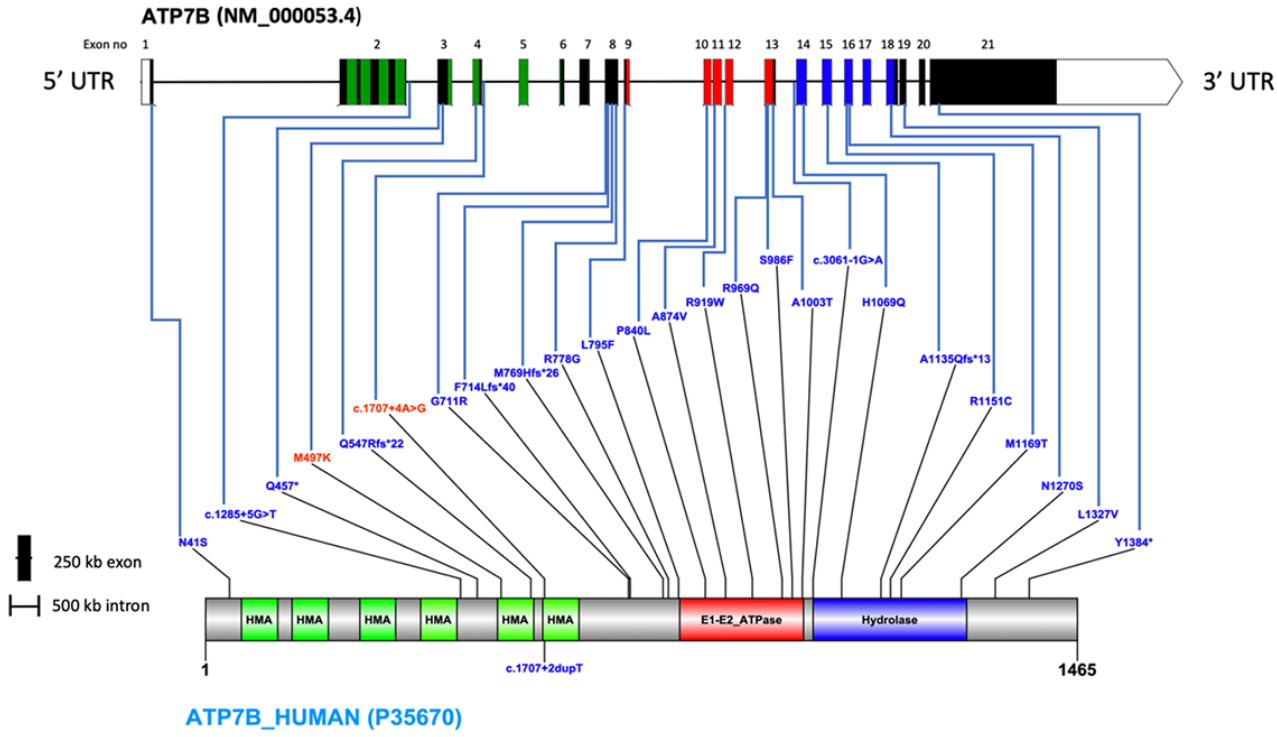

Figure 4. The locations of the variants on the *ATP7B* gene and protein domains were visualized. The novel variants are shown in red.

the *ATP7B* gene were shown to be distributed throughout the gene when exon 8 was excluded. Therefore, despite studies suggesting that a certain exon of the gene should be examined first, the most appropriate first step option for the molecular genetic diagnosis of WD in the Turkish population could be DNA sequence analysis of the whole gene.

Previous studies have shown that there is no significant difference in the prevalence of the disease between the genders in WD. Conversely, in our study, the ratio of male patients to female patients was significantly higher (22/34, 65%) (Figure 3), which is an unexpected situation in autosomal inherited genetic diseases (23). When we look at the other studies from Türkiye, we see slight male dominance there as well (18/30, 60% and 12/23, 52%) (17,18). This may be due to the limited number of patients evaluated in the studies from Türkiye, but it may also be due to the different genetic backgrounds of the Turkish population. In addition, although there is no difference in the occurrence of the disease between the sexes, there appears to be a significant gender difference in the clinical presentation of WD patients. It has been reported that liver findings in women and neuropsychiatric findings in men are more common in WD than in the opposite sex. This situation may also be reflected in the selected patient group in studies conducted in Türkiye.

Although WD was clinically considered, no variant was detected in the molecular genetic analysis of 15 of 49 patients. Possible explanations for the non-detection of variants include unknown variants in regulatory regions such as promoters or enhancers, deeper introns, or other DNA control regions outside the scope of DNA sequencing, and large deletions/duplications. In addition, other disease-related cellular factors may be responsible for the clinical findings. On the other hand, 4 patients had only one potential disease-causing variant. The reason for this could be a variant in the other allele that remains undetected because of the same reasons specified for completely normal molecular genetic analyses. RNA-seq and large deletion/duplication analysis should be performed in such patients. Further studies are needed to determine the cause of WD in patients without a variant in the *ATP7B* gene in molecular genetic analysis.

CONCLUSION

In most patients with WD, clinical manifestations are usually latent and may not be noticed until progressive liver failure and/or neurological dysfunction. Therefore, molecular genetic analysis of the ATP7B gene is important for the early diagnosis and treatment of clinically suspected patients. The molecular genetic spectrum of the ATP7B gene in WD differs between populations. In this study, it was observed that there were differences in the Turkish population. The variants we detected will contribute to our understanding of the molecular genetic structure of the disease in the Turkish population. In addition, the new variants detected will be added to the international databases of the disease. Thus, it will help to fully reveal the genetic mechanisms underlying the disease.

Acknowledgements: We thank all of the patients and their parents who contributed to the study.

Ethics

Ethics Committee Approval: This study was approved by the Ankara City Hospital Ethics Committee (approval number: EI/878/2020, date: 02.07.2020).

Informed Consent: Informed consent was obtained from the participants.

Authorship Contributions

Surgical and Medical Practices: A.B., V.T., B.Ç., Concept: A.B., V.T., B.Ç., Design: A.B., V.T., B.Ç., Data Collection or Processing: A.B., V.T., B.Ç., Analysis or Interpretation: A.B., V.T., B.Ç., Literature Search: A.B., V.T., B.Ç., Writing: A.B., V.T., B.Ç.

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

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Wilson S. Word Cloud contest. Brain 1912; 34: 295-507.
2. Sternlieb I. Perspectives on Wilson's disease. Hepatology 1990; 12: 1234-9.
3. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. Lancet 2007; 369: 397-408.
4. Perri RE, Hahn SH, Ferber MJ, Kamath PS. Wilson Disease--keeping the bar for diagnosis raised. Hepatology 2005; 42: 974.
5. Valentino PL, Roberts EA, Beer S, Miloh T, Arnon R, Vittorio JM, et al. Management of Wilson Disease Diagnosed in Infancy: An Appraisal of Available Experience to Generate Discussion. J Pediatr Gastroenterol Nutr 2020; 70: 547-54.
6. Chandra A, Bhattacharjee MS. Kayser-Fleischer Rings in Wilson's Disease. N Engl J Med 2021; 385: e46.
7. Ferenci P. Diagnosis of Wilson disease. Handb Clin Neurol 2017; 142: 171-80.
8. Figus A, Angius A, Loudianos G, Bertini C, Dessi V, Loi A, et al. Molecular pathology and haplotype analysis of Wilson disease in Mediterranean populations. Am J Hum Genet 1995; 57: 1318-24.
9. Coffey AJ, Durkie M, Hague S, McLay K, Emmerson J, Lo C, et al. A genetic study of Wilson's disease in the United Kingdom. Brain 2013; 136: 1476-87.
10. Kulkarni A, Sharma VK. Wilson's disease; 2017.
11. Peña MM, Lee J, Thiele DJ. A delicate balance: homeostatic control of copper uptake and distribution. J Nutr 1999; 129: 1251-60.
12. Rodriguez-Castro KI, Hevia-Urrutia FJ, Sturniolo GC. Wilson's disease: A review of what we have learned. World J Hepatol 2015; 7: 2859-70.
13. Tanzi RE, Petrukhin K, Chernov I, Pellequer JL, Wasco W, Ross B, et al. The Wilson disease gene is a copper-transporting ATPase with homology to the Menkes disease gene. Nat Genet 1993; 5: 344-50.
14. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405-23.
15. Schmidt HH-J. Role of genotyping in Wilson's disease. J Hepatol 2009; 50: 449-52.
16. Huong NTM, Hoa NPA, Ngoc ND, Mai NTP, Yen PH, Vân Anh HT, et al. Mutation spectrum of ATP7B gene in pediatric patients with Wilson disease in Vietnam. Mol Genet Metab Rep 2022; 31: 100861.
17. Simsek Papur O, Akman SA, Cakmur R, Terzioglu O. Mutation analysis of ATP7B gene in Turkish Wilson disease patients: identification of five novel mutations. Eur J Med Genet 2013; 56: 175-9.
18. Terzioğlu O. Clinical and genetic analysis of pediatric patients with Wilson disease. Turk J Gastroenterol 2015; 26: 397-403.
19. Zhang Y, Wu Z-Y. Wilson's disease in Asia. Neurology Asia 2011; 16: 103-9.
20. Ferenci P. Regional distribution of mutations of the ATP7B gene in patients with Wilson disease: impact on genetic testing. Hum Genet 2006; 120: 151-9.
21. Gomes A, Dedoussis GV. Geographic distribution of ATP7B mutations in Wilson disease. Ann Hum Biol 2016; 43: 1-8.
22. Balashova MS, Tuluzanovskaya IG, Glotov OS, Glotov AS, Barbitoff YA, Fedyakov MA, et al. The spectrum of pathogenic variants of the ATP7B gene in Wilson disease in the Russian Federation. J Trace Elem Med Biol 2020; 59: 126420.
23. Litwin T, Gromadzka G, Członkowska A. Gender differences in Wilson's disease. J Neurol Sci 2012; 312: 31-5.