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Novel Variants Associated with Wilson Disease in Saudi Families

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1. Abstract

1.1. Background: Wilson disease (WD) is an autosomal recessive inherited disorder caused by homozygous or compound heterozygous mutations in the *ATP7B* gene. The *ATP7B* gene encodes trans-membrane copper-transporting ATPase 2, which mediates the excretion of copper into bile and delivers copper to the transporting protein ceruloplasmin in blood. The genetic database reported over 782 pathogenic ATP7B variants that cause WD. In the Saudi population, several mutations are identified in the *ATP7B* gene associated with WD, such as p. Gln1399Arg, p. Ser744Pro, and p. Gly1341Ser.

1.2. Aim: We aimed to investigate and characterize WD's common and specific genetic variants in Saudi Arabia.

1.3. Methods: Blood samples were collected from four WD patients including three siblings (P(1), P(2), and P(3)) and one separate case P(4). Blood samples were also obtained from the unaffected parents of the siblings. Genomic DNA was extracted from blood samples using a Gentra Puregene Blood Kit. DNA was sequenced using massively parallel sequencing via Ion Torrent PGM with the Ion PI™ Hi-Q™ Sequencing 200 Kit.

1.4. Results: In three affected siblings, a novel homozygous frameshift variant (M851X) was identified in the *ATP7B* locus at position chr13:52524431delA. The parents were found to be heterozygous carriers for this variant. A homozygous frameshift placed at this location results in a premature stop codon and putative expression of a non-functional truncated ATP7B protein. In the fourth separate case, four homozygous missense variants were also identified: Val1140Ala, Thr977Lys, Arg952Lys, and Lys832Arg.

1.5. Conclusion: This study reported novel WD-associated *ATP7B* gene variants in the Saudi population.

2. Background

Wilson disease (WD) was first described in 1912 by Samuel Alexander Kinnier Wilson as a familial disease characterized by progressive, lethal neurologic dysfunction with liver cirrhosis, and the Kayser-Fleischer corneal ring [1, 2]. Over the past years, many studies have extended the knowledge of Wilson disease, leading to our current understanding that the fundamental mechanisms of Wilson

disease are related to impaired biliary excretion of copper. This is resulting in a gradual accumulation of copper and consequent tissues damages, initially within the liver but eventually in other organs, including the brain [3, 4]. WD (OMIM# 277900) is an autosomal recessive inherited disorder caused by homozygous or compound heterozygous mutations in ATP7B, encoded by the *ATP7B* gene located on chromosome 13q14.3. To this date, mutation in *ATP7B* gene was reported to be the principal player in the development of WD [5]. Some studies have identified the engagement of other mutated genes in the pathogenesis of WD, such as Patatin- like phospholipase domain-containing protein 3 (PNPLA3). Mutation in this gene was found to increase the severity of fatty liver in WD patient [6]. The *ATP7B* gene (OMIM# 606882) encodes trans-membrane copper-transporting ATPase 2, which mediates the excretion of copper into bile and delivers copper to the transporting protein ceruloplasmin in blood [7]. Dietary copper is mainly metabolized at the liver; in WD, the defective pathogenic ATP7B gene leads to hepatocyte copper overload that associated with liver pathology. Excess non-ceruloplasmin-bound copper is released into the circulation, leading to secondary pathological accumulation in other tissues [8]. Pathological accumulation of copper causes a broad spectrum of clinical signs and symptoms. Liver disease is the first clinical manifestation presented in 40–60% of patients with WD. Liver damages range from asymptomatic/subtle morphological changes manifested by simple acute self-limited hepatitis to severe hepatitis featured with recurrent jaundice, cirrhosis with or without portal hypertension, hepatobiliary malignancies, and even acute liver failure [9]. Neurological manifestations account for the second common clinical symptom of WD after hepatic manifestations. Initial neurological presentation occurs in 18–68% of patients. The main clinical signs include different movement disorders such as tremor, dystonia, parkinsonism, choreoathetosis, dysarthria, gait and posture disturbances, drooling, and dysphagia [3]. Additionally, psychiatric presentations are observed in higher prevalence in patients with WD, such as depression, psychosis, and manic/hypomanic episodes have. Other psychological disorders linked to WD are obsessive-compulsive disorder (OCD), anorexia, academic problems, and isolated irritability [10]. Moreover, ophthalmological signs are common presentations in WD due to pathological copper accumulation in the eyes, these include the Kayser-Fleischer ring (copper accumulation in the Descemet membrane) and sunflower cataract. Identification of Kayser- Fleischer rings requires a slit-lamp examination or anterior segment optical coherent tomography (OCT). This non-contact procedure is an alternative method for detecting copper depositions in the cornea [11]. Wilson disease may also affect other systems of the body such as hematologic, renal, bone, cardiac, endocrinologic, or dermatologic systems. Hematologic symptoms include acute Coombs-negative hemolytic anemia, leukopenia, and thrombocytopenia. Renal abnormalities presented mainly with tubular dysfunction (e.g., renal tubular acidosis, aminoaciduria) and nephrolithiasis. Osteopenia, osteoporosis, and arthropathy are the most common features of bone abnormalities in WD. Cardiac patients with WD can exhibited signs and symptoms related to arrhythmias, cardiomyopathy, and autonomic dysfunction. Endocrine manifestations include disorders of growth and puberty, hypothyroidism, hypoparathyroidism, infertility, or repeated miscarriages. Finally, dermatological manifestations involve hyperpigmentation of the lower extremities, azure lunulae of the nails, anetoderma, xerosis, and acanthosis nigricans [12]. Wilson disease has been reported at ages from 2 to 80 years, and most of the patients were diagnosed before the age of 40 [13]. To establish the diagnosis of WD, a combination of clinical features and laboratory parameters is required. In WD, the serum ceruloplasmin is typically reduced, and the concentration of non-ceruloplasmin-bound copper is increased. Obtaining a 24- hour urinary copper excretion (considered positive if the level is >100 μg over 24 hours) is essential to establish the diagnosis and guide the treatment plan of WD. Indeed, liver biopsy with hepatic parenchymal copper concentration measurement is considered the gold standard diagnostic tool, and the threshold value of ≥ 250 μg/g of dry liver weight has high sensitivity. Molecular genetic testing is another crucial diagnostic tool [14, 15]. However, none of the available laboratory tests is accurate and specific for WD. Therefore, a diagnostic scoring system known as the Leipzig score (Table 1) was proposed to provide accurate diagnostic accuracy [16]. Wilson disease considered as successfully treatable metabolic disorder if the patient we early diagnosed using accurate and solid diagnostic strategies [6]. Current management options for WD include different pharmacological therapies. The pharmacological treatment is based on drugs that produce a negative copper body balance, including copper chelators (d-penicillamine and trientine) to increase urinary copper excretion, and zinc salts to decrease copper absorption from the gastrointestinal tract [17]. Failure of pharmacological therapies mandates liver transplantation which is usually indicated in acute (fulminant) liver failure or decompensated liver cirrhosis. In acute liver failure, the indication for liver transplantation is generally based on the revised King's prognostic Wilson index, which is a score that depends on laboratory parameters [18]. Other new agents for WD have being investigated in clinical trials such as tetra thiomolybdate salts as well as genetic therapeutic modalities are being examined in animal studies [6]. To date, more than 700 pathogenic ATP7B variants have been reported, including missense (55%), nonsense (9%), frameshift deletion, insertion or substitution (23%), and splice site variant (6%) [19]. However, WD is a rare genetic disorder, and the prevalence of the disease is 13.9 per 100,000 (95% CI: 12.9-14.9). The most recent global population prevalence of WD was estimated between 1 in 17,000 to 1 in 20,000. WD has been reported in several countries, including Saudi Arabia [20]. Several mutations are identified in the ATP7B gene associated with WD in the Saudi population, such as p.Gln1399Arg, p.Ser744Pro, and p.Gly1341Ser [21]. Therefore, more investigations are required to detect and characterize WD's common and specific genetic variants in Saudi Arabia [22-24]. Herein, we aimed to improve our understanding of the genomic background of WD and other disease [25], using the advanced available genetic tech-

nologies [26]. Using a massively parallel sequencing genetic analysis strategy, the current study identified novel mutations in the ATP7B gene of four individuals with Wilson disease. Novel identified mutations in the ATP7B gene will help in improving the diagnosis of Wilson disease nationally and internationally to provide early effective therapeutic intervention for WD patients [27].

Table 1: Leipzig criteria: Diagnostic criteria for Wilson disease

Clinical or Laboratory Findings		Points
Kayser-Fleischer Rings	Present Absent	2 0
Neurological Symptoms or MRI Findings	Severe Mild Absent	2 1 0
Serum Ceruloplasmin Level (g/L)	<0.1 0.1-0.2 Normal (>0.2)	2 1 0
24-h Urinary Copper	>2x upper limit of normal 1-2x upper limit of normal Normal Normal, but >5x upper limit of normal after D-penicillamine	2 1 0 2
Coombs-negative Hemolytic Anemia	Present Absent	1 0
Total Liver Copper Level (Micromol/g)	>5x upper limit of normal (>4) Increased (0.8-4) Normal (<0.8) Rhodanine-positive granules present	2 1 -1 1
Genetic Mutation	Present on both chromosomes Present on 1 chromosome Absent	4 1 0
Total Score	Diagnosed established Diagnosis possible Diagnosis unlikely	4 3 2 or less

3. Case presentation

In this study, we have included four patients with WD from two different families. The first family consists of 3 siblings: patient (1), patient (2), and patient (3). We have investigated their parents, who are not affected by the disease and have no kinship. The second family includes the fourth patient (4) only with positive parental consanguinity. All four cases and their families were clinically diagnosed with WD in the internal medicine clinic at the King Faisal Specialist Hospital and Research Centre-Jeddah (KFSH&RC. Gen.Org). They were enrolled in the western province of Saudi Arabia. Genetic testing was completed in all patients on a clinical basis of WD. Clinical features and biochemical data were retrospectively collected from the electronic medical charts of the individual patients (**Table 2**).

4. Methods

4.1. Patient and family recruitment

This study includes four cases of Wilson disease that were evaluated in the internal medicine clinic at the King Faisal Specialist Hospital and Research Centre-Jeddah (KFSH&RC. Gen.Org) and their families, which were enrolled in the western province of Saudi Arabia. After signing the informed consent and documentation of family history forms, only confirmed cases with Wilson disease were selected based on clinical diagnosis. The diagnosis was based on the patient's clinical presentation. The retrieved demographic data included the age, sex, race, ethnicity, and previous & current treatment history. The patient demographic and clinical characteristics are provided in (**Table 2)**.

Table 2: Demographic data, clinical features, laboratory, and medications

Patient Number	1	2	3	4
Demographics				
Sex	Male	Female	Female	Male
Age (Years)	30	27	19	32
Age At Diagnosis (Years)	8	7	5	13
Clinical Features				
Hepatic	+ve	+ve	+ve	+ve
Neurological	-ve	-ve	-ve	+ve
Kayser-Fleischer Ring	-ve	-ve	-ve	-ve
Family History Of WD	+ve	+ve	+ve	+ve
Parental Consanguinity	-ve	-ve	-ve	+ve
Labs				
Ceruloplasmin Mg/L (150-300)	<3	<3	<3	<3
Serum Copper Umol/L (11-22)	0.9	0.8	1.4	3.8
24-H Urinary Copper *Mg/24 H (0.00-1.57)*	7.07	3.3	6.85	7.14
Hemoglobin G/L	150	116	128	152
Platelet 10^9/L	359	279	344	171
INR	1.1	1.1	1	1.1
Albumin G/L (39-49)	44	44	45	41
Alanine Aminotransferase U/L	81	14	8	21
Aspartate Aminotransferase U/L	41	17	13	24
Alkaline Phosphate U/L	67	69	56	90
Total Bilirubin Umol/L	7	6	5	10
Liver Biopsy *Mg/G*	+ve	+ve	+ve	ND
Meld Score	8	7	7	9
Imaging				
Abdomen US	Normal	Normal	Mild Hepatom-egaly	Cirrhosis
MRI	ND	ND	ND	+ve
Medications				
Penicillamine	500 mg BID	250 mg BID	250 mg BID	250 mg QID
Zinc Gluconate	60 mg BID	30 mg TID	30 mg BID	60 mg TID
Pyridoxine	25 mg OD	25 mg OD	25 mg OD	25 mg OD

WD= WILSON'S DISEASE, ND= NOT DONE, US= ULTRASOUND, MRI= MAGNETIC RESONANCE IMAGING

4.2. Ethical approval and participants' consent

This study was approved by the Institutional Review Board of King Faisal Specialist Hospital & Research Centre in Jeddah (IRB number 2018-36). Informed consent was routinely obtained from the participants. Prior to blood sample collection, all patients who met the inclusion and exclusion criteria completed the informed consent (ICF) and case report form (CRF). All participants in the current study agreed to publish the data generated from the survey.

4.3. DNA extraction

Peripheral blood samples were collected from each patient (approximately 2-3 cc) in an EDTA tube. Subsequently, the genomic DNA was extracted from all blood samples using a Gentra Puregene Blood Kit (Qiagen, Cat No: 158389) according to the manufacturer's instructions. Briefly, 20 µl of proteinase K was added to the bottom of a 1.5 ml Eppendorf tube. Then, 200 µl of whole blood samples were added to the Eppendorf tube, mixed with the proteinase K, and incubated for 1 min at room temperature (15–25°C), followed by 200 µl of buffer AL to the sample. Mixing then occurred by pulse-vertexing for 15 sec. Then, the mixture was incubated in a water bath at 56°C for 10 min, spun for 2 min at 2000 x g, and 200 µl of ethanol (96–100%) was added to the sample, followed by mixing by pulse-vertexing for 15 sec. Next, it was centrifuged for 1 min at 16,000 x g. Finally, 100 µl of the DNA Hydration Solution was added, and it was vortexed for 5 sec and incubated at 65°C for 5 min. The quantity and quality of extracted DNA were evaluated by using the Qubit® 2.0 Fluorometer (Life Technologies) with the Qubit™ dsDNA High Sensitivity Assay Kit (Life Technologies, Cat No Q32851) and Nanodrop 2000 system (Thermo Scientific), respectively, according to the manufacturer's instructions.

4.4. Library preparation for NGS

Extracted DNA (100 ng per sample) was used for library preparation using the Ion AmpliSeq™ Library Kit Plus (Life Technologies, Cat # 4488990) with the Ion AmpliSeq™ Exome RDY Kit (Life Technology, cat # 8849838) according to the manufacturer's instructions. Briefly, each library was barcoded with the Ion Xpress™ Barcode Adapters 1-96 Kit (Life Technologies) and then combined to a final concentration of 100 pM. Then 14 µl of HiFi solution was added to the 56 µl of diluted DNA with nuclease-free water to complete a total of 70 µl. In each well of the 96 healthy plates, 5 µl was loaded, vortex and amplified by PCR 40 min

4.5. Ligation

For the ligation step,1.5 µl of P1 adapter was added to1.5 µl barcode

adaptor and 3 µl of nuclease- free water. Then 4 µl of switch solution was added to the diluted barcode, and 2 µl of the DNA ligase was added, vortex and 80 µl of magnetic beads were used. After that,150 µl of 75% ethanol were added, left for 5 min at RT to dray. Then 50 µl of TE buffer was added and quantified by RT-PCR to do emulsion.

4.6. Emulsion (emPCR)

For template preparation by emulsion PCR (emPCR) using the Ion OneTouch 2 system with the Ion PI™ Hi-Q™ OT2 200 Kit (Life Technologies, Cat# A26428). According to the user guide. Briefly, 2000 of the master mix was added to 80 µl of nuclease-free water and 120 µl of P1 enzyme and 100 µl of ion sphere particles (ISPs) and 100 µl of the diluted library. So, the total 2400 µl was a mix, vortex, then 800 µl were loaded to the loading path. 200 µl of the Ion OneTouch oil and run for 5 hrs. The supernatant was discarded, and 100 µl were left.

4.7. Enrichment

The Ion OneTuch2 Supplies kit (Life Technologies, Cat# A26367) was used for enrichment. Briefly, the supernatant 100 µl was centrifuged at 15500 for 8 min, and the supernatant was removed. Only 20 µl was leftover.80 µl of the recovered template-positive ion sphere particles (ISPs) underwent an enrichment cycle using Dyna beads My One Streptavidin C1 Beads in an Ion OneTouch ES Instrument (Life Technologies).

4.8. Chip Loading

The annealing and flashing buffer were prepared according to the manufacturer's instructions. A 15 µl of annealing buffer was added to 20 µl of primer buffer and 10 µl of loading buffer. A total of 55 µl was loaded in the chip and centrifuged for 10 min, then 20 µl of the fuming solution was added. Then 100 µl of flushing solution was added. Finally, 6 µl of sequencing polymerase and 66 µl were loaded in the chip for sequencing.

4.9. Sequencing

DNA was sequenced by utilizing massive parallel sequencing via Ion Torrent PGM with the Ion PI™ Hi-Q™ Sequencing 200 Kit (Life Technologies, Cat# 4488651) and the Ion PI™ Chip Kit v3 (Life Technologies, Cat# A26770) according to the manufacturer's instructions. Briefly, the prepared DNA was loaded in Ion Torrent semiconductor chips and loaded into the Ion Torrent PGM machine.

4.10. Data analysis and variant filtration

The data analysis (phase I & II) was performed through Torrent Suite and the Saudi Human Genome Program (SHGP) bioinformatics pipeline. The Research Centre-Jeddah performed the tertiary analysis to predict putative causative variant (s) associated with the phenotype. The reads of the Ion Torrent-sequencing platform were aligned to the HG19 reference genome through the tMap program. The aligned reads were investigated for variant calling through the Torrent Suite Variant Caller TVC program. The variants were annotated using public and in-house databases and the Saudi Human Genome program. Then, genetic variants were filtered using minor allele frequency (MAF) of 0.01, mutation impact (nonsynonymous SNV, frameshift, deletion, and stop gain), and a high degree of consistency by four or more predictive models. Variants were further annotated, and functional significance was assessed using the Ensemble Variant Effect Predictor tool [22].

5. Results

5.1. Basic clinical characteristics of the studied subjects

All studied participants were of Saudi Arabian ethnicity and of both genders. The diagnostic clinical characteristics for WD of the included population are shown in (**Table 1)**. The clinical follow up was conducted in King Faisal Specialist Hospital & Research Centre from 2002–2019. As demonstrated in (**Table 2)**, the median age at diagnosis in the participants was 7.5 years. There were no significant variations in either demographic or other pertinent characteristics between the cases; however, there were some differences regarding some clinical features such as neurological symptoms, and abdominal ultra-sound findings. Other clinical data, including the patient age, nationality, stage, medications, and presence of other diseases, were collected, and are also indicated in (**Table 2).**

5.2. Mutation's analysis in patients with Wilson disease

Our current study enrolled four cases clinically diagnosed with Wilson disease and two unaffected parents from Saudi Arabia. Genomic DNA was extracted from peripheral blood samples collected from each patient. Next, genomic analysis was performed using NGS. Interestingly, our genetic data analysis showed a novel homozygous frameshift variant (pM851fs) at position chr13:52524431delA was identified in the ATP7B locus in the three affected siblings (P (1), P (2), and P (3)). The parents of the affected individuals (P (1), P (2), and P (3)) were heterozygous carriers for this variant (Figure 1). A homozygous frameshift identified at this location is predicted to result in a premature stop codon resulting in the expression of a non-functional truncated ATP7B protein (Figure 1). On the other hand, four homozygous missense variants were identified in the fourth individual (P (4)): Val1140Ala, Thr977Lys, Arg952Lys, and Lys832Arg. All variants have been reported previously in dbSNP or the human genome mutation database (Table 3) [29]. The Thr977Lys is categorized as harmful by the ENSEMBL VEP [28] implementation of the SIFT functional prediction method [30].

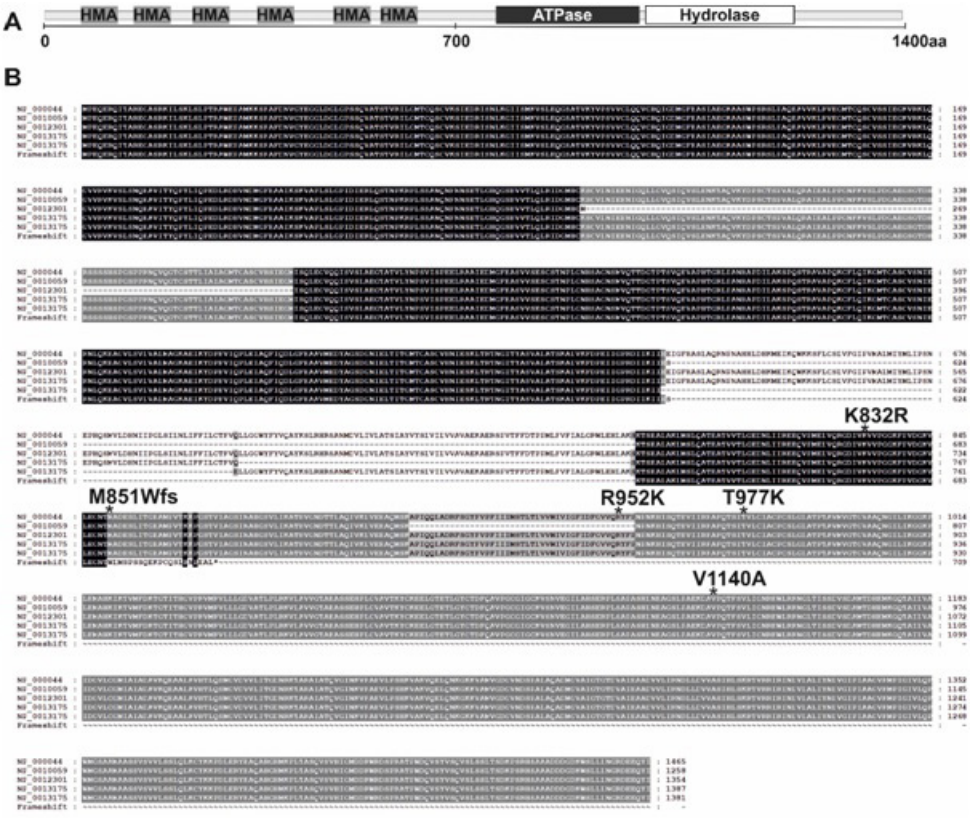

Figure 1: The functional domains of the ATP7B protein are indicated (A). Comparison between *ATP7B* amino acid sequences. An alignment of the protein sequence of the five reference sequences for ATP7B and one predicted protein sequence from a patient harbouring a frameshift was prepared using ClustalO and annotated using Genedoc (B). The mutations identified likely impact the ATPase/hydrolase functions of APT7B.

Table 3: *ATP7B* variants identified in P (4)

Location	Allele	Protein position	Amino acids	Codons	Existing variation	SIFT
13:52515354-52515354	G	1140	V/A	gTc/gCc	rs1801249,CD056134,CM044579,COSV54438812	tolerated(1)
13:52515354-52515354	G	933	V/A	gTc/gCc	rs1801249,CD056134,CM044579,COSV54438812	tolerated(1)
13:52515354-52515354	G	1029	V/A	gTc/gCc	rs1801249,CD056134,CM044579,COSV54438812	tolerated(1)
13:52515354-52515354	G	710	V/A	gTc/gCc	rs1801249,CD056134,CM044579,COSV54438812	tolerated(1)
13:52515354-52515354	G	351	V/A	gTc/gCc	rs1801249,CD056134,CM044579,COSV54438812	tolerated(0.93)
13:52515354-52515354	G	1075	V/A	gTc/gCc	rs1801249,CD056134,CM044579,COSV54438812	tolerated(1)
13:52515354-52515354	G	1062	V/A	gTc/gCc	rs1801249,CD056134,CM044579,COSV54438812	tolerated(1)
13:52520550-52520550	T	977	T/K	aCg/aAg	CM111989,CM960129	deleterious(0)
13:52520550-52520550	T	770	T/K	aCg/aAg	CM111989,CM960129	deleterious(0)
13:52520550-52520550	T	866	T/K	aCg/aAg	CM111989,CM960129	deleterious(0)
13:52520550-52520550	T	547	T/K	aCg/aAg	CM111989,CM960129	deleterious(0)
13:52520550-52520550	T	249	T/K	aCg/aAg	CM111989,CM960129	deleterious(0.01)
13:52520550-52520550	T	899	T/K	aCg/aAg	CM111989,CM960129	deleterious(0)
13:52523808-52523808	T	952	R/K	aGa/aAa	rs732774,CM127927,COSV54436520	tolerated(1)
13:52523808-52523808	T	841	R/K	aGa/aAa	rs732774,CM127927,COSV54436520	tolerated(1)
13:52523808-52523808	T	522	R/K	aGa/aAa	rs732774,CM127927,COSV54436520	tolerated(1)
13:52523808-52523808	T	224	R/K	aGa/aAa	rs732774,CM127927,COSV54436520	tolerated(1)
13:52523808-52523808	T	952	R/K	aGa/aAa	rs732774,CM127927,COSV54436520	tolerated(1)
13:52523808-52523808	T	874	R/K	aGa/aAa	rs732774,CM127927,COSV54436520	tolerated(1)
13:52524488-52524488	C	832	K/R	aAg/aGg	rs1061472,CM116966,COSV54438485	tolerated(0.07)
13:52524488-52524488	C	670	K/R	aAg/aGg	rs1061472,CM116966,COSV54438485	tolerated(0.14)
13:52524488-52524488	C	721	K/R	aAg/aGg	rs1061472,CM116966,COSV54438485	tolerated(0.08)
13:52524488-52524488	C	104	K/R	aAg/aGg	rs1061472,CM116966,COSV54438485	tolerated(0.1)
13:52524488-52524488	C	832	K/R	aAg/aGg	rs1061472,CM116966,COSV54438485	tolerated(0.08)
13:52524488-52524488	C	754	K/R	aAg/aGg	rs1061472,CM116966,COSV54438485	tolerated(0.06)

6. Discussion

Wilson disease is a potentially treatable genetic disorder manifested by defects in copper metabolism and accumulation in the liver and other organs. The severity of clinical presentations of WD varies among individuals based on the time of discovering the abnormality [6]. Consequently, this disease leads to liver damage, neurological degeneration, and other health complications [31]. Thus, early detection of WD before the onset of acute symptoms is essential to provide suitable intervention and ensure a better prognosis, as delayed diagnosis of WD was reported to be the primary cause of treatment failure [6, 32, 33]. WD is considered a high prevalent genetic disease, with 1 in 30,000 worldwide [34]. The incidence of WD in Saudi Arabia has been well documented [20]. For instance, a study of 56 patients published in 2003 described the genetic findings of patients with WD implying a solid association with family history in 72% of the cases, and 68% (38/56) were associated with consanguineous parents. In the study above, the genetic analysis identified pathogenic variants in three exons (exons 8, 19, and 21) of the ATP7B gene in 50% of the patients (28/56). Also, the same study reported that 32% of patients harbored a novel frameshift in exon 21 (p. Gln1399Arg), 16% had missense variant in exon 8 (p. Ser744Pro), and one patient had a novel missense variant in exon 19 (p. Gly1341Ser). Additionally, the variant found in exon 21 was novel and considered a founder mutation as it belonged to a single Saudi tribe. Overall, the study did not report any phenotype-genotype correlation [21]. Another cohort study of 71 WD patients, the most significant cohort documented in Saudi Arabia, showed that the main clinical features of the included patients are neurological, hepatic, and mixed symptomatic presentation in 54.9%, 16.9%, and 28.2%, respectively [20]. A family history of WD was found in 57.7% of patients, and 36.6% had consanguineous parents. Moreover, family screening discovered asymptomatic cases in 15.5% of cases. One of this study recommendations is that early WD diagnosis should be improved for any age to receive early effective therapy and prevent lifelong disabilities [20]. In the present study, six individuals were included in the downstream investigation; two unaffected parents and four diagnosed with WD. Interestingly, our study has identified a novel homozygous frameshift variant (p.M851fs) in the ATP7B locus in the three affected individuals (P (1), P (2), and P (3)). Moreover, the parents of these individuals were found to be heterozygous carriers for this variant. Indeed, a homozygous frameshift was identified at this location is predicted to result in a premature stop codon and the expression of a non-functional truncated ATP7B protein. Furthermore, four homozygous missense variants were identified in the unrelated case (P (4)): Val1140Ala, Thr977Lys, Arg952Lys, and Lys832Arg. Although this specific variant is novel, variants affecting this position (Thr977Arg; Thr977Met) have previously been found in westernized WD patients but not in the Saudi Arabian patients' population [22]. Molecular genetic analysis has shown that ATP7B mutations in patients with WD vary between countries and populations [7]. For example, in Northern or Eastern European ancestry, His1069Gln (H1069Q) is the most common substitution variant in 40% of patients. While in Asian countries (South Korea, Japan, and China), the Arg778Leu (R778L) missense mutation is detected in exon 8 with an allele frequency of 14–49%. Furthermore, the missense mutation Met645Arg (M645R) (exon 6) is common among WD patients in mainland Spain. Therefore, the findings of our study and others contribute to a better understanding of the molecular variants of WD in the Saudi population as well as globally [23]. As a result, discovering these most common and pathogenic mutations might eventually provide a foundation for developing an effective national screening program for WD genetic diagnosis by molecular and genomic tools using specific biomarkers [35]. In addition, this will improve the genetic diagnosis of WD based on the distribution of mutations among a particular population.

7. Conclusion

Wilson disease, among other genetic disorders that can be treated effectively if detected before the development of severe manifestations; yet, if missed, WD is a fatal condition [6]. Therefore, we recommend using advanced genetic tools such as NGS to uncover and characterize all mutations among WD patients in Saudi Arabia. Thus, it would assist in the early diagnosis of WD and improve the clinical management of the presenting manifestations. Furthermore, the early implication of clinical intervention of WD would avoid the development of hepatic, neuropsychiatric, and systemic disabilities. Additionally, further studies are needed, which will help identify the differences in common mutations in our population and can be used to design biomarkers for genetic testing of WD in the future.

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