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ORIGINAL ARTICLE

Hepatology

Genetic profiling of Wilson disease reveals a potential recurrent pathogenic variant of *ATP7B* in the Jordanian population

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Abstract

Objectives: Wilson disease (WD) is an autosomal-recessive disorder that disrupts copper homeostasis. ATPase copper transporting beta (*ATP7B)* gene is implicated as the disease-causing gene in WD. The common symptoms associated with WD include hepatic, neurological, psychiatric, and ophthalmic manifestations. The genetic landscape of WD is under-investigated in the Middle East and has never been studied in Jordan. We aimed to investigate the genetic profile of several unrelated Jordanian families with one or more patients affected by WD.

Methods: Twenty-four Jordanian families with WD underwent clinical evaluation and genetic profiling by whole-exome and Sanger sequencing.

Results: Surprisingly, the same variant (*ATP7B*:c.3551C>T;p.Ile1184Thr) was identified, for the first time, exclusively in the homozygous state, in eight consanguineous unrelated families. Before our study, the previous classification of this variant was either of uncertain significance (VUS) or likely pathogenic (LP). Interestingly, the patients harboring this variant displayed variable clinical manifestations on both the intra- and interfamilial levels, as previously described in cases with WD. The age of diagnosis, hepatic manifestations, neuropsychiatric involvements, and Kayser–Fleischer ring occurrence varied significantly in terms of existence and severity among the recruited individuals. Following our investigation, based on clinical data and co-segregation analysis, we re-classified the variant *ATP7B*:c.3551C>T;p.Ile1184Thr from VUS/LP to pathogenic, for the first time. Besides, our genetic analysis helped in resolving diagnostic ambiguity by either establishing or ruling out the diagnosis of WD.

Conclusion: Since the identified variant (ATP7B:p.Ile1184Thr) was discovered in multiple unrelated families, we create an avenue for the potential consideration of this variant as a recurrent, or possibly a founder variant, in the Jordanian population. Our work sheds light, for the first time, on the molecular underpinnings of WD in Jordan and compiles the WD-causing variants in the Middle East. Ultimately, the findings of our work can guide designing region-specific targeted genetic testing of WD in Jordan and provide valuable insights to direct clinical decisions for atypical WD presentations.

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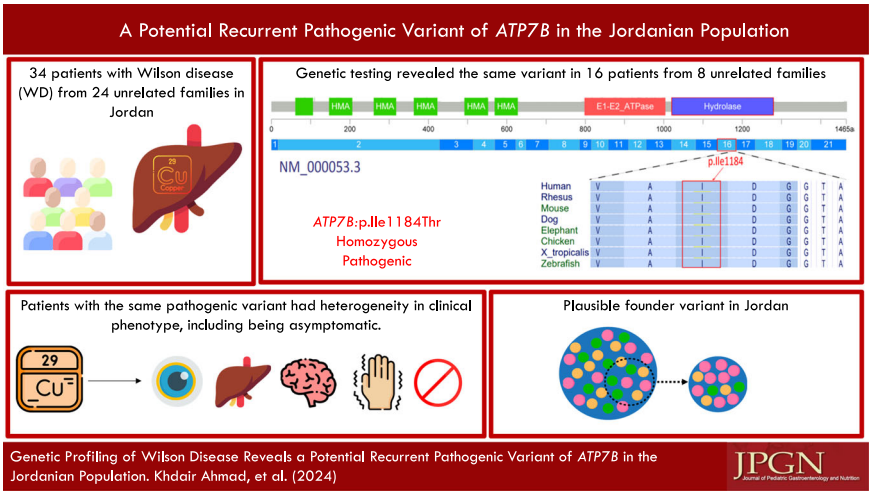

KEYWORDS
c.3551C>T, founder, genetic testing, Ile1184Thr, whole-exome sequencing

1 | INTRODUCTION

Wilson disease (WD; OMIM 277900) is an autosomal-recessive disorder disrupting copper homeostasis and it is caused by pathogenic variants in the ATPase copper transporting beta (*ATP7B) gene*.[1] WD can develop from an initially hepatic or neuropsychiatric disease into a multisystemic disorder.[1,2] Heretofore, according to the Human Gene Mutation Database, more than 900 variants have been identified in *ATP7B* to be associated with WD, mainly found in the compound heterozygous state.[3]

The worldwide clinical prevalence of WD is estimated to be 30 instances per million, with a carrier frequency of 1 in 90.[1,4] While WD's prevalence in the Middle East has not yet been investigated, it is anticipated to be higher, given the high consanguinity levels in such a population.[4,5] WD diagnosis is primarily based on a battery of assessments such as biochemical testing, ophthalmic assessment for corneal Kayser–Fleischer ring (KFR), as well as hepatic and neuropsychiatric evaluations.[6] The suggestive biochemical hallmarks of WD include abnormal liver function test (LFT), low ceruloplasmin, and high basal 24-h urinary copper levels.[6,7] Genetic testing of WD can aid in accurately diagnosing the disease in equivocal cases and presymptomatic immediate relatives of confirmed cases.[8] However, large-scale genetic testing is not affordable or widely used in certain countries with resource-constraint healthcare settings, such as Jordan.[9] Investigating WD's genetic landscape in Jordan and identifying potential recurrent variants may permit the development of a cost-effective genetic testing strategy into clinical practice. Additionally, unveiling founder variants offers a means to trace the common ancestry of certain Jordanian inhabitants, along with their expansion and migration over time. Hence, the characterization of founder variants provides a better understanding of WD evolution in Jordan and allows us to tailor population-specific genetic testing approaches.[10]

What is Known

- Wilson disease (WD) is an inherited disorder affecting copper metabolism, leading to various organ damage.
- Little is known about WD genetics in the Middle East, especially in Jordan.

What is New

- A specific disease-causing variant (ATP7B:c.3551C>T;p.Ile1184Thr) was found in eight unrelated Jordanian families with WD, suggesting it might be a founder variant.
- These findings can guide the development of targeted genetic tests in Jordan, enabling faster and more accurate diagnosis.

Only one study previously described the clinical characteristics of Jordanian pediatric patients with WD.[11] However, it did not delve into the molecular etiology or establish correlations between patients' clinical manifestations and their genetic backgrounds.

Here, we recruited 24 Jordanian families with a history of WD and identified the same pathogenic variant in 8 unrelated families. Our objective is to elucidate a potential founder variant in *ATP7B* within the Jordanian population and to delineate the associated clinical manifestation observed in patients harboring this pathogenic variant.

2 | METHODS

2.1 | Study subject and clinical evaluation

Upon enrollment, all participants or their legal guardians provided written informed consent forms. This study was approved by the Institutional Review Board of Jordan University Hospital (JUH), Amman, Jordan (Reference Number 67/2017/1732), and was performed in compliance with the Declaration of Helsinki's tenets.

Patients enrolled in the study were recruited from different hospitals in Jordan between the Years 2018 and 2023. Study investigators reached out to WD-treating physicians at the main referral hospitals for WD in Jordan and asked them to refer WD cases. These hospitals included JUH and Prince Hamza Hospital in Amman, along with the Royal Medical Services hospitals distributed across the country. Detailed medical records were collected for all patients, and the diagnosis was based on typical WD manifestations, including hepatic, neurologic, psychiatric, biochemical, and ophthalmic features. Inclusion in this study was confirmed based on previously stated criteria.[12] The Leipzig score was also calculated for all recruited cases after genetic testing (Table 1). A Leipzig score of ≥4 reflects an established diagnosis with WD, while a score of ≤2 indicates that the diagnosis with WD is unlikely.[13,14] To minimize the possibility of relatedness between the recruited families, we ascertained that these families had distinct last family names and originated from dispersed unrelated tribes across Jordan. Peripheral blood samples were collected from the patients and the available family members using EDTA tubes.

2.2 | Genetic testing

DNA was isolated from blood samples using QIAprep Spin Miniprep Kit following the manufacturer's guidelines (Qiagen). One proband from each family underwent whole-exome sequencing, as previously described by Azab et al.[15] Briefly, the exome library was constructed using SureSelect V-6 Post kit and then sequenced using NovaSeq. 6000 platform (Illumina Inc.). The generated data from the sequence reads of the probands from families F2, F3, and F5-8 were mapped into the National Center for Biotechnology Information reference sequence Genome Reference Consortium Human (GRCh) Build 38 using Burrows-Wheeler Aligner (bwa)-0.7.17. The sequence reads from family F1's proband were mapped to GRCh37 using bwa-0.7.12. The discovered variants were called from reads mapped to GRCh38 and GRCh37 using Genomic Analysis Tool Kit (GATK) version GATKv4.0.5.1, and GATKv3.4.0, respectively. Notably, the coding exons of the *ATP7B* canonical transcript (NM_000053) were 100% covered at 20× sequencing depth (Figure 1A). The variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[16]

The available family members from F1 to F4 underwent Sanger sequencing for the pathogenic variant (*ATP7B*:c.3551C>T), as previously described (Table S1).[17] The chromatograms were analyzed using ChromasPro software (Technolysium Ltd.). Noteworthy, only individual F4:II-5 underwent genetic testing before our investigation by an external diagnostic laboratory, but co-segregation analysis was performed in our laboratory.

3 | RESULTS

3.1 | Initial clinical assessment

Clinical evaluations were conducted on 43 patients with WD from 24 Jordanian-unrelated consanguineous families. Of these, 8 families, comprising 16 tested affected family members, harbored the same variant in *ATP7B* (Figure 2). The age of diagnosis of WD-related symptoms in these eight probands ranged between 8 and 20 years old. All the recruited patients exhibited low ceruloplasmin levels and high basal 24-h urinary copper collection. Nevertheless, their hepatic, neurologic, psychiatric, and other manifestations were heterogeneous (Table 1).

Family F1 had four patients with WD across two generations (Figure 2). The proband (F1:V-4) was diagnosed with WD at the age of 12 years. F1:IV-4 first presented with ascites, recurrent jaundice, elevated aspartate aminotransferase (AST) and alanine transaminase (ALT) levels, thrombocytopenia, and tested negative for KFR (Table 1). The proband's maternal uncle (F1:IV-7) was diagnosed with WD at the age of 12 but exhibited positive KFR. Furthermore, F1:IV-7 manifested several neurological and psychiatric manifestations, including increased tone in all limbs, ataxia, emotional lability, speech difficulties, choking episodes when eating, and increased salivation. The proband's maternal aunt (F1:IV-6), was also affected by WD and had bilateral KFRs. Interestingly, F1:IV-6 only displayed neuropsychiatric symptoms at the age of 16. These manifestations encompass increased tone in hands, speech difficulties, emotional liability, blurred vision, ataxic gait, and increased salivation. Another maternal aunt (F1:IV-2) was asymptomatic at the age of 9 years old. The only discernible features of WD shown by F1:IV-2 were bilateral KFRs, persistently elevated serum aminotransferase activity, low ceruloplasmin, and high urinary copper.

In family F2, three siblings had WD (Figure 2). The proband (F2:II-1) was first diagnosed with WD at age 20, presenting with hepatosplenomegaly and thrombocytopenia. F2:II-1 also featured several neurological

TABLE 1 Clinical features of the recruited individuals.

Family ID	ID—Zygosity of ATP7B:p.Ile1184Thr	Sex	Age at diagnosis (Y.O)	KFR	HM	NM	PM	OM	Serum ceruloplasmin (g/L**)	Basal 24-h urinary copper*** (μg/24 h)	Medications	Leipzig score
F1	V-4* — HOM variant	M	12	No	Ascites, recurrent jaundice, and persistently elevated serum aminotransferase activity	No	No	Thrombocytopenia	0.03	551	D-penicillamine (250 mg × 3)	8
	IV-2 — HOM variant	F	9	Yes	Persistently elevated serum aminotransferase activity	No	No	No	0.04	126	D-penicillamine (250 mg × 3) Zinc (1 × 3)	10
	IV-6 — HOM variant	F	16	Yes	No	Increased tone in hands, speech difficulties, ataxic gait, and increased salivation	Emotional lability	Blurring of vision	0.02	172	D-penicillamine (250 mg × 3) Zinc (1 × 3)	12
	IV-7 — HOM variant	M	12	Yes	No	Increased tone in all limbs, speech difficulties, increased salivation, and chocking episodes when eating	Emotional lability	No	0.02	110	D-penicillamine (250 mg × 3) Zinc (1 × 3)	12
F2	II-1* — HOM variant	M	20	No	Hepatomegaly	Tremor, drooling, dysarthria, and pseudobulbar palsy	No	Thrombocytopenia and splenomegaly	0.03	1828	Trientine	10
	II-2 — HOM variant	F	3	No	Abdominal pain, hepatomegaly, and high levels of LFT	No	No	No	0.04	186	D-penicillamine	8
	II-3 —	F	N/A	N/A	High levels of LFT	No	No	No	0.03	372	N/A	8

TABLE 1 (Continued)

Family ID	ID—Zygosity of ATP7B:p.Ile1184Thr	Sex	Age at diagnosis (Y.O)	KFR	HM	NM	PM	OM	Serum ceruloplasmin (g/L**)	Basal 24-h urinary copper*** (μg/24 h)	Medications	Leipzig score
	HOM variant											
F3	V-1* — HOM variant	F	15	No	Acute liver failure, hepatomegaly, and persistently elevated serum aminotransferase activity	No	No	No	0.073	274	N/A	8
	V-2 — HOM variant	F	30	No	N/A	N/A	N/A	N/A	0.009	47.3	N/A	7
F4	II-3* — HOM variant	F	16	No	Elevated LFT	Pseudobulbar palsy and seizures	No	No	0.022	167	D-penicillamine (250 mg × 4) Zinc (25 mg × 2)	10
	II-5 — HOM variant	M	9	No	Elevated LFT	Yes	No	No	0.031	134	N/A	10
	II-4 — HET	F	14	No	No	No	No	No	0.14	25.4	N/A	2
F5	IV-1* — HOM variant	M	8	N/A	Jaundice, elevated LFT, and fatty liver	No	No	Splenomegaly	0.079	744	D-penicillamine (250 mg × 5) Zinc (25 mg × 2)	8
F6	IV-1* — HOM variant	F	15	Yes	No	Increased salivation, increased drooling, slurred speech, abnormal hand movement, and brain atrophy by MRI	No	Menstrual irregularities	0.033	150	D-penicillamine	12

(Continues)

TABLE 1 (Continued)

Family ID	ID—Zygosity of ATP7B:p.Ile1184Thr	Sex	Age at diagnosis (Y.O)	KFR	HM	NM	PM	OM	Serum ceruloplasmin (g/L**)	Basal 24-h urinary copper*** (μg/24 h)	Medications	Leipzig score
F7	V-1* — HOM variant	F	12	Yes	Jaundice and elevated LFT	Tremor and involuntary movements	Emotional lability	Thrombocytopenia and splenomegaly	0.052	217	D-penicillamine	12
F-8	III-1* — HOM variant	M	8	No	Elevated LFT, acute hepatitis resembling autoimmune hepatitis, and compensated cirrhosis	No	No	Splenomegaly, hemolytic anemia upon initial presentation (Currently resolved)	0.041	1621	D-penicillamine (500 mg × 2)	9

Note: Asterisks (*) donate probands within the recruited families. ** normal range 0.2-0.6 g/L. *** Normal lab range <40 (μg/24 h). The values of basal 24-h urinary copper are at the initial diagnosis.
Abbreviations: F, family; HET, heterozygous; HM, hepatological manifestation; HOM, homozygous; KFR, Kayser–Fleischer ring; LFT, liver function test; MRI, magnetic resonance imaging; N/A, not available; NM, neurological manifestation; OM, other manifestations; PM, psychiatric manifestation; variant, for the pathogenic variant ATP7B:p.Ile1184Thr; Y.O, years-old.

manifestations, namely tremors, drooling, dysarthria, and pseudobulbar palsy. Noteworthy, F2:II-1 had dysphagia; hence, he underwent a gastrostomy procedure and was fed using a percutaneous endoscopic gastrostomy tube. The proband's sister (F2:II-2) was first diagnosed with WD at the age of 3 years old, suffering from abdominal pain and hepatomegaly. She did not show signs of KFR but revealed high levels of LFT, very low ceruloplasmin, and elevated urinary copper levels. Another sister (F2:II-3) exhibited high LFT levels and elevated basal 24-h urinary copper excretion but low ceruloplasmin.

Family F3 had four reportedly affected siblings (Figure 2). The proband (F3:V-1) had a then-reportedly unaffected fraternal twin sister (F3:V-2). Proband F3:V-1 was diagnosed with WD at 15 years, after presenting with acute liver injury (ALI). She first presented with jaundice, epistaxis, a history of gum bleeding while brushing, and amenorrhea. She also had prolonged prothrombin and partial thromboplastin times, as well as elevated levels of total bilirubin, AST, ALT, and lactate dehydrogenase. Additionally, her hematological findings showed decreased hemoglobin levels with low red blood cell count. Her abdominal ultrasound showed hepatomegaly (liver span 17 cm), and a partially contracted gallbladder with marked wall thickness. WD was sought as an underlying disease due to her family history of WD. Therefore, further biochemical and ophthalmic assessments were conducted, revealing low ceruloplasmin, high urinary copper levels, and negative KFR (Table 1). She was diagnosed with ALI due to WD (ALI-WD). The other affected family members (F3:V-4, F3:V-6, and F3:V-8) were diagnosed with WD in their adolescence. Noteworthy, the proband's sibling (F3:V-4) exhibited acute liver failure (ALF). No neurological manifestations were present in any of the F3 family members before or after treatment for WD.

Two individuals from family F4 were clinically diagnosed with WD (Figure 2). The proband (F4:II-3) was diagnosed at the age of 16 years, presenting with low ceruloplasmin levels, high urinary copper excretion, and elevated serum aminotransferase activity. Furthermore, her clinical history was significant for pseudobulbar palsy and seizures. However, no evidence of KFR or other neuropsychiatric manifestations was reported. Her younger brother (F4:II-5), at 12 years old, was asymptomatic but had increased LFT levels. Another sister (F4:II-4) was asymptomatic at the age of 14. Yet, F4:II-4 was suspected of having WD due to subnormal levels of ceruloplasmin, despite normal basal 24-h urinary copper levels (Table 1).

Proband (F5:IV-1), born into a consanguineous family, manifested hepatic features of WD at the age of 8 years (Figure 2). He presented with jaundice and elevated AST and ALT levels. Later, he developed splenomegaly and a fatty liver. His WD workup revealed low ceruloplasmin levels and a high basal

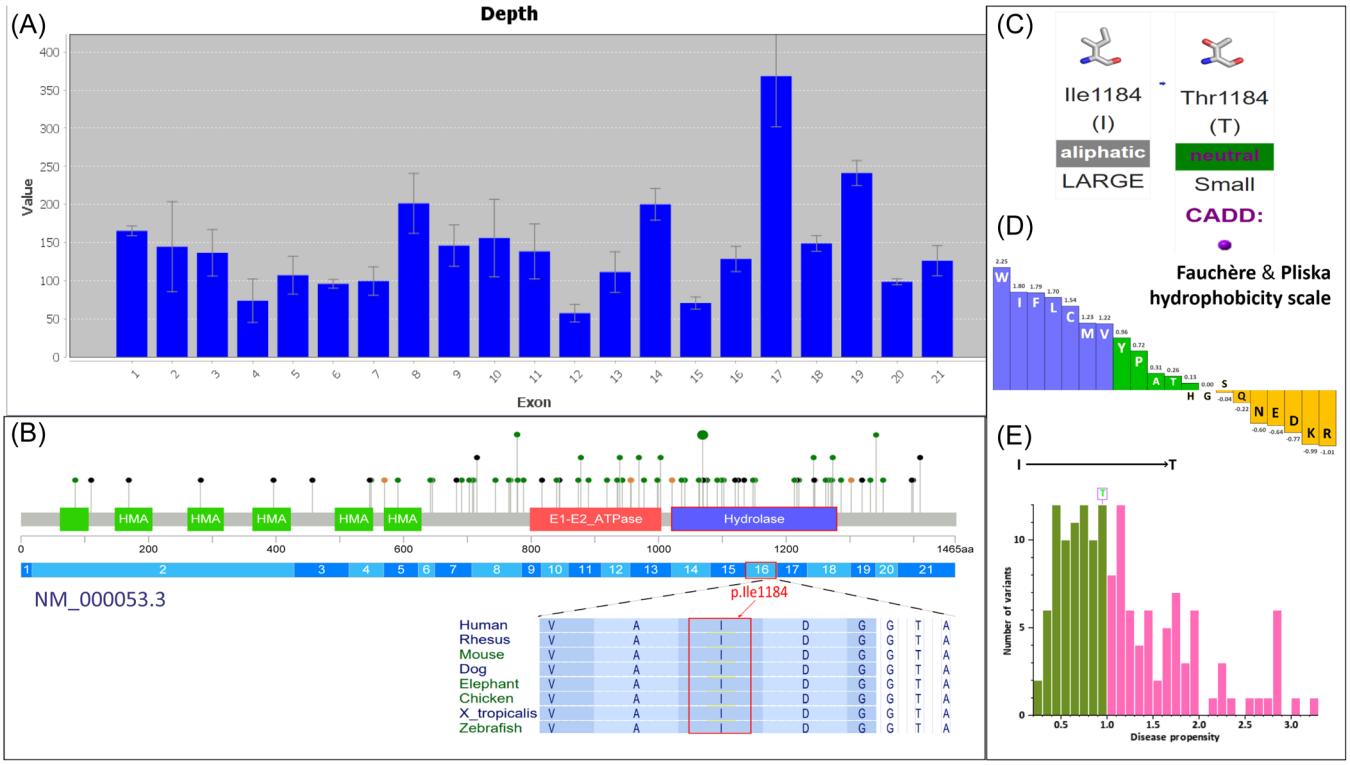

FIGURE 1 Genetic findings. (A) Representation of the coverage of the coding exons of the ATP7B (NM_000053) transcript in F01. (B) The upper panel depicts the distribution of the variants reported in ATP7B in the Middle East to be associated with causing Wilson disease. These variants are shown in Table S3. The lower panel shows the conservation of p.Ile1884 among different species. (C–E) VarSite output. (C) The structure and nature of the wild-type allele (Ile;I) and the allele with the change (Thr;T). (D) Hydrophobicity plot showing a decrease in hydrophobicity score upon Ile-to-Thr substitution. (E) Disease pyrogenicity histogram: based on the frequency of the variants reported in gnomAD compared to the disease-causing variants. CADD, combined annotation-dependent depletion; HMA, heavy metal-associated domain; I, isoleucine; T, threonine.

24-h urinary copper collection. He had four brothers and one sister; none was reportedly affected by WD.

Family F6 had a history of WD with several affected members across two generations (Figure 2). At age 15, the proband (F6:IV-1) first showed drooling, slurred speech, and abnormal hand movement. She was positive for KFR and had low ceruloplasmin and high basal 24-h urinary copper levels. Brain atrophy was revealed by magnetic resonance imaging.

Proband (F7:V-1) was born to consanguineous parents (Figure 2). She had three WD-free siblings. When F7:V-1 was 12 years old, she presented with jaundice, attacks of vomiting, tea-colored urine, and menstrual irregularities. Her labs showed abnormal LFTs, thrombocytopenia, low ceruloplasmin, and elevated basal 24-h urinary copper collection. She tested positive for KFR.

Proband (F8:III-1) was born into a non-consanguineous family and manifested hepatic features of WD at the age of 8 years (Figure 2). He presented with jaundice, ascites, enlarged spleen, elevated ALT and AST, coagulopathy, and prolonged international normalized ratio (INR). His hematology profile showed Coombs-negative hemolytic anemia and low platelets. He had low ceruloplasmin and high basal 24-h urinary copper levels. No neurological manifestations were present, and he did not have KFR. His serum ammonia level was normal, and his INR improved with vitamin K treatment. No other members of his family were affected.

3.2 | Molecular testing

To identify the genetic etiology of the recruited cases with WD, molecular testing was performed on one affected proband from 24 unrelated participating families. Remarkably, the same putative variant was identified in 8 families, consisting of 16 tested patients, exclusively in the homozygous state (Figure 2). The identified sequence change (c.3551T>C) introduced thymine-to-cytosine substitution in exon 16 of the transcript NM_000053.4 (Figure 1B).

Notably, ATP7B:p.Ile1184Thr replaces an aliphatic and hydrophobic residue, isoleucine, with a neutral amino acid, threonine (Figure 1C). Codon ATP7B:p.Ile1184 showed a highly conserved residue across various species (Figure 1B). Based on VarSite

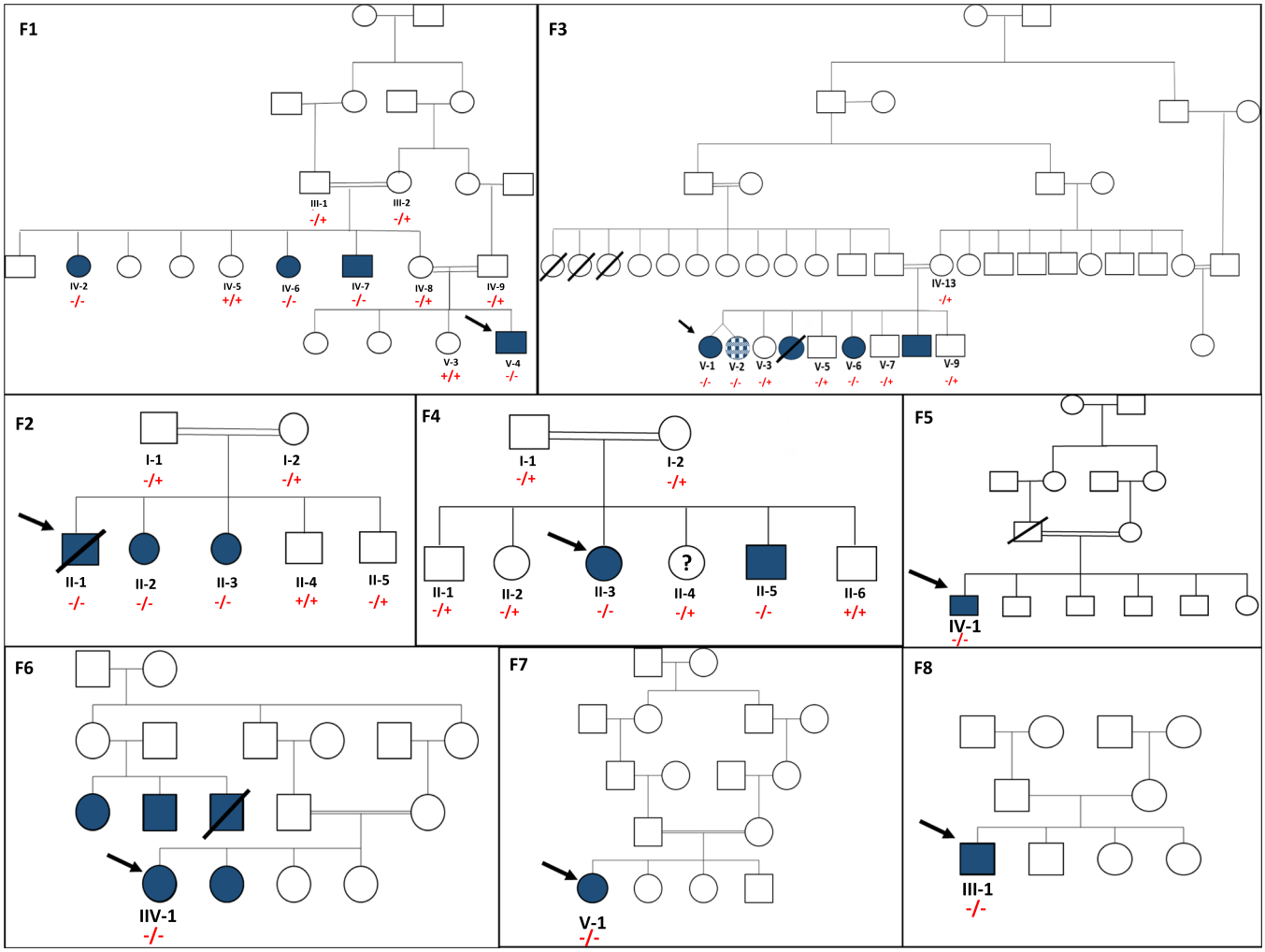

FIGURE 2 Pedigrees of eight participating families with a history of WD (F1–F8). Only recruited individuals are given genotypes. Circles, squares, and diagonal slashes indicate female, male, and deceased individuals, respectively. Double horizontal lines represent consanguinity. Proband patients are marked by black arrows. Blue shading and empty-filled symbols refer to affected and unaffected members, respectively. The plaid symbol indicates that the diagnosis of WD was established only after genetic testing. A question mark represents suspicion of having WD upon recruitment. Genotypes are written in red below each tested individual as follows: (+) indicates wild-type allele, whereas (–) represents allele with the variant (ATP7B:c.3551T>C). WD, Wilson disease.

prediction (Figure 1C–E), this variant has a disease propensity score of 0.9, indicating an amino acid change less commonly implicated in causing disease.[18] Nevertheless, several other in silico prediction tools were utilized and supported its plausible pathogenic effect (Table S2). The variant (ATP7B:p.Ile1184Thr) has an entry in ClinVar (Variation ID: 977075; last accessed 2024-06-08) with conflicting interpretations of likely pathogenic (LP) and variant of uncertain significance (VUS). The allele frequency of *ATP7B*:c.3551T>C is rare in the population databases and without declarable homozygosity. *ATP7B*:c.3551T>C has been previously reported in a compound heterozygous state in patients with WD.[19,20] Noteworthy, this is the first time for the variant *ATP7B*:c.3551T>C to be observed in the homozygous state in patients with WD.

3.3 | Co-segregation analysis and post-genotyping clinical reassessment

After Sanger sequencing of the available family members, the homozygous variant (*ATP7B*:c.3551T>C; p.Ile11841Thr) co-segregated with the disease phenotype (Figures 2 and 3). Collectively, based on the evidence above and in compliance with the ACMG guidelines, we reclassified the identified variant (c.3551T>C;p.Ile1184Thr) from VUS/LP to pathogenic for the first time.

Following genetic testing, we sought clinical re-evaluation for the two clinically ambiguous presentations, specifically F3:V-2 and F4:II-4. The fraternal twin of the proband in F3 (F3:V-2) was reportedly unaffected, albeit she harbored the pathogenic variant (*ATP7B*:c.3551T>C) in the homozygous form.

VARIANT DETAILS
Chromosomal variant coordinates: • GRCh38:chr13:51941086 • GRCh37:chr13:52515222
RefSeq: NM_000053.3
Exon: 16
HGVS: c.3551T>C; p.Ile1184Thr
ClinVar ID: VCV000977075
dbSNP: rs755817220
Maximum MAF (gnomADv4): 0.00004472 in admixed American
Classification: Pathogenic

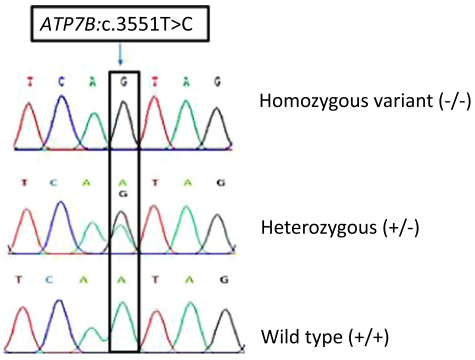

FIGURE 3 ATP7B:p.Ile1184Thr variant was identified in the recruited families. Left pane, details about the detected variant. Right pane, representative chromatograms of the sequence change (ATP7B:c.3551T>C) with different zygosity in the recruited participants. –, allele with the variant; +, wild-type allele; dbSNP, database for single nucleotide polymorphism; gnomADv4, Genome Aggregation Database version 4; GRCh, Genome Reference Consortium Human; HGVS, Human Genome Variation Society; MAF, minor allele frequency.

Therefore, we pursued investigating her laboratory workup. Her serum ceruloplasmin and basal 24-h urinary copper levels were low and high, respectively (Table 1). These findings corresponded to a Leipzig score of 7, confirming a diagnosis with WD. In contrast, the other case (F4:II-4) was suspected of manifesting WD due to showing subnormal levels of ceruloplasmin but normal basal 24-h urinary copper collection. Genotyping of F4:II-4 revealed her as a heterozygote for the pathogenic variant (*ATP7B*:c.3551T>C). This resulted in a Leipzig score of 2, indicating that F4:II-4 was highly unlikely to have WD. Ultimately, these reassessments helped resolve the clinical diagnoses of these cases through post-genetic testing.

4 | DISCUSSION

Here, 24 Jordanian families with WD underwent molecular testing to uncover the genetic underpinnings of WD in Jordan. Surprisingly, the same variant (ATP7B:p.Ile1184Thr), found exclusively in the homozygous state, was identified among eight unrelated families with a total of 16 tested patients (Figure 2). We helped reclassify the variant from VUS/LP to pathogenic for the first time. To our knowledge, this is the first molecular investigation to be conducted on patients with WD in Jordan.

The patients from the eight recruited families, all harboring the same homozygous variant (ATP7B:p.Ile1184Thr), manifested a spectrum of clinical presentation at both the hepatic and extrahepatic levels, as commonly described in cases with WD[1,19,21–23] (Table 1 and Figure 2). The age of diagnosis of the recruited probands with ATP7B:p.Ile1184Thr ranged between 8 and 20 years old, in concert with prior reports showing a wide age of diagnosis among patients with WD.[6,24] The hepatic manifestations ranged from asymptomatic liver biochemical abnormalities to hepatomegaly, liver cirrhosis, and ALI. Notably, the recruited patients with positive KFR initially presented with neurological and psychiatric manifestations of WD, rather than hepatic involvements. The KFR occurrence can be found in both the hepatic and neuropsychiatric sequelae of WD. Nevertheless, WD patients with KFR have been associated with exhibiting initial neuropsychiatric involvement more frequently than hepatic presentation, in alignment with the notion observed here.[23,25] Typically, WD starts with a hepatic presentation, whereas neurological symptoms emerge after a decade in undiagnosed or nonadherent patients.[7,23] The described variability imposes challenges in establishing clear genotype-phenotype correlations, even among patients with the same variant within the same family.

This is the first time for this variant (ATP7B:p.Ile1184Thr) to be observed in a homozygous state. In two earlier studies, the identified variant (c.3551T>C;p.Ile1184Thr) has been described, albeit in a compound heterozygous state.[19,20] One study by Coffey et al. reported a female patient from the United Kingdom harboring three LP variants in *cis*, and *trans* states, p.[(Ile381Ser;Ile1184Thr)];[(Leu722fs)].[19] The patient presented with ascites and was KFR positive. Although her ceruloplasmin level was normal, her liver biopsy revealed a high copper concentration.[19] Moreover, this variant (ATP7B:p.Ile1184Thr) was reported in a French male patient in a compound heterozygous state with another LP deep intronic variant (NG_008806.1:c.2866-1521G>A).[20] He was first diagnosed with WD at the age of 23 years without showing KFR but presenting hemolytic anemia and ALF. He received a liver transplant as an initial treatment.[20]

Furthermore, an investigation by Schushan et al. conducted homology-modeling of the Ile1184Thr-mutated ATP7B core, but it was unable to determine the impact of the p.Ile1184Thr on the protein structure and function.[26]

These earlier studies reporting the variant (ATP7B:p.Ile1184Thr), while informative, lacked definitive evidence of pathogenicity.[19,20,26] Before our analysis, according to the ACMG guidelines, the variant (ATP7B:p.Ile1184The) met the classification of either VUS or LP.[16] This can be attributed to several factors, including the previous lack of co-segregation evidence in multiple affected family members.[16] Our investigation, using clinical data and co-segregation analysis, contributed to reclassifying the pathogenicity of the variant (ATP7B: p.Ile1184Thr) from VUS/LP to pathogenic for the first time.

We assisted in discriminating against the clinical diagnosis in two atypical cases triggered by the cascade genotyping of at-risk relatives. Surprisingly, the then-reportedly unaffected twin sibling of F3's proband (F3:V2) turned out to have the homozygous variant (Figures 2 and 3). This sole discovery of biallelic variants in *ATP7B* is sufficient to establish the diagnosis of WD.[13] However, our genetic analysis prompted laboratory workup, which revealed low ceruloplasmin levels and high basal 24-h urinary copper collection in F3:V2. Besides homozygosity, these clinical findings also confirmed that F3:V2 is indeed affected by WD, though currently asymptomatic. Discovering "paucisymptomatic" siblings during the recommended genetic screening of first-degree relatives is common and was previously reported.[20] In another case, the sister of the proband in F4, (F4:II-4), was suspected of having WD due to showing subnormal ceruloplasmin levels yet normal basal 24-h urinary copper excretion and negative KFR. After Sanger analysis, F4:II-4 was identified as a heterozygote for the pathogenic variant (ATP7B:p.Ile1184Thr). These findings corresponded to a Leipzig score of 2 points, implying that F4:II-4 is unlikely to have WD.[13] In concordance, manifesting mild presentation of WD, especially modestly subnormal ceruloplasmin levels in heterozygotes, has been previously described.[27] These findings demonstrate the importance of implementing targeted genetic testing into screening protocols for at-risk family members with WD, especially in resource-constraint countries like Jordan. This can ultimately reduce the burden of misdiagnosis and ensure an effective healthcare strategy.

Interestingly, in the Middle East, the WD-causing variants were distributed across all domains of ATP7B, with heavy clustering in the E1-E2 ATPase and hydrolase domains (Figure 1B and Table S3).[5] However, the pathogenic variant (ATP7B:p.Ile1184Thr) has never been reported in any other studies from the Middle East region. Several criteria should be met to establish a variant as a founder in a certain population: (1) a high allele frequency of the variant in a specific population; (2) evidence of identical haplotypes surrounding the variant in multiple patients; (3) and historical or demographic data supporting a single ancestral origin.[28,29] Since the homozygous pathogenic variant (ATP7B:p.Ile1184Thr) has been identified among 16 Jordanian patients from eight unrelated families, we propose this variant as a potential founder or recurrent variant in the Jordanian population. Future genetic studies of WD in the region and haplotype analysis are needed to support this candidate variant (ATP7B:p.Ile1184Thr) as a founder variant.

In conclusion, this is the first genetic investigation of WD in Jordan. Sixteen patients, from eight unrelated families, were found to share the same homozygous pathogenic variant in *ATP7B* (c.3551T>C;p.Ile1184The). Notably, this variant was observed exclusively in the homozygous state for the first time in the affected individuals. Patients with ATP7B:p.Ile1184Thr variant exhibited diverse clinical manifestations at both the intra- and interfamilial levels, emphasizing the typical variable expressivity associated with WD. We underlined the importance of genetically testing at-risk family members with WD, guiding the clinical diagnosis in ambiguous and asymptomatic cases. We also highlighted the potential implication of ATP7B:p.Ile1184Thr as a founder variant in the Jordanian population. Our findings can enable the development of region-specific targeted genetic testing of patients with WD in Jordan. This can provide valuable insights guiding clinical decisions in suspected WD cases in this region.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All the data supporting the findings of this work are available within the paper. Upon a reasonable request, any further required data supporting the findings of this work can be provided by the corresponding author.

ETHICS STATEMENT

This study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Jordan University Hospital (protocol code 67/2017/1732). Informed consent was obtained from all individual participants or their legal guardians.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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