Wilson’s disease in Lebanon and regional countries: Homozygosity and hepatic phenotype predominance

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AIM
To determine the phenotypes and predominant disease-causing mutations in Lebanese patients with Wilson’s disease, as compared to regional non-European data.

METHODS
The clinical profile of 36 patients diagnosed in Lebanon was studied and their mutations were determined by molecular testing. All patients underwent full physical exam, including ophthalmologic slit-lamp examination ultrasound imaging of the liver, as well as measurement of serum ceruloplasmin and 24-h urinary-Cu levels. In addition, genetic screening using PCR followed by sequencing to determine disease-causing mutations and polymorphisms in the ATP7B gene was carried on extracted DNA from patients and immediate family members. Our phenotypic-genotypic findings were then compared to reported mutations in Wilson’s disease patients from regional Arab and non-European countries.

RESULTS
Patients belonged to extended consanguineous families. The majority were homozygous for the disease-causing mutation, with no predominant mutation identified.
The most common mutation, detected in 4 out of 13 families, involved the ATP hinge region and was present in patients from Lebanon, Egypt, Iran and Turkey. Otherwise, mutations in Lebanese patients and those of the region were scattered over 17 exons of \textit{ATP7B}. While the homozygous exon 12 mutation Trp939Cys was only detected in patients from Lebanon but none from the region, the worldwide common mutation H1069Q was not present in the Lebanese and was rare in the region. Pure hepatic phenotype was predominant in patients from both Lebanon and the region (25%-65%). Furthermore, the majority of patients, including those who were asymptomatic, had evidence of some hepatic dysfunction. Pure neurologic phenotype was rare.

CONCLUSION
Findings do not support presence of a founder effect. Clinical and genetic screening is recommended for family members with index patients and unexplained hepatic dysfunction.

Key words: Wilson Disease; Cu-metabolism; Phenotype; Genotype; ATP7B; Hepatic manifestations

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Core tip: We report on the genotype-phenotype of 36 Lebanese patients with Wilson’s disease from 13 different families. The majority were homozygous for disease-causing mutations. The most common mutation worldwide, His1069Trp, was absent in our patients. The ATP hinge region may comprise a hot spot for mutations, as it was detected in 4 families. Hepatic phenotypes were predominant in both symptomatic and asymptomatic patients. Neurologic phenotypes were rare. Compared to findings reported in regional Arab and non-European countries, our results do not support a founder effect. Mutations are scattered over 17 exons, with no common or frequent mutation characterizing the region.

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INTRODUCTION
Wilson’s disease (WD) is an autosomal recessive disorder of copper (Cu) metabolism, resulting from defects in the \textit{ATP7B} gene protein. It is characterized by failure of Cu incorporation into ceruloplasmin (Cp) and decreased biliary Cu excretion. As a consequence, Cu accumulates in various organs, primarily liver and brain. The clinical presentations of WD are characterized by substantial diversity. Patients can present at any age in variable combinations of liver impairment, neurologic dysfunction and/or osseomuscular symptom. Hepatic manifestations include asymptomatic transaminitis, acute or chronic hepatitis, fulminant hepatic failure and/or cirrhosis, while neurologic symptoms vary from mild tremors, articulating problems, dysarthria, Parkinson-like features, seizures and cognitive dysfunction. Some patients have mixed hepato-neurologic presentation\cite{1}. Ophthalmologic involvement with Kaiser-Fleischer (KF) rings is common.

Traditionally, the diagnosis of WD is based on low serum-Cp (<20 mg/dL), high 24-h urinary Cu and high hepatic Cu content (250 \(\mu\)g/g dry tissue)\cite{2,3}. Recent guidelines for the diagnosis of WD were published by the European Association for the Study of the Liver (EASL)\cite{4}. Nonetheless, the diagnosis of WD may be difficult based on clinical and laboratory criteria, and in some patients it is delayed, leading to detrimental consequences\cite{5}. This is why molecular testing and genotypic analysis may be warranted for confirming and/or supporting a diagnosis of WD, particularly in asymptomatic patients\cite{3}.

More than 500 mutations have been identified in WD with a very high allelic heterogeneity. Most patients are compound heterozygous, rendering it difficult to ascribe a phenotype to a specific genotype\cite{6}. Furthermore, a large number of mutations are rare, making it impractical to screen populations for all WD-causing mutations\cite{7}. Some mutations, however, are relatively frequent and population-specific, like the p.His1069Gln on exon 14 in Northern and Eastern European patients\cite{8}, the p.Arg778Leu and the p.Arg778Gly mutations on exon 8 among Chinese and Taiwanese patients respectively\cite{9}, and the deletion in the 5’ regulatory region in Sardinian patients\cite{10}. These findings facilitate molecular diagnosis based on patients’ ethnic background. In the Arab World, consanguinity and marriage among individuals belonging to the same ethnic background is very common, thereby increasing the prevalence of genetic disorders, including WD\cite{11}. However, it is not known whether there is a predominant WD mutation in the Arab world, and if so what its phenotypic associations are.

In a cohort of Egyptian patients, genotypic and phenotypic profiles were described, but no prevalent mutation was identified\cite{12}. Moreover, previous reports from Lebanon on a limited number of families suggested an association of liver presentation with homozygous missense mutations: Gly691Arg and non-His1069Trp in exons 7 and 14 of the \textit{ATP7B} gene respectively\cite{13,14}. Whether a specific WD mutation prevails in Lebanon is not known.

In this study, we described the spectrum and frequency of mutations and phenotypes in 36 Lebanese WD patients. We also conducted a comprehensive
literature search for regional studies on WD in Arab and non-European countries in the Middle East. In order to determine whether there is a frequent mutation characterizing the region, a comparative study was undertaken to identify common mutations in the region, and to compare them to our data. We also determined if common mutations in the region were associated with similar clinical phenotypes.

MATERIALS AND METHODS
A total of 36 patients (P1-P36) from 13 unrelated Lebanese families (U, Or, S, Ah, T, B, H, Ha, Is, Z, Ri, Sc and Gh) were enrolled in the study. Most patients were diagnosed at the American University of Beirut (AUB) Medical Center, a major tertiary referral center in Lebanon. All participating subjects were asked to sign a written consent form (Protocol No. BioCh.JU.01) that was approved by both the Institutional Review Board and Research Committee at AUB.

Clinical testing
Patients’ data for evaluation included: history, date of birth, age of onset of symptoms, age at diagnosis, and findings from full physical exam, ophthalmologic slit-lamp examination, and biochemical tests, including liver function tests, serum-Cp and 24-h urinary-Cu levels. Abdominal ultrasound imaging of the liver was performed on all patients and, when necessary, brain magnetic resonance imaging was done. Phenotypic classifications were designated following Ferenci’s classification as hepatic, neurologic, mixed or asymptomatic[15]. Diagnosis was further established by computing total WD score developed at the 8th international meeting[15]. Family members (siblings, parents) of all WD-confirmed index patients were also subjected to physical, biochemical and genotypic testing.

Genotypic screening
DNA screening for disease-causing mutations and single nucleotide polymorphisms was performed on all recruited subjects and their immediate family members. Extraction of DNA from blood samples followed by amplification (using PCR) of the 21 ATP7B exons was carried out as described[6,14]. Amplified PCR products were purified, sequenced and compared to published normal sequences in the following data-banks: Blat at University of California Santa-Cruz, Genome Bio-informatics (http://www.Genome.ucsc.edu/cgi-bin/hgBlat) or Blast at National Center for Biotechnology Information (http://www.ncbi.nih.gov/blast).

WD in regional countries
After identifying the disease-causing mutations in our patients, we compared them to reported mutations in WD patients from regional Arab and Non-European countries. A comprehensive literature search of PubMed and Medline, as well as of the University of Alberta database (http://www.wilsondisease.med.ualberta.ca/database.asp), was conducted for articles published from the regional Arab and non-European countries. Index terms used were Wilson Disease, genotype, phenotype, and each of the following countries: Lebanon, Syria, Jordan, Egypt, Iraq, Saudi Arabia, Kuwait, Bahrain, Qatar, UAE, Yemen, Tunisia, Morocco, Libya, Mauritania, Turkey, Iran and Oman. We included studies in which both the genotype and phenotype were identified. In some studies, it was not clearly indicated whether patients presenting to one medical center with a certain mutation belonged to the same family or to different ethnic groups[16,17]. This made it difficult to estimate the most frequent genotype. We, therefore, opted to identify common mutations between Lebanon and the region, and to determine the frequent regional mutations as indicated by the authors of the various reports.

RESULTS
Clinical presentation
In this study, 36 Lebanese WD patients, including 15 females and 21 males, were recruited from different regions in Lebanon. Patients belonged to 13 unrelated families, referred to as: U (P1-P9); Or (P10-P12); S (P13-P15); Ah (P16-P19); T (P20-P23); B (P24-P26); H (P27-P28); Ha (P29-P30); Is (P31); Z (P32-P33); Ri (P34); Sc (P35); and Gh (P36) families. Consanguinity was present in the parents of 27 of the patients (75%), who belonged to the U, S, B, H, Ha, and Z families (Table 1). WD scores computed following EASL guidelines ranged between 4 and 12 (Table 2), confirming the diagnosis.

The clinical profiles of affected subjects are summarized in Table 2. Age at diagnosis ranged between 1 and 39 years. All patients had low Cp level (< 0.2 g/L), except for P4 and P15. Out of 31 patients, 18 (58%) had KF rings (5/36 were NAV). Out of the 36 WD patients, 24 were symptomatic (67%; 16 males and 8 females) and presented clinically at an average age of 14.5 years. Data on P1-P6 were not available. Twelve patients were asymptomatic (33%), diagnosed by genetic screening of family members of index patients. Their average age was 7.6 years.

Pure hepatic phenotype was the most common in our symptomatic patients (9/32: P1, P12-P17, P18, P20, P25, P27-P28 and P30). Neurologic presentation was noted in 12.5% of patients (4/32: P13-P14, P16 and P18). Mixed presentation was observed in 25% of patients (7/32: P2, P17, P24, P25, P29, P31 and P32), two of whom had suicidal attempts/disposition (P10 and P25). Notably, liver cirrhosis was present in 12 symptomatic (38%) patients (P1, P5, P10, P12-P14, P18, P20, P24, P25-P30 and P30), including 4 patients with mixed presentation.

Of the asymptomatic subjects who were diagnosed by screening, 10/12 patients had evidence of liver disease, ranging from transaminitis (P3-P5, P11, P12,
Mutation(s) were detected in 51:16:3 chromosomes at 72.8%:22.8%:4.3% frequency respectively. No mutation was identified in P6, who had been diagnosed based on KF rings, and clinical and biochemical testing.

Out of 35 patients, 29 were homozygous (82.8%) for a disease-causing mutation and 6 were compound heterozygous (17.1%). Parents of our index patients were carriers for the disease-causing mutations. Mutations were most frequent in the exon 18 motif encoding the conserved ATP hinge region of WD gene product. Four out of the 13 unrelated families (H, Ha, Is and Z) had, in this motif, missense mutations in the homozygous state; these were Asn1270Ser in 6 patients (P21-P23; P21 and P23) and hepatomegaly detected by abdominal ultrasound (P11, P13 and P12) to full blown cirrhosis (P22-P23). Overall, 27/32 patients (84%) on whom we had clinical information presented with some form of hepatic dysfunction.

Patients with the neurologic phenotype presented at an average age of 22.3 years, while those with hepatic and mixed phenotypes presented at 12.2 and 14 years, respectively. KF rings were present in 17 symptomatic patients (5 symptomatic were NAV) and absent in two (P12, P13). They were not identified in the asymptomatic patients, except for patient P31 who had KF rings with no evidence of hepatic or neurologic dysfunctions.

**Mutation analysis**

Sequencing of the ATP7B gene revealed (Table 1) 9 different disease-causing mutations in 70 chromosomes (35 patients), which were distributed as: 7 missense (exons: 7, 12, 10, 13, 15 and 18), 1 nonsense (exon 19), and 1 frame-shift (exon 8). Out of 70 chromosomes, missense/frameshift and/non-sense mutation(s) were detected in 51:16:3 chromosomes at 72.8%:22.8%:4.3% frequency respectively. No mutation was identified in P6, who had been diagnosed based on KF rings, and clinical and biochemical testing.

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Table 2 Phenotypic and genotypic profiles of Lebanese patients with Wilson’s disease

| ID  | Mutation(s) | Gl manifestation(s) | Neurological manifestations | KF rings | Cp | Urinary Cu | Score |
|-----|-------------|---------------------|-----------------------------|----------|----|-----------|-------|
| P1  | Gly691Arg   | Liver cirrhosis     | Absent                      | Present  | NAV| 718.8     | 8     |
| P2  | Gly691Arg   | Liver cirrhosis     | Change in school performance| Present  | 0.11| 1998      | 10    |
| P3  | Gly691Arg   | Asymptomatic        | Absent                      | Absent   | 0.03| 148.5     | 8     |
| P4  | Gly691Arg   | Asymptomatic        | Absent                      | Absent   | 0.22| 304       | 6     |
| P5  | Gly691Arg   | Asymptomatic        | Absent                      | Absent   | 0.02| 65.9      | 7     |
| P6  | Gly691Arg   | NAV                 | NAV                         | NAV      | NAV| NAV       | 4     |
| P7  | Gly691Arg   | NAV                 | NAV                         | NAV      | NAV| NAV       | 4     |
| P8  | Gly691Arg   | NAV                 | NAV                         | NAV      | NAV| NAV       | 4     |
| P9  | Gly691Arg   | NAV                 | NAV                         | NAV      | NAV| NAV       | 4     |
| P10 | Gly691Arg/  | Liver cirrhosis     | Suicidal attempts           | Present  | 0.08| 2184      | 12    |
|     | Val8455er   |                     |                             |          |    |           |       |
| P11 | 2299insC    | Asymptomatic        | Absent                      | Absent   | 0.04| 99        | 7     |
| P12 | 2299insC    | Absent              | Slurred speech, ataxia, tremors| Present  | 0.072| 512       | 12    |
| P13 | 2299insC    | Asymptomatic        | Absent                      | Absent   | 0.05| 152.8     | 8     |
| P14 | 2299insC    | Absent              | Choreoathetosis, tremors, rigidity| Present  | 0.423| 2300      | 10    |
| P15 | 2299insC    | Asymptomatic        | Absent                      | Absent   | 0.019| 10        | 6     |
| P16 | 2299insC/   | Liver cirrhosis     | Absent                      | Present  | 0.096| 775       | 10    |
|     | p.Ala1003Thr|                     |                             |          |    |           |       |
| P17 | 2299insC/   | Liver cirrhosis     | Absent                      | Present  | 0.096| 590       | 10    |
|     | p.Ala1003Thr|                     |                             |          |    |           |       |
| P18 | 2299insC/   | Liver cirrhosis     | Absent                      | Present  | 0.17| 645       | 9     |
|     | p.Ala1003Thr|                     |                             |          |    |           |       |
| P19 | 2299insC/   | Absent              | Absent                      | Present  | 0.12| 487       | 9     |
|     | p.Ala1003Thr|                     |                             |          |    |           |       |
| P20 | 2299insC    | Liver cirrhosis     | Absent                      | NAV      | 0.023| 651       | 8     |
| P21 | Trp939Cys   | Asymptomatic        | Absent                      | Absent   | 0.02| 77.6      | 7     |
| P22 | Trp939Cys   | Asymptomatic        | Absent                      | Absent   | 0.02| 20        | 6     |
| P23 | Trp939Cys   | Asymptomatic        | Absent                      | Absent   | 0.02| 41.5      | 6     |
| P24 | Trp939Cys   | Liver cirrhosis, ascites| Jaw drooping, hypersalivation, slurred speech, narrow based gait, intention tremors| Present  | 0.021| 744       | 12    |
| P25 | Trp939Cys   | Liver cirrhosis, Hepatic encephalopathy, Hepatomegaly, Mild to moderate ascites| Absent| Absent | 0.04| NAV       | 6     |
| P26 | Asn1270Ser  | Liver cirrhosis     | Psychiatric symptoms and suicidal attempts| Present  | 0.03| 27.6      | 10    |
| P27 | Asn1270Ser  | Liver cirrhosis     | Absent                      | Present  | 0.03| 65.1      | 9     |
| P28 | Asn1270Ser  | Ascites, liver cirrhosis| Absent| Present  | 0.04| 55        | 9     |
| P29 | Asn1270Ser  | Transaminitis       | Neurodevelopmental          | Present  | 0.078| 171       | 11    |
| P30 | Asn1270Ser  | Asymptomatic        | Absent                      | Present  | 0.03| 116       | 8     |
| P31 | Asn1270Ser  | Chronic liver parenchymal disease| Dysarthria and left-sided dystonia| Present  | 0.029| 402.3     | 12    |
| P32 | Prol2371Leu | Ascites, Liver cirrhosis, Hepatic encephalopathy| Absent| Present  | 0.17| 1041.1    | 9     |
| P33 | Prol2371Leu | Asymptomatic        | Absent                      | Absent   | 0.19| 89.7      | 6     |
| P34 | Arg1319stop | Asymptomatic        | Absent                      | Absent   | 0.02| 92        | 8     |
| P35 | Thr1092Met/ | Chronic liver disease and early portal hypertension| Clenching of mandible, left side dysonia, sialorrhea, dystaoria, head tremors| Present  | 0.025| 199       | 12    |
|     | Arg1319stop |                     |                             |          |    |           |       |
| P36 | None identified | Absent | Drooling, dysarthria, difficulty concentrating, dysphagia| Present  | 0.085| NAV       | 6     |

1Developed later. Normal range: Serum ceruloplasmin: 0.2 to 0.6 g/L; Urine copper: 15 to 50 µg/24 h. Score = Ferrenci Score of diagnosis. 2 or less: Very unlikely; 3: Possible, more tests needed; 4 or more: Established.

4.3% respectively. Compound heterozygous mutations were identified in exons 10 (Or: P10), 13 (S: P13-P15) and 15 (P15).

Eight polymorphisms were detected in exons 2, 3, 10, 12, 13 and 16 (Table 3) in patients and normal chromosomes obtained from related and unrelated individuals. Three polymorphisms (Lys832Arg, Arg952Lys and Val1140Ala) were present in the homozygous state in 94% (34/36) of patients and in the heterozygous state in 5% (P13 and P15), in addition to others in exons 2, 3 and 13 (Table 3).

**WD patients: Lebanon vs regional countries**

A search of the literature for population studies on the spectrum of mutations in WD patients in the region, including Arab and non-European countries,
was conducted. A total of 77 articles on WD patients were initially identified, but only those reporting the genotypes and/or the phenotypes were considered. Consanguinity, homozygosity and frequency of mutation were also noted when indicated.

Seventeen articles were included and distributed as follows: Saudi Arabia [18–21], Egypt [12, 22–24], Turkey [25, 26], Iran [27, 28], Oman [29] and Lebanon [6, 13, 14, 30]. Two reports on WD from Iraq were not included, as they had no genotypic information. There were no reports on WD from Jordan, Libya, Tunisia, Morocco or Syria.

Homozygosity was highly prevalent in Lebanese WD patients (83%), and ranged between 68%–85.7% and 50%–53% in Egyptian and Saudi Arabian patients respectively. This finding is attributed to high consanguinity (Table 4) that is common in our societies, or the high prevalence of the same mutation in carriers. Frequency of asymptomatic cases was relatively similar in Lebanon, Egypt and Saudi Arabia. Similar to Lebanese patients, many of asymptomatic patients had evidence of hepatic dysfunction on laboratory and/or imaging studies. Hepatic phenotype was more common than neurologic phenotype in patients from Lebanon, Egypt, Turkey, Iran and Saudi Arabia. Taking into account patients who are asymptomatic and those with mixed phenotype, the vast majority of patients in those countries have some form of hepatic dysfunction. A minority of patients had pure neurologic phenotype. Also, the frequency of patients having KF rings was high and was similar in the 5 countries (Table 4). In a report on a single family from Oman, 78% of patients were asymptomatic and 21% had neurologic phenotype. No patients had a hepatic phenotype in that study.

In conducting our analysis of genotypes, we considered a mutation to be frequent if it was present in multiple unrelated families. We compared genotypic changes in the ATP7B gene of Lebanese patients with those from regional Arab and non-European patients. In our patients, the conserved ATP hinge region (exon 18) was the most frequently mutated region identified in 4 unrelated families (Table 1).

Table 3 shows that Lebanese patients share in common with: (1) Egypt, Iran and Turkey, the Val845Ser and Asp1270Ser mutations in exons 10 and 12 respectively; (2) Egypt, the Pro1273Leu mutation in exon 18; (3) Egypt and Turkey, the Arg1319X mutation in exon 19; and (4) Turkey, the Ala1003Thr mutation in exon 13 and the exon 7 mutation (Gly691Arg) reported in one Turkish patient [26]. More interestingly, the mutation in exon 12 (Trp939Cys) was only detected in Lebanese patients and in none of the searched/listed countries. Whereas the worldwide exon 14 mutation (His1069Gln) was detected in some patients from Egypt, Iran and Turkey, it was not identified in Lebanese or in Saudi Arabian patients.

DISCUSSION
The diagnosis of WD based on clinical grounds alone is often difficult. Thus, it may be necessary to resort to genetic testing. In this study, involving more than 500 patients from Lebanon and the region, we found a great deal of genetic heterogeneity with no common or population specific mutation. This reflects the extensive ethnic diversity of people in this part of the world and argues against the presence of a founder gene, even in highly consanguineous populations. It also implies that patients suspected to have WD without a family history, i.e., without a known mutation...
in their family, may need to be screened for mutations in all exons of the ATP7B gene. In view of clustering of WD patients within families, their members should be screened for mutations identified in index patients. This is important as it could prevent the silent progression of WD, which may occur as early as 1 year of age, and facilitate management.

Based on the recently published EASL criteria for diagnosis, all our symptomatic and asymptomatic patients had a composite score > 4 (range: 6-12), confirming the diagnosis of Wilson's disease.
the diagnosis beyond doubt. In many of our patients, confirmation of the diagnosis required mutation analysis. Traditionally WD was diagnosed on the basis of low Cp level, KF ring presence and increased 24-h urine Cu level in the context of hepatic and/or neurologic manifestations[31]. In our experience, many patients with WD do not satisfy all these criteria. For example, patients P6 and P4 u had normal Cp, 13 did not have KF rings and 4 had normal 24-h urinary Cu. This highlights the difficulties and challenges of making a diagnosis of WD based on clinical grounds alone, particularly in asymptomatic patients.

Worldwide, the majority of WD patients are compound heterozygous[32]. In contrast, in our community, the high rate of consanguinity increases the chance of homozygosity, which is present in 83% of our patients. Only 17% of our patients were compound heterozygous. Missense mutations were the most predominant in Lebanese patients, as worldwide[33]. These occurred in 8 exons of ATP7B. One possible hot spot of the WD gene in our patients is that of the conserved ATP hinge region in exon 18. Two mutations in the homozygous state, Pro1273Leu and Asn1270Ser, were the most frequent, being identified in 8 patients from 4 unrelated families. None of the possible hot spot mutations in Lebanon were shared with those of Asia, Latin America or Europe.

One of our WD patients had no identifiable mutations in the coding region of the ATP7B gene. Mutations may be present in the promoter or the transcription factor regions which control protein translation and function. In such cases, detailed clinical testing and family history may be of help in diagnosis, such as P6, in whom the diagnosis was based on clinical assessment showing low Cp and presence of KF rings in the context of neurologic manifestation. Finally, all our patients had multiple genetic polymorphisms that may influence the final folded conformation, affinity and/or the function of the Wilson protein and possibly the phenotype of WD patients[34].

Remarkable differences in phenotypes and age at diagnosis were noted among patients and even among family members carrying the same genotype. The age of onset of the disease varied between 1-22 years, with one (P39) diagnosed at 39 years. In the 8 family, patient P6 was diagnosed at 5 and passed away before the onset of his brother’s symptoms at the age of 13 (P34). Variation in age at diagnosis was also observed in asymptomatic cases. During a checkup at the age of 7, the female index patient in family T (P21) was found to have transaminitis and hepatomegaly. She was confirmed to have WD and was homozygous for a mutation in exon 12. Genetic screening of her 2 brothers, P2 (8 years) and P3 (3 years), confirmed WD. Though they were asymptomatic, it was surprising to find that both already had evidence of liver cirrhosis on liver imaging. This raises the question as to whether sex plays a role in the clinical manifestations of disease[35]. Verification of this, however, requires a cohort study with a larger number of patients. Such phenotypic diversity has been reported even among monozygotic twins[36], suggesting a role for epigenetic and/or environmental factors in the expression of WD[36-38].

Diversity in clinical presentation introduces yet another obstacle in the diagnosis of WD, regardless of whether the patient is symptomatic or asymptomatic. A patient, at age of diagnosis, may have mild to severe hepatic and/or neurologic symptoms with or without KF rings. This emphasizes, again, the limitations of pure clinical evaluation and argues for genetic testing of all family members of an affected sibling. In our patients, 28% had pure hepatic manifestations ranging from transaminitis and hepatomegaly to clinically unapparent or overt cirrhosis and portal hypertension. On the other hand, only 9% of our patients had pure neurologic symptoms ranging from weak school performance, slurred speech, tremors, drooling, dysarthria, dysphagia and ataxia to suicidal attempts in some (P10 and P11).

Our asymptomatic patients (36%) were found to have liver involvement (transaminitis, fatty liver and cirrhosis) with no KF rings, except for P15. Changes such as fatty liver were detected at the age of 1 year in P15, diagnosed by genetic screening. Therefore, early diagnosis is important in families with index patient(s), to mitigate against progression of the disease. This is in line with the EASL recommendations to perform genetic testing for WD, in individuals with liver disease or neurologic movement disorders of unclear etiology. Whether genetic testing for WD in patients with unexplained hepatic dysfunction will turn out to be cost effective or not in this part of the world is unclear.

Few studies from the Arab world on WD from Lebanon, Egypt, Saudi Arabia and Oman have been published. Similar to Lebanon, the majority of patients from Egypt and Saudi Arabia have consistently shown a high prevalence of consanguinity and homozygosity, with a great deal of genetic heterogeneity, and no mutation characteristic of the region identified. The predominant phenotype of WD in the region was also hepatic, suggesting the benefits of screening for WD in patients with unexplained hepatic dysfunction.

Lebanese and Egyptian patients share missense mutations in exons 8, 10, 18 and 19 (Table 4). However, mutations Gly691Arg and Trp939Cys were identified in Lebanese patients but not in Saudi Arabian or Egyptian ones. There were also common mutations with Turkish WD patients, including exon 7 (Gly691Arg), exon 10 (Val8455Ser), exon 13 (Ala1003Thr), exon 18 (Asp1270Ser) and exon 19 (Arg1319stop). Only exon 10 (Val845Ser), and exon 18 (Asp1270Ser) were shared with Iranian patients. Interestingly, the exon 12 mutation of Trp939Cys was detected in Lebanon but not in any regional country. We reported this mutation in the homozygous state in 5 Lebanese patients, while worldwide it was only detected in 1 Hungarian patient in the heterozygous state[39]. This extensive genotypic diversity argues for testing patients suspected to have WD for mutations in all exons of ATP7B. The shared
mutations with the region may be attributed to common ancestors (Turkey and Egypt) who ruled Lebanon in the past. The origin of the Trp939Cys mutation, however, remains undetermined.

To our surprise, the His1069Gln mutation which is common in diverse populations in North America, Europe and several Mediterranean countries was not present in Lebanese patients, but was reported in a minority of patients from Egypt, Iran and Turkey. We did not identify a predominant mutation in Lebanon or the region. Whether mutations in the ATP hinge region in exon 18 may turn out to be a hot spot in this part of the world requires further studies on larger numbers of WD patients.

One major strength of our study is that it involves more than 500 patients from Lebanon and the region. It includes a comprehensive clinical and genetic assessment of WD patients in Lebanon, as well as studies from the region clearly stating the genotype and phenotype. Our patients belonged to extended consanguineous families having similar environmental exposures and dietary habits, which helped in reducing the effects of confounding factors on the genotype and phenotype of patients. In addition, our study has some limitations including the absence of true population studies and the lack of long-term follow-up to determine reliably the true phenotype of patients. It is possible that many WD patients in Lebanon and the region remain undiagnosed or unreported, hence missing new mutations and other genotype-phenotype associations.

In conclusion, WD in Lebanon and the region is characterized by extensive genotypic and phenotypic diversity, and by high rates of consanguinity and homozygosity. No predominant mutation has been identified in the region, while the predominant phenotype seems to be hepatic. Clinical and/or genetic testing of all family members for WD, as well as those with unexplained hepatic dysfunction may increase the detection rate of the disease. This could facilitate early institution of therapy and reduce the mortality and morbidity of this condition.

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COMMENTS
Background
Wilson’s disease (WD) is an autosomal recessive disorder of copper metabolism, characterized by extensive phenotypic diversity. Most of the patients are compound heterozygotes, having different mutation on each of the ATP7B alleles. Attempts to establish genotype-phenotype correlations was hampered by the large number of mutations in the ATP7B gene and difficulty in ascribing a phenotype to one allele. This, however, may be overcome by examining WD in homozygous patients. In Lebanon, consanguinity is quite prevalent, increasing the probability of homozygosity and possibility of establishing a phenotype-genotype correlation. They hereby report the spectrum of mutations and phenotypes of 36 Lebanese patients diagnosed with WD. In addition, we examine if a frequent mutation characterizing the region exists by comparing our findings with those reported from regional studies on WD in Arab and non-European countries.

Research frontiers
This manuscript examines whether genotype-phenotype correlation exists in Lebanese patients diagnosed with WD. It also determines if a frequent mutation characteristic of the Lebanese patients and/or the region occurs.

Innovations and breakthroughs
This is the first comparative study that attempts to identify a frequent mutation characterizing WD patients from Lebanon and regional Arab and non-European countries. Although this region is characterized by high rates of consanguinity and homozygosity, no frequent mutation has been identified in the region but predominance of the hepatic phenotype was noted.

Applications
This study improves our understanding of WD pathogenesis and the genetic determinants of patients’ phenotype. It emphasizes the importance of genetic screening for WD in family members with index patients, as well in patients with unexplained hepatic dysfunction. This would surely facilitate diagnosis and early management prior to onset of symptoms, thereby preventing the progressive clinical deterioration of the patient.

Terminology
WD is a rare disease of copper homeostasis that results from a defect in the ATP7B gene encoding a copper transporter. Ceruloplasmin is the major copper carrying protein in blood with ferroxidase activity. Kaiser-Fleischer rings refer to copper deposition circumscribing the iris of the eye, diagnostic of WD.

Peer-review
It is a very interesting manuscript.

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