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***Observational Study***

**Wilson disease in lebanon and regional countries: Homozygosity and hepatic phenotype predominance**

Barada K *et al.* Wilson disease in Lebanon

Kassem Barada, Aline El Haddad, Meghri Katerji, Mustapha Jomaa, Julnar Usta

**Kassem Barada, Aline El Haddad,** Department of Internal Medicine, American University of Beirut Medical Center, 110236 Beirut, Lebanon

**Meghri Katerji, Mustapha Jomaa, Julnar Usta,** Department of Biochemistry and Molecular Genetics, Faculty of Medicine, American University of Beirut, 110236 Beirut, Lebanon

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**Correspondence to:** **Julnar Usta, PhD, Professor**, Department of Biochemistry and Molecular Genetics, American University of Beirut, Riad el Solh, 110236 Beirut, Lebanon. justa@aub.edu.lb

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**Abstract**

***AIM***

To determine the phenotypes and predominant disease-causing mutations in Lebanese Wilson disease patients, comparing it with regional non-European countries.

***METHODS***

The clinical profile of 36 patients diagnosed in Lebanon was studied and their mutations were determined by molecular testing. All patients had full physical exam, including ophthalmologic slit-lamp examination ultrasound imaging of the liver, as well as their serum ceruloplasmin and 24-h urinary-Cu levels determined. In addition, genetic screening, using PCR followed by sequencing, for 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 on Wilson 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 Lebanon, Egypt, Iran and Turkey. Otherwise, mutations in Lebanon and the region were scattered over 17 exons of ATP7B. While the homozygous exon 12 mutation Trp939Cys was only detected in patients from Lebanon but none of the regional countries, the worldwide common mutation H1069Q was not present in Lebanon 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 wilson disease patients from 13 different families. Majority were homozygous for disease causing mutations. The worldwide most common mutation His1069Trp was absent in our patients. ATP hinge region may comprise a hot spot for mutations detected in 4 families. Hepatic phenotypes are predominant in both symptomatic and asymptomatic patients. Neurologic phenotypes are rare. We compare our findings with those reported in regional Arab and non-European countries, and they do not support the presence of a founder effect. Mutations are scattered over 17 exons with no common and frequent mutation characterizing the region.

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**INTRODUCTION**

Wilson disease (WD) is an autosomal recessive disorder of copper (Cu) metabolism resulting from defects in the ATP7B gene protein. It is characterized by failure of copper incorporation into ceruloplasmin (Cp) and decreased biliary copper excretion. As a consequence, copper 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[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-hr urinary Cu, and high hepatic Cu content (250 µg/g dry tissue)[2,3]. Recent guidelines for the diagnosis of WD were published by the European Association for the study of the Liver (EASL)[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[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[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[6]. Furthermore, a large number of mutations are rare, making it impractical to screen populations for all WD-causing mutations[7]. Some mutations however, are relatively frequent and population-specific, like the p.His1069Gln on exon 14 in Northern and Eastern Europe[8], the p.Arg778Leu and the p.Arg778Gly mutations on exon 8 among Chinese and Taiwanese patients respectively[9], and the deletion in the 5 prime regulatory region in Sardinia[10]. This facilitates 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, increasing thus the prevalence of genetic disorders, including WD[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[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 ATP7B gene respectively[13,14]. Whether a specific WD mutation prevails in Lebanon is not known.

In this study, we describe 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 ours. 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 Medical Center, a major tertiary referral center in Lebanon. All participating subjects were asked to sign a written consent form (protocol#: BioCh.JU.01) that was approved by both the Institutional Review Board and Research Committee at AUB.

***Clinical testing***

Patients’ evaluation included: history, date of birth, age of onset of symptoms, age at diagnosis, 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 MRI was done. Phenotypic classifications were designated following Ferenci’s classification as hepatic, neurologic, mixed or asymptomatic[3]. 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, were carried on, as described before[6,14]. Amplified PCR products were purified, sequenced and compared to published normal sequences in various 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 on WD patients from regional Arab and Non-European countries. A comprehensive literature search of PubMed and Medline including 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 WD Lebanese patients, 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); S (P11-P19); Ah (P20); T (P21-P23); B (P24-P25); H (P26-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 patients (75%) belonging to 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 (< 0.2g/L) except for P4 and P14. Eighteen patients out of 31 (58%) had KF rings (5/36 were NAV).

Out of the 36 WD patients, 24 were symptomatic (67%; 16 males, 8 females) and presented clinically at an average age of 14.5 years. Data on P6-P9 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, P16-P17, P18, P20, P25, P27-P28, P32]. Neurologic presentation was noted in 12.5% of patients (4/32: P12, P14, P34, P36). Mixed presentation was observed in 25% of patients (7/32: P2, P10, P24, P26, P29, P31, P35) two of whom had suicidal attempts/disposition (P10, P26). Notably, liver cirrhosis was present in 12 symptomatic (38%) patients: P1, P2, P10, P16-P18, P20, P24, P26-P28, andP32, 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, P13, P21-P23, P30, P33), hepatomegaly detected by abdominal ultrasound (P11, P13, P21), to full blown cirrhosis (P22-P23). Overall, 27/32 patients (84%) on whom we had clinical information had some form of hepatic dysfunction.

Patients with 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 (P25, P34). They were not identified in the asymptomatic patients except for patient P19 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) distributed as: 7 missense (exons: 7, 12, 10, 13, 15, and 18), 1 non-sense (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 P36 who was diagnosed based on KF rings, clinical and biochemical testing.

Out of 35 patients, 29 patients 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, Z) had, in this motif, missense mutations in the homozygous state: Asn1270Ser in 6 patients (P26-P31) and Pro1273Leu in 2 patients (P32-P33) accounting for 17% and 5.7% of chromosomes, respectively. Other identified mutations (Table 1) include: missense mutations in exon 7: Gly691Arg (10 patients: U, Or) and exon 12: Trp939Cys (5 patients: T, B); frameshift in exon 8: 2299insC (10 patients: S, Ah); and nonsense mutation in exon 19: Arg1319stop (2 patients: Ri, Sc) accounting for a chromosome frequency of: 27%; 14%; 23% and 4.3% respectively. Compound heterozygous mutations were identified in exons: 10 (Or: P10); 13 (S: P16-P19) and 15 (P35).

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% (P11 and P19), in addition to othersin exons 2, 3, and 13 (Table 3).

***WD patients: Lebanon vs regional countries***

Search of the literature for population studies on 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 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, and Syria.

Homozygosity was highly prevalent in Lebanese WD-patients (83%), and ranging between 68%-85.7% and 50-53% in Egyptian and Saudi-Arabia patients, respectively. This 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 each of 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 18 respectively; (2) Egypt, the Pro1273Leu mutation in exon 18; and (3) Egypt and turkey the Arg1319X mutation in Exon-19; d) 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, on 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. 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 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 don’t satisfy all these criteria. For example, patients P4 and P14 had normal Cp, 13 did not have KF rings and 4 had normal 24-hour 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 it is worldwide[33]. These occurred in 8 exons of ATP7B. One possible hot spot of 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 has no identifiable mutations in the coding region of the ATP7B. 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 P36 in whom the diagnosis was based on clinical assessment with 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 (P36) diagnosed at 39 years. In the B-family, patient P25 was diagnosed at 5 and passed away before the onset of his brother’s symptoms at the age of 13 (P24). Variation in age at diagnosis was also observed in asymptomatic ones. 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 P22 (8y), and P23 (3y) confirmed WD. Though they were asymptomatic, it was surprising to find that both had already evidence of liver cirrhosis on liver imaging. This raises the question as to whether gender plays a role in the clinical manifestations of disease[15]. Verification of this, however, requires a cohort study with larger number of patients. Such phenotypic diversity has been reported even among monozygotic twins[35] 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 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, 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 and tremors, drooling, dysarthria, dysphagia, ataxia, to suicidal attempts in some (P10, P26). Our asymptomatic patients (36%) were found to have liver involvement (transaminitis, fatty liver, cirrhosis) with no KF rings except for P19. 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 were published. Similar to Lebanon, the majority of patients from Egypt and Saudi Arabia had consistently 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 Saudi Arabian or Egyptian ones. There were also common mutations with Turkish WD patients including: exon 7 (Gly691Arg); exon 10 (Val845ser); exon 13 (Ala1003Thr); exon 18 (Asp1270Ser) and exon 19 (Arg1319stop). Only exon 10 (Val845ser), and exon 18 (Asp1270Ser) were shared with Iranian patients. Interestingly, exon 12 mutation 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[40] was not present in Lebanese patients, but was reported in a minority of patients from Egypt, Iran and Turkey. We didn’t 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 compounding 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 on 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 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. We 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***

The 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 while predominance of hepatic phenotype was noted.

***Applications***

The 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, preventing thus 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 the major copper carrying protein in blood with ferroxidase activity. Kaiser Fleischer rings refers to copper deposition circumscribing the iris of the eye, diagnostic of WD.

***Peer-Review***

It is a very intresting manuscript.

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**Table 1 Spectrum of mutations in the *ATP7B* gene of Lebanese Wilson disease patients**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **ID** | **Sex** | **Birth Date** | **AD** | **Exon #** | **Mutations** | **Region of Protein** |
| U | P1 | M | 1966 | 7 | 7 | Gly 691 Arg | TM2 |
| P2 | M | 1985 | 9 | 7 | Gly 691 Arg | TM2 |
| P3 | F | 1986 | 131 | 7 | Gly 691 Arg | TM2 |
| P4 | F | 1990 | 91 | 7 | Gly 691 Arg | TM2 |
| P5 | M | 1996 | 31 | 7 | Gly 691 Arg | TM2 |
| P6 | M | NAV | 32 | 7 | Gly 691 Arg | TM2 |
| P7 | M | NAV | 72 | 7 | Gly 691 Arg | TM2 |
| P8 | M | NAV | 122 | 7 | Gly 691 Arg | TM2 |
| P9 | F | NAV | NAV | 7 | Gly 691 Arg | TM2 |
| Or | P10 | F | 1986 | 21 | 7/10 | Gly 691 Arg/Val 845 Ser | TM2/Td |
| S | P11 | F | 1993 | 51 | 8 | 2299insC/2299insC | TM4 |
| P12 | M | 1973 | 12 | 8 | 2299insC/2299insC | TM4 |
| P13 | F | 1997 | 101 | 8 | 2299insC/2299insC | TM4 |
| P14 | M | 1980 | 16 | 8 | 2299insC/2299insC | TM4 |
| P15 | M | 2007 | 11 | 8 | 2299insC/2299insC | TM4 |
| P16 | M | 1981 | 16 | 8/13 | 2299insC/p.Ala1003Thr | TM4/Ch-TM6 |
| P17 | F | 1983 | 14 | 8/13 | 2299insC/p.Ala1003Thr | TM4/Ch-TM6 |
| P18 | F | 1993 | 12 | 8/13 | 2299insC/p.Ala1003Thr | TM4/Ch-TM6 |
| P19 | F | 1989 | 15† | 8/13 | 2299insC/p.Ala1003Thr | TM4/Ch-TM6 |
| Ah | P20 | M | 1992 | 15 | 8 | 2299insC/2299insC | TM4 |
| T | P21 | F | 1998 | 7 | 12 | Trp 939 Cys | Td |
| P22 | M | 2001 | 81 | 12 | Trp 939 Cys | Td |
| P23 | M | 2006 | 31 | 12 | Trp 939 Cys | Td |
| B | P24 | M | 1992 | 13 | 12 | Trp 939 Cys | Td |
| P25 | M | 2002 | 53 | 12 | Trp 939 Cys | Td |
| H | P26 | F | 1985 | 18 | 18 | Asn 1270 Ser | ATP hinge |
| P27 | F | 1987 | 181 | 18 | Asn 1270 Ser | ATP hinge |
| P28 | F | 1991 | 8 | 18 | Asn 1270 Ser | ATP hinge |
| Ha | P29 | F | 1998 | 14 | 18 | Asn 1270 Ser | ATP hinge |
| P30 | F | 2002 | 11 | 18 | Asn 1270 Ser | ATP hinge |
| Is | P31 | M | 1995 | 13 | 18 | Asn 1270 Ser | ATP hinge |
| Z | P32 | M | 1990 | 15 | 18 | Pro 1273 Leu | ATP hinge |
| P33 | M | 2000 | 61 | 18 | Pro 1273 Leu | ATP hinge |
| Ri | P34 | M | 2009 | 3 | 19 | Arg 1319 stop | TM7 |
| Sc | P35 | M | 1979 | 22 | 15/19 | Thr 1092 Met/Arg 1319 stop | ATP loop/ TM7 |
| Gh | P36 | M | 1970 | 39 | - | None identified | - |

AD: Age at Diagnosis; NAV: Not available. 1Screening; 2Died at age; 3Deceased.

**Table 2 Phenotypic and genotypic profiles of Lebanese Wilson disease patients**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Mutations** | **GI manifestations** | **Neurological manifestations** | **KF rings** | **Cp** | **Urinary Cu** | **Score** |
| P1 | Gly 691 Arg | Liver cirrhosis | Absent | Present | NAV | 718.8 | 8 |
| P2 | Gly 691 Arg | Liver cirrhosis | Change in school performance | Present | 0.11 | 1998 | 10 |
| P3 | Gly 691 Arg | Asymptomatic1 | Absent | Absent | 0.03 | 148.5 | 8 |
| P4 | Gly 691 Arg | Asymptomatic1 | Absent | Absent | 0.22 | 304 | 6 |
| P5 | Gly 691 Arg | Asymptomatic1 | Absent | Absent | 0.02 | 65.9 | 7 |
| P6 | Gly 691 Arg | NAV | NAV | NAV | NAV | NAV | 4 |
| P7 | Gly 691 Arg | NAV | NAV | NAV | NAV | NAV | 4 |
| P8 | Gly 691 Arg | NAV | NAV | NAV | NAV | NAV | 4 |
| P9 | Gly 691 Arg | NAV | NAV | NAV | NAV | NAV | 4 |
| P10 | Gly 691 Arg/Val 845 Ser | Liver cirrhosis | Suicidal attempts | Present | 0.08 | 2184 | 12 |
| 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 | 0.03 | 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/p.Ala1003Thr | Liver cirrhosis | Absent | Present | 0.096 | 775 | 10 |
| P17 | 2299insC//p.Ala1003Thr | Liver cirrhosis | Absent | Present | 0.096 | 590 | 10 |
| P18 | 2299insC/p.Ala1003Thr | Liver cirrhosis | Absent | Present | 0.17 | 645 | 9 |
| P19 | 2299insC/p.Ala1003Thr | Absent | Absent | Present | 0.12 | 487 | 9 |
| P20 | 2299insC | Liver cirrhosis | Absent | NAV | 0.023 | 651 | 8 |
| P21 | Trp 939 Cys | Asymptomatic | Absent | Absent | 0.02 | 77.6 | 7 |
| P22 | Trp 939 Cys | Asymptomatic | Absent | Absent | 0.02 | 20 | 6 |
| P23 | Trp 939 Cys | Asymptomatic | Absent | Absent | 0.02 | 41.5 | 6 |
| P24 | Trp 939 Cys | Liver cirrhosis, ascites | Jaw Drooping, hypersalivation, slurred speech, narrow based gait, intention tremors | Present | 0.021 | 744 | 12 |
| P25 | Trp 939 Cys | Liver cirrhosis, Hepatic encephalopathy, Hepatomegaly, Mild to moderate ascites | Absent | Absent | 0.04 | NAV | 6 |
| P26 | Asn 1270 Ser | Liver cirrhosis | Psychiatric symptoms and suicidal attempts | Present | 0.03 | 27.6 | 10 |
| P27 | Asn 1270 Ser | Liver cirrhosis | Absent | Present | 0.03 | 65.1 | 9 |
| P28 | Asn 1270 Ser | Ascites, liver cirrhosis | Absent | Present | 0.04 | 55 | 9 |
| P29 | Asn 1270 Ser | Transaminitis | Neurodevelopmental | Present | 0.078 | 171 | 11 |
| P30 | Asn 1270 Ser | Asymptomatic | Absent | Absent | 0.03 | 116 | 8 |
| P31 | Asn 1270 Ser | Chronic liver parenchymal disease | Dysarthria and left sided dystonia | Present | 0.029 | 402.3 | 12 |
| P32 | Pro 1273 Leu | Ascites, Liver cirrhosis, Hepatic encephalopathy | Absent | Present | 0.17 | 1041.1 | 9 |
| P33 | Pro 1273 Leu | Asymptomatic | Absent | Absent | 0.19 | 89.7 | 6 |
| P34 | Arg 1319 stop | Asymptomatic | Delay in speech | Absent | 0.02 | 92 | 8 |
| P35 | Thr 1092 Met/Arg 1319 stop | Chronic liver disease and early portal hypertension | Clenching of mandible, left side dystonia, sialorrhea, dysarthia, head tremors | Present | 0.025 | 199 | 12 |
| P36 | None identified | Absent | Drooling, dysathria, difficulty concentrating, dysphagia | Present | 0.085 | NAV | 6 |

Cp: Ceruloplasmin (g/L); Urinary Cu: 24 h urine copper (µg/24h); NAV: Not available; KF: Keiser Fleischer. 1developed later. Normal range: Serum ceruloplasmin: 0.2 to 0.6 g/L; Urine copper: 15 to 50 µg/24h. Score = Ferrenci Score of diagnosis. 2 or less: Very unlikely; 3: Possible, more tests needed; 4 or more: Established[4].

**Table 3 Identified Polymorphisms in the *ATP7B* gene of Lebanese Wilson disease patients**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Polymorphism | Asp 96 Gly | Ser406Ala | Val 456 Leu | Lys 832 Arg | Arg 952 Lys | Ala 1003 Ala | Val 1140 Ala | Ser 1166 Ser |
| Exon | 2 | 2 | 3 | 10 | 12 | 13 | 16 | 16 |
| Base Change | GAC 🡪 GGC | TCT 🡪 GCT | GTG 🡪 CTG | AAG 🡪 AGG | AGA 🡪 AAA | GCG 🡪 GCA | GTC 🡪 GCC | AGC 🡪 AGT |
| Domain | Cu1-4 | Cu4 binding | Cu4/ Cu5 | Td | Tm5 | ATP binding/ Tm6 | ATP loop | ATP loop |
| Family | U |  |  |  | HM | HM |  | HM |  |
| Or |  | HM | HM | HM | HM |  | HM |  |
| S | P1, P2, P31, P41,P59 |  |  |  | HM | HM |  | HM |  |
| P7, P8 |  |  |  | HT | HT |  | HT |  |
| P3, P4 |  |  |  |  | HM |  | HM |  |
| AH |  |  | HM | HM | HM |  | HM |  |
| TF |  |  |  | HM | HM | HM | HM | HM |
| B |  |  |  | HM | HM | HM | HM |  |
| H |  | HM | HM | HM | HM |  | HM |  |
| Ha |  |  |  |  |  |  |  | HM |
| Is |  |  | HM | HM | HM |  | HM |  |
| Z |  | HM | HM | HM | HM |  | HM |  |
| Ri | HM |  |  | HM |  |  | HM |  |
| Sc |  |  | HT | HM | HM |  | HM |  |
| Gh |  | HM | HM | HM | HM |  | HM |  |
| Ah |  |  | HM | HM | HM |  | HM |  |

**Table 4 Lebanese *vs* regional Arab and non-European Wilson disease patients: Genotype-Phenotype**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|   | **Lebanon** | **Egypt** | **Iran** | **Turkey** | **Saudi Arabia** | **Oman** |
| Number of patients  | *n* = 36 | 198 | 88\* | 46 | 152\*\* | 14 |
| Number of families | *n* = 13 | 135 | - | 46 | 53 | 1 |
| % Homozygosity | 83% | 68.4% - 85.7% | NAV | NAV | 50% - 53% | NAV |
| % Consanguinity | 75% | 39.5% - 78.9% | NAV | NAV | 36.6% - 88.8% | NAV |
| % Hepatic manifestation | 28% | 45.5% - 84.2% | 65.20% | 43.50% | 25% - 54.9% | 0% |
| % Neurologic manifestation | 12.50% | 4.2% - 15.8% | 4.30% | 34.80% | 0% - 25% | 21.40% |
| % Mixed manifestation | 21.80% | 0% - 20.9% | 21.70% | 21.70% | 19.6% -55.6% | 0% |
| % Asymptomatic  | 37% | 0% - 35.1% | - | 0% | 30.35% | 78.60% |
| % KF Rings | 58% | 26.3% - 69.2% | 65.20% | 67.40% | 50.7% - 59% | NAV |
| Mutation | E2 |   | Glu 396 stop |   |   |   |   |
| E3 |   |   |   | Gly 457 stop |   |   |
| E4-6 | No common mutations identified |
| E7 | Gly 691 Arg |   |   | Gly 691 Arg |   |   |
| E8 | 2299insC | c. 2304-5insC | Trp 779 Gly | Gly 710 Ser | Ser744Pro |   |
| Cys 703 Tyr | Pro 767 Arg |   |
| E9 | No common or frequent mutations identified |
| E10 | Val 845 Ser | Val 845 Ser | Val 845 Ser | Val 845 Ser |   |   |
| E11 | No common or frequent mutations identified |
| E12 | Trp 939 Cys |   |   |   |   |   |
| E13 | Ala 1003 Thr |   | 3061-1G>A sp | Ala 1003 Thr |   | Deletion of E13 |
| E14 |   | Thr 1076 Ile |   |   |   |   |
|   | His 1069 Gln | His 1069 Gln | His 1069 Gln |   |   |
| E15 | Thr 1092 Met | His 1126 fs | Ile 1102 Thr |   |   |   |
| E16-17 | No common or frequent mutations identified |
| E18 | Asn 1270 Ser | Asn 1270 Ser | Asn 1270 Ser | Asn 1270 Ser |   |   |
| Pro 1273 Leu | Pro 1273 Leu |   |   |  |   |
|   | IVS18-2A>G |   |   |   |   |
| E19 | Arg 1319 stop | Arg 1319 stop |   | Arg 1319 stop |   |   |
| E20 |   |   |   |   | Gly 1341 Ser |   |
| E21 |   |   |   |   | Gln 1399 Arg |   |
| Ref. | Barada *et al*[13,30] | Abdelghaffar *et al*[12,22]  | Dastsooz *et al*[27] | Simsek Papur *et al*[25] | Al Jumah *et al*[18] | Al Tobi *et al*[29] |
| Al Fadda *et al*[19] |
| Usta *et al*[6,14] | El-Karaksy *et al*[23] | Zali *et al*[28] | Loudianos *et al*[26] | Majumdar *et al*[20,21] |
| El-Mougy *et al*[24] |