ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2017 September 28; 23(36): 6549-6746

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

Contents

Weekly Volume 23 Number 36 September 28, 2017

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORIAL OFFICE
Jin-Lei Wang, Director
Yuan Qi, Vice Director
Ze-Mao Gong, Vice Director
World Journal of Gastroenterology
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
September 28, 2017

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Gastroenterology

Submit a Manuscript: http://www.f6publishing.com

World J Gastroenterol 2017 September 28; 23(36): 6715-6725

DOI: 10.3748/wjg.v23.i36.6715

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

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

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, Beirut 110236, Lebanon

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

ORCID number: Kassem Barada (0000-0002-9629-1708); Aline El Haddad (0000-0001-7527-0935); Meghri Katerji (0000-0003-0971-8177); Mustapha Jomaa (0000-0003-3807-6839); Julnar Usta (0000-0002-6191-6763).

Author contributions: El Haddad A, Katerji M, Jomaa M compiled the data and helped in the literature review; Barada K and Usta J coordinated and supervised the research and the write up of the manuscript.

Supported by the Medical Practice Plan and University Research Board grants to J Usta at the American University of Beirut.

Institutional review board statement: The study was reviewed and approved by the American University of Beirut (AUB) Institutional Review Board (IRB Protocol No. BioCh.JU.01). Blood samples were drawn after seeking approval from each participant and obtaining their signature on the consent form.

Conflict-of-interest statement: The authors state they have no conflicts to declare.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Julnar Usta, PhD, Professor, Department of Biochemistry and Molecular Genetics, American University of Beirut, Riad el Solh, Beirut 110236, Lebanon. justa@aub.edu.lb
Telephone: +961-1-350000

Received: May 4, 2017
Peer-review started: May 5, 2017
First decision: June 7, 2017
Revised: June 16, 2017
Accepted: July 12, 2017
Article in press: July 12, 2017
Published online: September 28, 2017

Abstract

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 *ATP7B*. While the homozygous exon 12 mutation Trp939Cys was only detected in patients from Lebanon but none from the regional countries, 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

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.

Barada K, El Haddad A, Katerji M, Jomaa M, Usta J. Wilson's disease in Lebanon and regional countries: Homozygosity and hepatic phenotype predominance. *World J Gastroenterol* 2017; 23(36): 6715-6725 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i36/6715.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i36.6715

INTRODUCTION

Wilson's 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 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[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 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 European patients[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' regulatory region in Sardinian patients[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[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 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 (P_1-P_{36}) 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[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 was carried out as described[6,14]. Amplified PCR products were purified, sequenced and compared to published normal sequences in the following databanks: 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 (P_1-P_9); Or (P_{10}); S (P_{11}-P_{19}); Ah (P_{20}); T (P_{21}-P_{23}); B (P_{24}-P_{25}); H (P_{26}-P_{28}); Ha (P_{29}-P_{30}); Is (P_{31}); Z (P_{32}-P_{33}); Ri (P_{34}); Sc (P_{35}); and Gh (P_{36}) 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 P_4 and P_{14}. 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 P_6-P_9 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: P_1, P_{16}-P_{17}, P_{18}, P_{20}, P_{25}, P_{27}-P_{28} and P_{32}]. Neurologic presentation was noted in 12.5% of patients (4/32: P_{12}, P_{14}, P_{34} and P_{36}). Mixed presentation was observed in 25% of patients (7/32: P_2, P_{10}, P_{24}, P_{26}, P_{29}, P_{31} and P_{35}), two of whom had suicidal attempts/disposition (P_{10} and P_{26}). Notably, liver cirrhosis was present in 12 symptomatic (38%) patients (P_1, P_2, P_{10}, P_{16}-P_{18}, P_{20}, P_{24}, P_{26}-P_{28} and P_{32}), 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 (P_3-P_5, P_{11}, P_{13},

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

Family	ID	Sex	Birth date	AD	Exon	Mutation(s)	Region of protein
U	P_1	M	1966	7	7	Gly691Arg	TM2
	P_2	M	1985	9	7	Gly691Arg	TM2
	P_3	F	1986	13[1]	7	Gly691Arg	TM2
	P_4	F	1990	9[1]	7	Gly691Arg	TM2
	P_5	M	1996	3[1]	7	Gly691Arg	TM2
	P_6	M	NAV	3[2]	7	Gly691Arg	TM2
	P_7	M	NAV	7[2]	7	Gly691Arg	TM2
	P_8	M	NAV	12[2]	7	Gly691Arg	TM2
	P_9	F	NAV	NAV	7	Gly691Arg	TM2
Or	P_{10}	F	1986	21	7/10	Gly691Arg/Val845Ser	TM2/Td
S	P_{11}	F	1993	5[1]	8	2299insC/2299insC	TM4
	P_{12}	M	1973	12	8	2299insC/2299insC	TM4
	P_{13}	F	1997	10[1]	8	2299insC/2299insC	TM4
	P_{14}	M	1980	16	8	2299insC/2299insC	TM4
	P_{15}	M	2007	1[1]	8	2299insC/2299insC	TM4
	P_{16}	M	1981	16	8/13	2299insC/p.Ala1003Thr	TM4/Ch-TM6
	P_{17}	F	1983	14	8/13	2299insC/p.Ala1003Thr	TM4/Ch-TM6
	P_{18}	F	1993	12	8/13	2299insC/p.Ala1003Thr	TM4/Ch-TM6
	P_{19}	F	1989	15[1]	8/13	2299insC/p.Ala1003Thr	TM4/Ch-TM6
Ah	P_{20}	M	1992	15	8	2299insC/2299insC	TM4
T	P_{21}	F	1998	7	12	Trp939Cys	Td
	P_{22}	M	2001	8[1]	12	Trp939Cys	Td
	P_{23}	M	2006	3[1]	12	Trp939Cys	Td
B	P_{24}	M	1992	13	12	Trp939Cys	Td
	P_{25}	M	2002	5[3]	12	Trp939Cys	Td
H	P_{26}	F	1985	18	18	Asn1270Ser	ATP hinge
	P_{27}	F	1987	18[1]	18	Asn1270Ser	ATP hinge
	P_{28}	F	1991	8	18	Asn1270Ser	ATP hinge
Ha	P_{29}	F	1998	14	18	Asn1270Ser	ATP hinge
	P_{30}	F	2002	11	18	Asn1270Ser	ATP hinge
Is	P_{31}	M	1995	13	18	Asn1270Ser	ATP hinge
Z	P_{32}	M	1990	15	18	Pro1273Leu	ATP hinge
	P_{33}	M	2000	6[1]	18	Pro1273Leu	ATP hinge
Ri	P_{34}	M	2009	3	19	Arg1319stop	TM7
Sc	P_{35}	M	1979	22	15/19	Thr1092Met/Arg1319stop	ATP loop/TM7
Gh	P_{36}	M	1970	39	-	None identified	-

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

P_{21}-P_{23}, P_{30} and P_{33}) and hepatomegaly detected by abdominal ultrasound (P_{11}, P_{13} and P_{21}) to full blown cirrhosis (P_{22}-P_{23}). 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 (P_{25}, P_{34}). They were not identified in the asymptomatic patients, except for patient P_{19} 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 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 P_{36}, 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 (P_{26}-P_{31}) and Pro1273Leu in 2 patients (P_{32}-P_{33}), accounting for 17% and 5.7% of chromosomes respectively. Other identified mutations (Table 1) included missense mutations in exon 7 (Gly691Arg; 10 patients: U and Or) and exon 12 (Trp939Cys; 5 patients: T and B), frameshift in exon 8 (2299insC; 10 patients: S and Ah), and nonsense mutation in exon 19 (Arg1319stop; 2 patients: Ri and Sc), accounting for a chromosome frequency of 27%, 14%, 23% and

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

ID	Mutation(s)	GI manifestation(s)	Neurological manifestations	KF rings	Cp	Urinary Cu	Score
P_1	Gly691Arg	Liver cirrhosis	Absent	Present	NAV	718.8	8
P_2	Gly691Arg	Liver cirrhosis	Change in school performance	Present	0.11	1998	10
P_3	Gly691Arg	Asymptomatic[1]	Absent	Absent	0.03	148.5	8
P_4	Gly691Arg	Asymptomatic[1]	Absent	Absent	0.22	304	6
P_5	Gly691Arg	Asymptomatic[1]	Absent	Absent	0.02	65.9	7
P_6	Gly691Arg	NAV	NAV	NAV	NAV	NAV	4
P_7	Gly691Arg	NAV	NAV	NAV	NAV	NAV	4
P_8	Gly691Arg	NAV	NAV	NAV	NAV	NAV	4
P_9	Gly691Arg	NAV	NAV	NAV	NAV	NAV	4
P_{10}	Gly691Arg/ Val845Ser	Liver cirrhosis	Suicidal attempts	Present	0.08	2184	12
P_{11}	2299insC	Asymptomatic	Absent	Absent	0.04	99	7
P_{12}	2299insC	Absent	Slurred speech, ataxia, tremors	Present	0.072	512	12
P_{13}	2299insC	Asymptomatic	Absent	Absent	0.03	152.8	8
P_{14}	2299insC	Absent	Choreoathetosis, tremors, rigidity	Present	0.423	2300	10
P_{15}	2299insC	Asymptomatic	Absent	Absent	0.019	10	6
P_{16}	2299insC/ p.Ala1003Thr	Liver cirrhosis	Absent	Present	0.096	775	10
P_{17}	2299insC/ p.Ala1003Thr	Liver cirrhosis	Absent	Present	0.096	590	10
P_{18}	2299insC/ p.Ala1003Thr	Liver cirrhosis	Absent	Present	0.17	645	9
P_{19}	2299insC/ p.Ala1003Thr	Absent	Absent	Present	0.12	487	9
P_{20}	2299insC	Liver cirrhosis	Absent	NAV	0.023	651	8
P_{21}	Trp939Cys	Asymptomatic	Absent	Absent	0.02	77.6	7
P_{22}	Trp939Cys	Asymptomatic	Absent	Absent	0.02	20	6
P_{23}	Trp939Cys	Asymptomatic	Absent	Absent	0.02	41.5	6
P_{24}	Trp939Cys	Liver cirrhosis, ascites	Jaw drooping, hypersalivation, slurred speech, narrow based gait, intention tremors	Present	0.021	744	12
P_{25}	Trp939Cys	Liver cirrhosis, Hepatic encephalopathy, Hepatomegaly, Mild to moderate ascites	Absent	Absent	0.04	NAV	6
P_{26}	Asn1270Ser	Liver cirrhosis	Psychiatric symptoms and suicidal attempts	Present	0.03	27.6	10
P_{27}	Asn1270Ser	Liver cirrhosis	Absent	Present	0.03	65.1	9
P_{28}	Asn1270Ser	Ascites, liver cirrhosis	Absent	Present	0.04	55	9
P_{29}	Asn1270Ser	Transaminitis	Neurodevelopmental	Present	0.078	171	11
P_{30}	Asn1270Ser	Asymptomatic	Absent	Absent	0.03	116	8
P_{31}	Asn1270Ser	Chronic liver parenchymal disease	Dysarthria and left-sided dystonia	Present	0.029	402.3	12
P_{32}	Pro1273Leu	Ascites, Liver cirrhosis, Hepatic encephalopathy	Absent	Present	0.17	1041.1	9
P_{33}	Pro1273Leu	Asymptomatic	Absent	Absent	0.19	89.7	6
P_{34}	Arg1319stop	Asymptomatic	Delay in speech	Absent	0.02	92	8
P_{35}	Thr1092Met/ Arg1319stop	Chronic liver disease and early portal hypertension	Clenching of mandible, left side dystonia, sialorrhea, dysarthia, head tremors	Present	0.025	199	12
P_{36}	None identified	Absent	Drooling, dysathria, difficulty concentrating, dysphagia	Present	0.085	NAV	6

[1]Developed 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]. Cp: Ceruloplasmin (g/L); KF: Keiser-Fleischer; NAV: Not available; Urinary Cu: 24-h urine copper (μg/24 h).

4.3% respectively. Compound heterozygous mutations were identified in exons 10 (Or: P_{10}), 13 (S: P_{16}-P_{19}) and 15 (P_{35}).

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% (P_{11} and P_{19}), 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,

Table 3 Identified polymorphisms in the *ATP7B* gene of Lebanese patients with Wilson's disease

Polymorphism	Asp96Gly	Ser406Ala	Val456Leu	Lys832Arg	Arg952Lys	Ala1003 Ala	Val1140Ala	Ser1166Ser
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	

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 18 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

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

	Lebanon	Egypt	Iran	Turkey	Saudi Arabia	Oman
Number of patients	36	198	88	46	152[1]	14
Number of families	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		Glu396stop				
E3				Gly457stop		
E4-6			No common mutations identified			
E7	Gly691Arg			Gly691Arg		
E8	2299insC	c. 2304-5insC	Trp779Gly	Gly710Ser	Ser744Pro	
		Cys703Tyr		Pro767Arg		
E9			No common or frequent mutations identified			
E10	Val845Ser	Val845Ser	Val845Ser	Val845Ser		
E11			No common or frequent mutations identified			
E12	Trp939Cys					
E13	Ala1003Thr		3061-1G>A sp	Ala1003Thr		Deletion of E13
E14		Thr1076Ile				
		His1069Gln	His1069Gln	His1069Gln		
E15	Thr1092Met	His1126fs	Ile1102Thr			
E16-17			No common or frequent mutations identified			
E18	Asn1270Ser	Asn1270Ser	Asn1270Ser	Asn1270Ser		
	Pro1273Leu	Pro1273Leu				
		IVS18-2A>G				
E19	Arg1319stop	Arg1319stop		Arg1319stop		
E20					Gly1341Ser	
E21					Gln1399Arg	
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]				

[1]152 patients described in articles from Saudi Arabia, of which 5 patients were from yemen and syria.

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 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 P_4 and P_{14} 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 P_{36}, 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 (P_{36}) diagnosed at 39 years. In the B family, patient P_{25} was diagnosed at 5 and passed away before the onset of his brother's symptoms at the age of 13 (P_{24}). 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 (P_{21}) 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, P_{22} (8 years) and P_{23} (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[15]. Verification of this, however, requires a cohort study with a 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 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 (P_{10} and P_{26}).

Our asymptomatic patients (36%) were found to have liver involvement (transaminitis, fatty liver and cirrhosis) with no KF rings, except for P_{19}. Changes such as fatty liver were detected at the age of 1 year in P_{15}, 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 (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, 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[40] 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 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 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.

ACKNOWLEDGMENTS

The authors would like to thank all patients who agreed to participate in the study. They also thank the Medical Research Volunteer Program at AUB for matching the joining of Mr Mustapha Jomaa, the Laboratory of Dr Julnar Usta.

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.

REFERENCES

1 **Schilsky ML**. Wilson disease: Clinical manifestations, diagnosis, and natural history. Available from: URL: https://www.uptodate.com/contents/wilson-disease-clinical-manifestations-diagnosis-and-natural-history
2 **Sternlieb I**. The outlook for the diagnosis of Wilson's disease. *J Hepatol* 1993; **17**: 263-264 [PMID: 8315256]
3 **Ferenci P**, Caca K, Loudianos G, Mieli-Vergani G, Tanner S, Sternlieb I, Schilsky M, Cox D, Berr F. Diagnosis and phenotypic classification of Wilson disease. *Liver Int* 2003; **23**: 139-142 [PMID: 12955875]
4 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: Wilson's disease. *J Hepatol* 2012; **56**: 671-685 [PMID: 22340672 DOI: 10.1016/j.jhep.2011.11.007]
5 **Hahn SH**. Population screening for Wilson's disease. *Ann N Y Acad Sci* 2014; **1315**: 64-69 [PMID: 24731025 DOI: 10.1111/nyas.12423]
6 **Usta J**, Wehbeh A, Rida K, El-Rifai O, Estiphan TA, Majarian T, Barada K. Phenotype-genotype correlation in Wilson disease in a large Lebanese family: association of c.2299insC with hepatic and of p. Ala1003Thr with neurologic phenotype. *PLoS One* 2014; **9**: e109727 [PMID: 25390358 DOI: 10.1371/journal.pone.0109727]
7 **Gupta A**. Low-density oligonucleotide microarrays - A major step in Wilson's disease diagnosis. *Indian J Med Res* 2015; **141**: 145-147 [PMID: 25900946]
8 **Thomas GR**, Forbes JR, Roberts EA, Walshe JM, Cox DW. The Wilson disease gene: spectrum of mutations and their consequences. *Nat Genet* 1995; **9**: 210-217 [PMID: 7626145 DOI:

10.1038/ng0295-210]
9 **Chuang LM**, Wu HP, Jang MH, Wang TR, Sue WC, Lin BJ, Cox DW, Tai TY. High frequency of two mutations in codon 778 in exon 8 of the ATP7B gene in Taiwanese families with Wilson disease. *J Med Genet* 1996; **33**: 521-523 [PMID: 8782057]
10 **Gialluisi A**, Incollu S, Pippucci T, Lepori MB, Zappu A, Loudianos G, Romeo G. The homozygosity index (HI) approach reveals high allele frequency for Wilson disease in the Sardinian population. *Eur J Hum Genet* 2013; **21**: 1308-1311 [PMID: 23486543 DOI: 10.1038/ejhg.2013.43]
11 **Tadmouri GO**, Nair P, Obeid T, Al Ali MT, Al Khaja N, Hamamy HA. Consanguinity and reproductive health among Arabs. *Reprod Health* 2009; **6**: 17 [PMID: 19811666 DOI: 10.1186/1742-4755-6-17]
12 **Abdelghaffar TY**, Elsayed SM, Elsobky E, Bochow B, Büttner J, Schmidt H. Mutational analysis of ATP7B gene in Egyptian children with Wilson disease: 12 novel mutations. *J Hum Genet* 2008; **53**: 681-687 [PMID: 18483695 DOI: 10.1007/s10038-008-0298-7]
13 **Barada K**, Nemer G, ElHajj II, Touma J, Cortas N, Boustany RM, Usta J. Early and severe liver disease associated with homozygosity for an exon 7 mutation, G691R, in Wilson's disease. *Clin Genet* 2007; **72**: 264-267 [PMID: 17718866 DOI: 10.1111/j.1399-0004.2007.00853.x]
14 **Usta J**, Abu Daya H, Halawi H, Al-Shareef I, El-Rifai O, Malli AH, Sharara AI, Habib RH, Barada K. Homozygosity for Non-H1069Q Missense Mutations in ATP7B Gene and Early Severe Liver Disease: Report of Two Families and a Meta-analysis. *JIMD Rep* 2012; **4**: 129-137 [PMID: 23430908 DOI: 10.1007/8904_2011_91]
15 **Ferenci P**. Whom and how to screen for Wilson disease. *Expert Rev Gastroenterol Hepatol* 2014; **8**: 513-520 [PMID: 24650289 DOI: 10.1586/17474124.2014.899898]
16 **Kalinsky H**, Funes A, Zeldin A, Pel-Or Y, Korostishevsky M, Gershoni-Baruch R, Farrer LA, Bonne-Tamir B. Novel ATP7B mutations causing Wilson disease in several Israeli ethnic groups. *Hum Mutat* 1998; **11**: 145-151 [PMID: 9482578 DOI: 10.1002/(SICI)1098-1004(1998)11:2<145::AID-HUMU7>3.0.CO;2-I]
17 **Hussein H**, Jabbar A. Mutation analysis in Iraqi patients with Wilson's disease: Identification of four novel mutations. *Wasit J Sci Med* 2014; **7**: 149-158
18 **Al Jumah M**, Majumdar R, Al Rajeh S, Awada A, Al Zaben A, Al Traif I, Al Jumah AR, Rehana Z. A clinical and genetic study of 56 Saudi Wilson disease patients: identification of Saudi-specific mutations. *Eur J Neurol* 2004; **11**: 121-124 [PMID: 14748773]
19 **Al Fadda M**, Al Quaiz M, Al Ashgar H, Al Kahtani K, Helmy A, Al Benmousa A, Abdulla M, Peedikayil M. Wilson disease in 71 patients followed for over two decades in a tertiary center in Saudi Arabia: a retrospective review. *Ann Saudi Med* 2012; **32**: 623-629 [PMID: 23396027 DOI: 10.5144/0256-4947.2012.623]
20 **Majumdar R**, Al Jumah M, Al Rajeh S, Fraser M, Al Zaben A, Awada A, Al Traif I, Paterson M. A novel deletion mutation within the carboxyl terminus of the copper-transporting ATPase gene causes Wilson disease. *J Neurol Sci* 2000; **179**: 140-143 [PMID: 11054498]
21 **Majumdar R**, Al Jumah M, Fraser M. 4193delC, a common mutation causing Wilson's disease in Saudi Arabia: rapid molecular screening of patients and carriers. *Mol Pathol* 2003; **56**: 302-304 [PMID: 14514926]
22 **Abdel Ghaffar TY**, Elsayed SM, Elnaghy S, Shadeed A, Elsobky ES, Schmidt H. Phenotypic and genetic characterization of a cohort of pediatric Wilson disease patients. *BMC Pediatr* 2011; **11**: 56 [PMID: 21682854 DOI: 10.1186/1471-2431-11-56]
23 **El-Karaksy H**, Fahmy M, El-Raziky MS, El-Hawary M, El-Sayed R, El-Koofy N, El-Mougy F, El-Hennawy A, El-Shabrawi M. A clinical study of Wilson's disease: The experience of a single Egyptian Paediatric Hepatology Unit. *Arab J Gastroenterol* 2011; **12**: 125-130 [PMID: 22055589 DOI: 10.1016/j.ajg.2011.07.007]
24 **El-Mougy FA**, Sharaf SA, Elsharkawy MM, Mandour IA, El-Essawy RA, Eldin AM, Helmy HM, Soliman DH, Selim LH, Sharafeldin HM, Mogahed EA, El-Karaksy HM. Gene mutations in Wilson disease in Egyptian children: report on two novel mutations. *Arab J Gastroenterol* 2014; **15**: 114-118 [PMID: 25465132 DOI: 10.1016/j.ajg.2014.10.005]
25 **Simsek Papur O**, Akman SA, Cakmur R, Terzioglu O. Mutation analysis of ATP7B gene in Turkish Wilson disease patients: identification of five novel mutations. *Eur J Med Genet* 2013; **56**: 175-179 [PMID: 23333878 DOI: 10.1016/j.ejmg.2013.01.003]
26 **Loudianos G**, Dessì V, Lovicu M, Angius A, Nurchi A, Sturniolo GC, Marcellini M, Zancan L, Bragetti P, Akar N, Yagci R, Vegnente A, Cao A, Pirastu M. Further delineation of the molecular pathology of Wilson disease in the Mediterranean population. *Hum Mutat* 1998; **12**: 89-94 [PMID: 9671269 DOI: 10.1002/(SICI)1098-1004(1998)12:2<89::AID-HUMU3>3.0.CO;2-G]
27 **Dastsooz H**, Imanieh MH, Dehghani SM, Haghighat M, Moini M, Fardaei M. Multiplex ARMS PCR to Detect 8 Common Mutations of ATP7B Gene in Patients With Wilson Disease. *Hepat Mon* 2013; **13**: e8375 [PMID: 24003324 DOI: 10.5812/hepatmon.8375]
28 **Zali N**, Mohebbi SR, Esteghamat S, Chiani M, Haghighi MM, Hosseini-Asl SM, Derakhshan F, Mohammad-Alizadeh AH, Malek-Hosseini SA, Zali MR. Prevalence of ATP7B Gene Mutations in Iranian Patients With Wilson Disease. *Hepat Mon* 2011; **11**: 890-894 [PMID: 22308153 DOI: 10.5812/kowsar.1735143X.762]
29 **Al-Tobi M**, Kashoob M, Joshi S, Bayoumi R. A Novel Splice-site Allelic Variant is Responsible for Wilson Disease in an Omani Family. *Sultan Qaboos Univ Med J* 2011; **11**: 357-362 [PMID: 22087377]
30 **Barada K**, El-Atrache M, El-Hajj II, Rida K, El-Hajjar J, Mahfoud Z, Usta J. Homozygous mutations in the conserved ATP hinge region of the Wilson disease gene: association with liver disease. *J Clin Gastroenterol* 2010; **44**: 432-439 [PMID: 20485189 DOI: 10.1097/MCG.0b013e3181ce5138]
31 **Das SK**, Ray K. Wilson's disease: an update. *Nat Clin Pract Neurol* 2006; **2**: 482-493 [PMID: 16932613 DOI: 10.1038/ncpneuro0291]
32 **Ferenci P**. Wilson's Disease. *Clin Gastroenterol Hepatol* 2005; **3**: 726-733 [PMID: 16233999]
33 **Lv T**, Li X, Zhang W, Zhao X, Ou X, Huang J. Recent advance in the molecular genetics of Wilson disease and hereditary hemochromatosis. *Eur J Med Genet* 2016; **59**: 532-539 [PMID: 27592149 DOI: 10.1016/j.ejmg.2016.08.011]
34 **Lu CX**, Qing Lin, Huang WQ, Tzeng CM. New mutations and polymorphisms of the ATP7B gene in sporadic Wilson disease. *Eur J Med Genet* 2014; **57**: 498-502 [PMID: 24878384 DOI: 10.1016/j.ejmg.2014.04.016]
35 **Członkowska A**, Gromadzka G, Chabik G. Monozygotic female twins discordant for phenotype of Wilson's disease. *Mov Disord* 2009; **24**: 1066-1069 [PMID: 19306278 DOI: 10.1002/mds.22474]
36 **Fraga MF**, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suñer D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, Esteller M. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci USA* 2005; **102**: 10604-10609 [PMID: 16009939 DOI: 10.1073/pnas.0500398102]
37 **Leggio L**, Gasbarrini G, Addolorato G. Wilson's disease. *Lancet* 2007; **369**: 902 [PMID: 17368142 DOI: 10.1016/S0140-6736(07)60437-1]
38 **Takeshita Y**, Shimizu N, Yamaguchi Y, Nakazono H, Saitou M, Fujikawa Y, Aoki T. Two families with Wilson disease in which siblings showed different phenotypes. *J Hum Genet* 2002; **47**: 543-547 [PMID: 12376745 DOI: 10.1007/s100380200082]
39 **Folhoffer A**, Ferenci P, Csak T, Horvath A, Hegedus D, Firneisz G, Osztovits J, Kosa JP, Willheim-Polli C, Szonyi L, Abonyi M, Lakatos PL, Szalay F. Novel mutations of the ATP7B gene among 109 Hungarian patients with Wilson's disease.*Eur J Gastroenterol*

Hepatol 2007; **19**: 105-111 [PMID: 17272994 DOI: 10.1097/01.meg.0000223904.70492.0b]

40 **Shah AB**, Chernov I, Zhang HT, Ross BM, Das K, Lutsenko S, Parano E, Pavone L, Evgrafov O, Ivanova-Smolenskaya IA, Annerén G, Westermark K, Urrutia FH, Penchaszadeh GK, Sternlieb I, Scheinberg IH, Gilliam TC, Petrukhin K. Identification and analysis of mutations in the Wilson disease gene (ATP7B): population frequencies, genotype-phenotype correlation, and functional analyses. *Am J Hum Genet* 1997; **61**: 317-328 [PMID: 9311736 DOI: 10.1086/514864]

P- Reviewer: Gumerova A, Samy Kohla MA **S- Editor**: Qi Y
L- Editor: Filipodia **E- Editor**: Zhang FF

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

ISSN 1007-9327