The His1069Gln Mutation in the ATP7B Gene in Romanian Patients with Wilson’s Disease Referred to a Tertiary Gastroenterology Center

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Abstract

Background & Aim. Wilson’s disease (WD) is a rare autosomal recessive disease. More than 500 mutations have been described so far, out of which 29 in exon 14. H1069Q mutation in the exon 14 of ATP7B gene is the most frequently encountered in Europe. The aim of the present study was to evaluate the incidence of mutations occurring in exon 14 of ATP7B gene in Romanian patients referred to a tertiary gastroenterology center, with known or suspected WD and in asymptomatic first degree relatives of index cases.

Methods: 93 patients were included in the study. Exon 14 of ATP7B gene has been amplified by PCR from genomic DNA and mutations identified by sequencing. Results: Only H1069Q missense mutation was detected in our study group. In patients with an established diagnosis of WD (38 cases), 34.2% were heterozygous for H1069Q and 21.1% were homozygous, with an allelic frequency of 38.1%. In paediatric WD patients (12 cases) 25% were heterozygous and 16.7% were homozygous (not significant versus adult population). Among asymptomatic first degree relatives of patients with WD (12 siblings, 25 parents) there were 40.5% cases heterozygous for H1069Q. In patients with suspected WD (17 cases), only 5.9% were heterozygous and no homozygous patient was identified. In our study group, H1069Q screening alone could not raise the Leipzig score to confirm diagnosis in patients with suspected WD or in asymptomatic first degree relatives. Conclusion: H1069Q mutation is highly prevalent in Romanian WD patients and first degree relatives, similar to other central and continental western European populations.

Key words

Wilson’s disease – genetic testing – molecular analysis – H1069Q.

Introduction

Wilson’s disease is an autosomal recessive disorder of copper metabolism with a prevalence of 1:30,000-1:100,000 in general population. It leads to abnormal copper accumulation in various organs, having a heterogeneous clinical presentation, usually with hepatic and/or neurologic manifestation [1, 2]. Fulminant hepatic failure requiring liver transplantation as the first manifestation of the disease is encountered as high as 20-30% in some referral centers [3]. The clinical heterogeneity of WD is paralleled by significant genetic heterogeneity and, since the cloning of WD gene - ATP7B - in 1993 [4], there have been over 500 mutations identified [5]. One of the most comprehensive WD mutation database - University of Alberta Wilson’s Disease Mutation Database - is accessible online [6]. According to this database, there are currently 29 disease-causing mutations cited in exon 14 of ATP7B gene – 23 substitutions and 6 deletions [6]. H1069Q mutation in the exon 14 of ATP7B gene is the most frequently encountered mutation in Europe, but with a large variation in prevalence according to the geographic area and ethnical background [7-11]. These missense mutations lead to a substitution of the aminoacid at position 1069 (histidine to glutamine) leading to a temperature sensitive defect in protein folding followed by degradation or an impaired ATP binding in the SEHPL domain [12].

Genetic testing is an important element of early WD diagnosis at least in some categories of patients: when clinical finding are not sufficient to support a definite WD diagnosis, for familial screening, for differential diagnosis of fulminant hepatitis [13]. Especially in siblings of known WD cases, genetic testing can sometimes establish an early diagnosis of WD, even in the presymptomatic period of the disease [14]. However, ATP7B is a large gene, with 21 exons, spanning over 80kb of genomic sequence and producing a
mRNA of 7.5 kb. Full sequencing is difficult to conduct in routine clinical practice; thus different study groups have focused on investigating genetic features of WD in specific populations, in order to identify common mutations, facilitating molecular diagnosis in the clinical setting.

The aim of our study was to investigate the mutations present in exon 14 of ATP7B gene in Romanian WD patients addressed to a tertiary Gastroenterology Unit, in their asymptomatic first degree relatives and in patients with suspected WD.

Materials and Methods

Study population

Between 1st January 2009 and 1st December 2011, 93 patients were included in the study. Thirty eight patients had an established diagnosis of WD according to the Leipzig score [15], 12 of them were paediatric cases. Patients were consecutively included in the study, upon presentation at our Gastroenterology Department. In 17 cases, the diagnosis of WD was suspected (possible), according to the Leipzig score and 37 patients were asymptomatic first degree relatives of patients with an established diagnosis of WD (12 siblings and 25 parents). Six of the first degree relatives had already an established diagnosis of WD based on clinical data and were included in this study group accordingly. Phenotypic classification of patients with established WD was considered according to the recommendations of the final report of the Proceedings of the Working Party at the 8th International Meeting on Wilson Disease and Menkes Disease [15]: H1 - acute hepatic WD, H2 - chronic hepatic WD, N1 - neurologic and hepatic presentation, N2 - neurologic presentation without symptomatic liver disease, O - other presentations. The study was approved by the Hospital Ethics Committee and all patients provided informed consent for participation in the study.

DNA isolation and purification

Eight ml whole blood was collected from all patients foruffy coat preparation. Genomic DNA was isolated from 200 μl buffy coat and purified using QIAamp DNA blood kit (Qiagen) according to manufacturer’s instructions.

Exon 14 amplification and sequencing

The DNA sequence corresponding to exon 14 of ATP7B gene was amplified by PCR from genomic DNA using Phusion (R) High Fidelity DNA Polymerase (Phusion High Fidelity PCR Master mix with HF Buffer, ThermoScientific) and sequence specific primers (Table I). The following PCR cycling conditions were used, for 50 μl PCR reactions, according to manufacturer’s instructions: initial denaturation 98°C for 30 sec, followed by 35 cycles comprised of 98°C denaturation for 10 sec, 62 °C annealing for 20 sec and 72°C extension for 30 sec - and a final extension step at 72°C for 10 min.

The presence of an amplicon was documented by gel electrophoresis using 1.5% Tris-acetate-EDTA agarose gel. PCR products were purified using QIAquick PCR purification kit (Qiagen) according to the manufacturer’s instructions. Purified PCR products were sequenced by Seqlab - Sequence Laboratories Göttingen GmbH, Germany (capillary electrophoresis, 3730 ABI automatic sequencer) using forward and reverse primers (Table II). The polymorphic state interpretation was conducted by editing each DNA sequence using ChromasPro v1.5 (Technelysium Pty Ltd) and aligning to the published human genomic database using BLAST function - Basic Local Alignment Search Tool - from Pubmed.

Statistical analysis

Categorical variables were compared using the chi-square test. A p value < 0.05 was considered for statistical significance. Data was analysed using the NCSS-PASS 2000 Software package.

Results

The characteristics of patients with established WD are depicted in Table II.

Table I. Primers used for PCR amplification and sequencing of exon 14 of ATP7B gene

<table>
<thead>
<tr>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR amplification of exon 14 of ATP7B gene from genomic DNA</td>
<td>5-TGCCTGTGACACTGAACTCC-3</td>
</tr>
<tr>
<td>Sequencing of Exon 14 of ATP7B gene</td>
<td>5-TAACCTGGAACTGTGGCAAG-3</td>
</tr>
</tbody>
</table>
One hundred and eighty six human chromosomes were analysed, out of which 76 patients were evidenced with an established WD diagnosis. In our study population, out of 29 disease-causing mutations cited in exon 14 of ATP7B gene [6], only H1069Q polymorphism was identified (Fig 1).

In patients with an established diagnosis of WD, H1069Q polymorphism was detected in 21 patients (55.2%): 8 patients (21.1%) were homozygous carriers and 13 patients (34.2%) were heterozygous carriers of the mutation, with an allelic frequency 38.1% (29 of the 76 analysed chromosomes). In the paediatric population (16 cases), 12 patients (75%) had an established diagnosis of WD. The frequency of H1069Q polymorphism in the paediatric WD population was 37.5%: 2 patients (12.5%) were homozygous carriers and 4 patients (25%) were heterozygous carriers of the mutation. When comparing paediatric with adult populations, the distribution of H1069Q polymorphism was not statistically significantly different (p=0.7).

The phenotypic classification of patients with WD in our study group was: 7.9% H1, 73.7% H2, 15.8% N1 and 2.6% N2. The distribution of H1069Q polymorphism according to the phenotypic classification is depicted in Table III and did not differ statistically significant (p=0.24).

In asymptomatic first degree relatives of patients with an established WD, only heterozygous carriers of the H1069Q mutation have been detected – 15 patients (40.5%). H1069Q mutation was identified only in one patient with suspected WD (heterozygote) (5.9%).

The distribution of H1069Q polymorphism according to the Leipzig score calculated before genetic testing is presented in Table IV. In our study group, there were 8 patients which had a Leipzig score of 2 points and 5 patients which had a Leipzig score of 3 points (in this setting). The detection of H1069Q mutation alone could not further raise the Leipzig score to the level of established WD – 4 points (see Table IV).

### Table III. Distribution of H1069Q polymorphism according to phenotypic classification of WD

<table>
<thead>
<tr>
<th>WD Phenotype</th>
<th>H1069Q</th>
<th>H1069Q</th>
<th>H1069Q</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>heterozygote</td>
<td>0</td>
<td>20 (69%)</td>
</tr>
<tr>
<td>H1</td>
<td>3 (100%)</td>
<td>0</td>
<td>20 (69%)</td>
</tr>
<tr>
<td>H2</td>
<td>8 (28.6%)</td>
<td>6 (21.4%)</td>
<td>14 (50%)</td>
</tr>
<tr>
<td>N1</td>
<td>2 (33.3%)</td>
<td>2 (33.3%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>N2</td>
<td>0</td>
<td>0</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>13 (34.2%)</td>
<td>8 (21.1%)</td>
<td>17</td>
</tr>
</tbody>
</table>

### Table IV. Distribution of H1069Q polymorphism according to Leipzig score calculated before genetic testing

<table>
<thead>
<tr>
<th>Leipzig score</th>
<th>H1069Q</th>
<th>H1069Q</th>
<th>H1069Q</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9 (31.0%)</td>
<td>0</td>
<td>20 (69%)</td>
<td>29 (100%)</td>
</tr>
<tr>
<td>1</td>
<td>6 (46.2%)</td>
<td>0</td>
<td>7 (53.8%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>2</td>
<td>1 (12.5%)</td>
<td>0</td>
<td>7 (87.5%)</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>≥ 4</td>
<td>13 (34.2%)</td>
<td>8 (20.5%)</td>
<td>17 (44.7%)</td>
<td>38 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>29 (31.2%)</td>
<td>8 (8.6%)</td>
<td>56 (60.2%)</td>
<td>93 (100%)</td>
</tr>
</tbody>
</table>

### Discussion

H1069Q is considered the most prevalent mutation in Central and Eastern Europe, some authors even suggesting that it could have arisen originally from Eastern Europe [16]. A gradient has been described for H1069Q allele frequency declining from northeast to southwest [16,17].

A high prevalence was reported for a group of 85 Polish WD patients, where the allele frequency reached 73% - 53% homozygous carriers and 41% heterozygous [7] but also in Lithuania, where even 92.3% of WD patients with hepatic presentation of the disease were homozygous or compound heterozygotes for the H1069Q mutation [8]. A high prevalence of H1069Q mutation was documented also in former East Germany: 39% homozygous patients and 48% heterozygous with an allelic frequency of 63% [18].

In a slavonic population from the European part of Russia the allelic frequency of H1069Q polymorphism was 48.7% with 32.5% homozygote carriers and 32.5% heterozygotes [19]. Similar allelic frequencies have been documented for German (42%), Austrian (43.9%) and Hungarian patients (42.8%) [7, 20, 21]. On the other hand, in the British population, H1069Q mutation was detected only in 12/42 patients (2 homozygotes) with an allelic frequency of 17% [11]. The same allelic frequency (17.5%) was detected...
also in the continental Italian population [22]. In our study group, in patients with WD, H1069Q polymorphism was detected with a frequency of 55.2%, 8 patients (21.1%) were homozygous carriers and 13 patients (34.2%) were heterozygous carriers of the mutation, with an allelic frequency 38.1%. To our knowledge this is the first study to document H1069Q polymorphism frequency in Romanian patients. The allelic frequency is thus closer to central and western European populations rather than north eastern European populations and, despite our common ancestry with the Italian population, the allelic frequency is much higher than in the Mediterranean basin.

Although the frequency of H1069Q mutation was high (55.2%), homozygotes were detected only in patients with already an established diagnosis of WD. In patients with suspected WD or in asymptomatic first degree relatives of patients with established WD, there were only heterozygous carriers of the mutation and determination of H1069Q alone did not further contribute to diagnosis, adding just one point to the Leipzig score. Screening for other WD mutations common in Central and Eastern Europe, located on exon 8 (2299insC, G710S), exon 15 (3400delC) and exon 13 (R969Q) might be recommended also in Romanian patients, as previously suggested [17]. Together with H1069Q, the detection of other mutations could raise the Leipzig score to the required 4 points to confirm the diagnosis even in asymptomatic patients, identifying homozygous or compound heterozygous carriers.

Genotype-phenotype associations have been described for H1069Q polymorphism in European populations. In homozygotes, symptoms started earlier than in compound heterozygotes or H1069Q negatives; there was an increased frequency of neurologic symptoms and higher frequency of Keiser Fleischer rings [18]. In the Hungarian population homozygous H1069Q carriers were older at onset and had neuropsychiatric symptoms more frequently than compound heterozygous or patients with other mutations [16]. In our study we did not find any significant association of the H1069Q polymorphic state with WD phenotype or with age at diagnosis. This might be explained by a referral bias, resulting from a relatively low number of patients with neuropsychiatric symptoms among patients who are referred for WD to a tertiary gastroenterology center (18.4% in our study group). In a recent study conducted in 117 Czech WD patients, the frequency of H1069Q variant did not significantly differ between patients with either the hepatic or the neurological presentation, also [23].

Conclusion

H1069Q mutation is highly prevalent in Romanian WD patients addressed to a tertiary gastroenterology referral center and in their first degree relatives, similar to other central and Western European populations. This mutation should be included in the panel used for molecular testing in Romanian WD patients.

Conflicts of interest

None to declare.

Acknowledgement

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References


