



RAPID COMMUNICATION

High frequency of the c.3207C>A (p.H1069Q) mutation in *ATP7B* gene of Lithuanian patients with hepatic presentation of Wilson's disease

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whole venous blood using a salt precipitation method. Firstly, the semi-nested polymerase chain reaction (PCR) technique was used to detect the c.3207C>A (p.H1069Q) mutation. Patients not homozygous for the c.3207C>A (p.H1069Q) mutation were further analyzed. The 21 exons of the WD gene were amplified in a thermal cycler (Biometra T3 Thermocycler, Göttingen, Germany). Direct sequencing of the amplified PCR products was performed by cycle sequencing using fluorescent dye terminators in an automatic sequencer (Applied Biosystems, Darmstadt, Germany).

RESULTS: Total of 13 WD patients (mean age 26.4 years; range 17-40; male/female 3/10) presented with hepatic disorders and 16 their first degree relatives (including 12 siblings) were studied. Some of WD patients, in addition to hepatic symptoms, have had extrahepatic disorders (hemolytic anemia 3; Fanconi syndrome 1; neuropsychiatric and behavioural disorder 2). Liver biopsy specimens were available in all of 13 WD patients (8 had cirrhosis; 1-chronic hepatitis; 3-acute liver failure, 1-liver steatosis). Twelve of 13 (92.3%) WD patients had the c.3207C>A (p.H1069Q) mutation, 6 of them in both chromosomes, 6 were presented as compound heterozygotes with additional c.3472-82delGGTTTAACCAT, c.3402delC, c.3121C>T (p.R1041W) or unknown mutations. For one patient with liver cirrhosis and psychiatric disorder (Leipzig score 6), no mutations were found. Out of 16 first degree WD relatives, 11 (68.7%) were heterozygous for the c.3207C>A (p.H1069Q) mutation. Two patients with fulminant WD died from acute liver failure and 11 are in full remission under penicillamine or zinc acetate treatment. Three women with WD successfully delivered healthy babies.

CONCLUSION: The c.3207C>A (p.H1069Q) missense mutation is the most characteristic mutation for Lithuanian patients with WD. Even 92.3% of WD patients with hepatic presentation of the disease are homozygous or compound heterozygotes for the p.H1069Q mutation.

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Key words: Wilson disease; *ATP7B* gene; c.3207C>A (p.H1069Q) mutation; Cirrhosis; Urine copper; Copper in liver biopsies

Abstract

AIM: To investigate the prevalence of the *ATP7B* gene mutation in patients with hepatic presentation of Wilson's disease (WD) in Lithuania.

METHODS: Eleven unrelated Lithuanian families, including 13 WD patients were tested. Clinically WD diagnosis was established in accordance to the Leipzig scoring system. Genomic DNA was extracted from

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INTRODUCTION

Wilson's disease (WD) is a rare autosomal recessive disorder of copper metabolism with prevalence of 1/30 000 and a carrier frequency of 1/90^[1,2]. The prevalence is as high as one in 10 000 in China, Japan, and Sardinia^[3,4]. The disease is characterized by hepatic, neurological or psychiatric disturbances and decreasing biliary copper excretion with consequent copper accumulation in the liver and extrahepatic tissue^[5]. The gene currently known to be associated with WD is the *ATP7B* gene, which was identified in 1993^[6,7]. The *ATP7B* gene has 21 exons and encodes a copper-transporting *P*-type adenosine triphosphatase. A copper-transporting *P*-type ATPase incorporates copper into the serum ferroxidase, ceruloplasmin, and excretes by biliary tree. More than 360 mutations in the *ATP7B* gene have been identified as summarised in the human gene mutation database of the institute of medical genetics in Cardiff (accession date May 2008; <http://www.hgmd.cf.ac.uk/ac/gene.php?gene=ATP7B>). *ATP7B* gene mutations are rare and the majority of patients are compound heterozygotes. Consequently, identification of mutations in WD patients often requires sequencing of a significant proportion of the *ATP7B* gene. It is important to know regional distribution of mutations of the *ATP7B* gene to design appropriate diagnostic strategies for the disease^[8]. As no data exist about WD genetic characteristics in the Lithuanian population, the purpose of the current study was to carry out a mutation analysis of Lithuanian WD patients.

MATERIALS AND METHODS

Patients

Lithuanian cohort, drawn from 11 unrelated families, consisted of 29 individuals and was comprised of 13 WD patients (mean age at onset of symptoms 26.4 years; range 17-40; male/female 3/10) and 16 of their first degree relatives. Patients were admitted at the Department of Gastroenterology, Hospital of Kaunas University of Medicine, Lithuania. The diagnosis of WD was established using the scoring system developed at the 8th International Meeting on WD, Leipzig/Germany, 2001. The diagnosis was accepted if the WD Leipzig score was ≥ 4 ^[9].

ATP7B gene mutation analysis

Genomic DNA was extracted from whole venous blood using a salt precipitation method^[10]. Firstly, rapid, semi-nested PCR technique was used to detect the c.3207C>A (p.H1069Q) mutation as described previously^[11]. Patients not homozygous for c.3207C>A (p.H1069Q) mutation were further analyzed. The 21 exons of the WD gene were amplified in a thermal cycler (Biometra T3 Thermocycler, Göttingen, Germany) as described elsewhere^[12]. Direct sequencing of the amplified polymerase chain reaction (PCR) products was performed by cycle sequencing using fluorescent dye terminators in an automatic sequencer (Applied Biosystems, Darmstadt, Germany). Mutations were quoted according to the guidelines from <http://www.HGVS.org/mutnomen/>, using the reference sequence with the GenBank accession number NM_00053.1.

RESULTS

Total of 13 WD patients presented with hepatic disorders and 16 their first degree relatives (including 12 siblings) were studied. All WD patients and their relatives were consulted in Department of Gastroenterology of Kaunas University of Medicine during 1999-2008. WD patient's clinical, laboratory data, and mutation type are summarized in Table 1.

Some of WD patients, in addition to hepatic symptoms, have had extrahepatic disorders (hemolytic anemia 3; Fanconi syndrome 1; neuropsychiatric and behavioural disorder 2). Specimens of liver biopsy (either percutaneous or transjugular) were available in all of 13 WD patients (8 case of cirrhosis; chronic hepatitis-1; fulminant hepatitis-3; steatosis-1). Kayser-Fleischer ring was present in 6 out of 13 patients (46%). Hepatic copper content was measured in 7 WD patients and was above 250 $\mu\text{g/g}$ dry weight in 4 cases.

Twelve of 13 (92.3%) WD patients had the c.3207C>A (p.H1069Q) mutation, 6 of them in both chromosomes, and 6 were presented as compound heterozygotes with additional c.3472-82delGGTTTAACCAT, c.3402delC, c.3121C>T (p.R1041W) or unknown mutation. For 1 patient with liver cirrhosis only mutation (p.H1069Q) in one allele was revealed, and for another patient with cirrhosis and psychiatric disorder, (Leipzig score 6) no mutations were found (Table 1). Out of 16 first degree WD relatives, 11 (68.7%) were heterozygous for the c.3207C>A (p.H1069Q) mutation. Two patients with fulminant WD died from acute liver failure (ALF), and 11 are in full remission under penicillamine or zinc acetate treatment. Three women with WD successfully delivered healthy baby.

DISCUSSION

WD might be a potentially life threatening disorder; however, with early diagnosis and consequent treatment the prognosis of WD is excellent and usually the need for liver transplantation can be prevented^[13]. None of

Table 1 Clinical and genetic characteristics of Lithuanian WD patients

Patient	Gender	Age (yr) at disease diagnosis	Clinical presentation	Kayser- Fleischer ring	Ceruloplasmin lower than 0.2 g/L	Urine cooper (μg/24 h)	Cooper in liver biopsy (μg/g)	Mutation	Outcome
1	F	27	Cirrhosis	Yes	Yes	120	264	[p.His1069Gln] + [p.His1069Gln]	Alive
2	F	18	ALF	No	Yes	144	ND	[p.His1069Gln] + [p.His1069Gln]	Alive
3	M	35	Cirrhosis	Yes	Yes	84	ND	[p.His1069Gln] + [p.His1069Gln]	Alive
4	F	34	ALF, HA	No	No	280	536	[p.His1069Gln] + [p.Arg1041Trp]	Death
5	F	26	Asymptomatic CH	No	Yes	38	35	[p.His1069Gln] + [p.Arg1041Trp]	Alive
6	F	19	ALF, HA	No	No	324	326	[p.His1069Gln] + [c.3472-82 del- GGTTTAACCAT]	Death
7	F	17	Cirrhosis	No	Yes	120	126	[p.His1069Gln] + [c.3472-82 del- GGTTTAACCAT]	Alive
8	F	18	Cirrhosis, FS, HA	No	Yes	182	258	[p.His1069Gln] + [c.3402delC]	Alive
9	M	24	Cirrhosis, NPD	Yes	Yes	116	188	NK/NK	Alive
10	F	23	Cirrhosis	Yes	Yes	130	ND	[p.His1069Gln] + [p.His1069Gln]	Alive
11	F	27	Cirrhosis	Yes	Yes	145	ND	[p.His1069Gln] + [p.His1069Gln]	Alive
12	M	40	Liver steatosis, NPD	Yes	Yes	350	ND	[p.His1069Gln] + [p.His1069Gln]	Alive
13	F	36	Cirrhosis	No	Yes	126	ND	[p.His1069Gln] + NK	Alive

Mutational analysis was performed only for presence of p.H1069Q. 4th and 5th patients were siblings; 6th and 7th patients were siblings. ALF: Acute liver failure; CH: Chronic hepatitis; HA: Hemolytic anaemia (Coombs-negative); FS: Fanconi syndrome; NPD: Neuropsychiatric disorder; ND: Not determined; NK: Not known.

the commonly used parameters alone allows a certain diagnosis of WD^[14]. In our study, only in 6 (46%) of our patients was Kayser-Fleischer ring present and serum ceruloplasmin levels were even higher than 0.3 g/L in 2 patients with ALF. Urine cooper excretion exceeded 100 μg/24 h in 11 (84.6%) of 13 patients and in one asymptomatic sibling with WD it was even lower than 40 μg/24 h. Therefore, urinary cooper excretion and hepatic copper concentration, because of potential errors in evaluation, should always be assessed in the context of other diagnostic criteria^[9,15].

WD is found worldwide with different geographical distribution of *ATP7B* gene mutations. The c.3207C>A (p.His1069Gln) missense mutation is the most frequent in Northern, Eastern and Central Europe^[2,8,16], the c.3400delC mutation is the most common in Brazil^[17], and c.2333G>T (p.Arg778Leu) in Asia countries^[18-21].

Knowledge of the regional distribution of mutations in the WD gene is important to design appropriate screening strategies^[8]. In Central and Eastern Europe, the frequency of the c.3207C>A (p.H1069Q) mutation in exon 14 previously has been reported as the highest in Poland and Eastern Germany and decreases in Western and Southern European countries^[8,22]. In Poland 72% of WD patients carry at least one allele with the c.3207C>A (p.H1069Q) mutation^[23]. Caca *et al*^[2] revealed that in East Germany 39% of WD patients were homozygous and 48% heterozygous for the c.3207C>A (p.H1069Q) mutation. Our study was the first to analyze genetic mutations of WD patients in Lithuania, a country in Baltic Sea area with 3.4 million inhabitants. The prevalence of the c.3207C>A (p.H1069Q) mutation in the *ATP7B* gene in WD patients in Lithuania was 92.3% (with 46.2% homozygotes and 46.2% compound heterozygotes). These findings were even higher than in Poland, a neighbour state, and were considerable higher

than in Russia (49%) or Sweden (38%)^[24]. In compound heterozygotes Lithuanian WD patients additional c.3472-82delGGTTTAACCAT, c.3402delC, and never mentioned new c.3121C>T (p.R1041W) mutations were detected. Only patients with hepatic or hepatic-neuropsychiatric presentation of WD were included in our study; however, other authors^[25] have shown that the c.3207C>A (p.H1069Q) mutation is also associated with a late and neurological presentation of the disease.

In conclusion, the results of our study showed that the c.3207C>A (p.H1069Q) missense mutation is characteristic for Lithuania patients of WD. Even 92.3% of WD patients with hepatic presentation of the disease are homozygous or compound heterozygote for this mutation. Therefore, limited genetic testing of c.3207C>A (p.H1069Q) mutation might be of high value both to confirm WD disease diagnosis and for familial genetic screening in Lithuanian population.

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COMMENTS

Background

Wilson disease (WD) is a life threatening autosomal recessive genetic disorder with impaired cooper metabolism and associated with hepatic, neurological and psychiatric symptoms. The diagnosis of WD is usually established using clinical signs and biochemical (hepatic copper concentration, ceruloplasmin concentration, copper urine excretion) tests; however, the most sensitive and

specific test is the detection of *ATP7B* gene mutations. The genetic testing is becoming the most important for confirmation of diagnosis and presymptomatic diagnostics in WD patients.

Research frontiers

The research was done to obtain data about the frequency and type of *ATP7B* gene mutations in WD patients in Lithuania.

Innovations and breakthroughs

It is important to know regional distribution of mutations of the *ATP7B* gene to design appropriate diagnostic strategies of WD. We have found an extremely high percentage of Lithuanian WD patients having the c.3207C>A (p.H1069Q) mutation. This is a new and interesting finding, showing that this mutation is typical for Lithuania WD patients, similar as was reported in some other Middle-Eastern European countries.

Applications

The present study indicates that due to high frequency of the c.3207C>A (p.H1069Q) mutation in Lithuania, even limited genetic testing of this mutation might be of high value both to confirm WD diagnosis and for familial genetic screening in Lithuanian population.

Terminology

ATP7B encodes a Cu-transporting P-type ATPase protein common to P-type ATPases. p.H1069Q missense mutations causes an amino acid substitution and disrupts ATP binding.

Peer review

This short report investigates the frequency and type of *ATP7B* gene mutations in Lithuanian patients with WD. The detected frequency (92.3%) of the c.3207C>A (p.H1069Q) mutation is one of the highest reported from Middle-Eastern European countries. This study is interesting.

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