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Novel Mutations of ATP7B Gene in Iranian Patients with Wilson's Disease

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Abstract

Background: Wilson's disease is a rare autosomal recessive disorder characterized by toxic accumulation of copper in liver and brain. The disorder is caused by mutations in the ATP7B gene, encoding a copper transporting P-type ATPase. Characterization of the spectrum of mutations in this gene is important both for diagnosis and genetic counseling of the families.

Materials and Methods: We enrolled 30 definitely diagnosed patients (ages ranging from 3 to 33). Genomic DNA was extracted from peripheral blood samples. All the exons of the gene were amplified by polymerase chain reaction using specified primers for each exon. The amplification products were then analyzed by direct automated sequencing.

Results: 87% of our patients had liver problems while 47% of suffered from neurological problems. In this study we will report the spectrum of mutation found among Iranian families, which are mainly different from other reports.

Conclusion: By performing the present study, some new mutations in ATP7B gene, Del C 3696(1232) and S1369L were identified for the first time in Wilson's disease patients.

Keywords: Wilson's disease; ATP7B; Mutation detection; Iran

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Introduction

Wilson's disease (WD) is a rare genetic disorder of copper metabolism with a worldwide incidence of 30 cases per million, a gene frequency of 0.56% and a carrier frequency of 1 in 90 (1). The disorder is caused by mutations in the ATP7B gene, which is located on chromosome 13q14.3 (2).

ATP7B gene regulates copper transportation into bile from hepatocytes. Copper incorporates into apoceruloplasmin to form ceruloplasmin (major copper carrier in blood). ATP7B dysfunction cause toxic accumulation of copper in liver and brain (3) that resulted in neurological and hepatic manifestations. Therefore, ATP7B dysfunction forms unstable apoceruloplasmin that is rapidly degraded (4) and the level of ceruloplasmin will be decreased. WD manifestations include bradykinesia, tremor, chorea, dystonia, rigidity and especially Kayser-Fleischer ring (KF ring) in eye; in addition other neurological presentations are myoclonic epilepsy, cerebellar ataxia, cognitive deterioration and proximal muscle weakness (5). Also WD has heterogenous neurological manifestation and is similar to neurological disorders that resulted in delay diagnosis (6).

Diagnosis of the disease is based on clinical findings, laboratory tests and good response to medication, but none of these findings are pathogonomic.

Characteristics of the Wilson's disease gene (OMIM 277900) which codes for a copper transporting P-type ATPase (ATP7B) has resulted in a major breakthrough for understanding the pathophysiology of WD, but the role of genetic testing in the clinical management of the patients is not yet established and for clinical purposes, the use of mutation analysis is limited by the occurrence of more than 200 mutations related to WD worldwide (2, 7). ATP7B mutations are associated with lower ceruloplasmin oxidase levels and earlier age of onset WD disease, serum ceruloplasmin can help to predict ATP7B mutations and facilitate the mutations analysis (8). Screening of patient's first degree relatives is valuable in early

diagnosis and management and avoidance of irreversible complications (9). Although WD has monogenic inheritance but sometimes genetic prevalence and the number of clinical diagnosis are different, but due to reduced penetrance of ATP7B mutation and difficulty in diagnosis, some patients are missed (10).

As regards to high prevalence of WD in Iran because of consanguinity (11), in this study we will report on the mutational spectrum of ATP7B gene among Iranian populations with WD.

Materials and Methods

Between 200-2001, 33 patients with a diagnosis of WD were enrolled. Patients were found either based on review of previous registries or by referral from other colleagues. These patients were selected according to routine diagnostic criteria for WD (Table1) and those patients who had suspicious laboratory results underwent another laboratory tests.

Table 1. Diagnostic criteria for our patients to enter the study.

1	↓ Serum Cereloplasmin
2	↓ Serum Copper
3	↑ 24 hour Urinary Copper Excretion > 100 µg/24h
4	Liver biopsy: ↑ hepatic copper content

Also patients were fully examined and referred to an ophthalmologist. We performed blood sampling of 10 cc on each of our patients and after centrifuge, serum component was sent to DNA extraction center, where two specialists performed the genomic DNA extraction procedure. Genomic DNAs were extracted in 24-36 hours.

And because at that time, assessment of mutations in ATP7B gene was only done in some limited centers through the world, our samples were sent to Charite Campus Mitte Center in Berlin, Germany (fifteen samples for the first time and the other eighteen samples for the second time) and final results were reported to us after 5 months.

Table 2. Prevalence of liver and neurological problems according to patients' sex.

Problem	Male	Female
Liver problems	13	13
Neurological problems	8	6

Results

During these procedures, 3 patients were excluded from the study because of poor DNA sampling; so our final cases were 30 patients with an equal sex distribution and a mean age of 16 years (ranging 3 to 33 years). Liver problems including icter, hepatomegaly, ascites, acute hepatic failure and encephalopathy was reported in 26 (87%) patients. This prevalence was equal in both sexes with 13 female and 13 male patients. Also 47% of our patients (14 patients including 6 females and 8 males) had neurological problems like dystonia, muscular spasm, rigidity, mood disorders and mental retardation (Table.2).

Kayser-Fleischer ring was detected in 21 (70%) patients. 43% of the patients had a positive family history that was always their brothers and sisters in addition to two separate cases in cousins and one case in an uncle and none of the parents had the disease. All of our patients except one of them were single.

Table 3. Identified mutations in ATP7B gene of Iranian patients.

Patient	Sex	Age	Mutation
1	Female	21	D642 H / -
2	Female	14	N 1270 S / N 1270 S
3	Male	16	- / -
4	Female	12	R 778 W / -
5	Female	11	- / -
6	Male	17	R 778 G / -
7	Female	17	(1232)3696 Del C / (1232)3696 Del C
8	Male	26	N 1270 S / D 642 H
9	Female	18	R 148 W / R 148 W
10	Male	14	Q 1142 X / R 919 W
11	Male	30	-75A ->C / -75A ->C (IVS12 -2A/C) 12
	Female	9	D 642 H / D 642 H
13	Female	36	S 1369 L/(546)1638 DelC(-75A -> C)
14	Female	18	- / -
15	Male	18	N 1270 S / N 1270 S
16	Female	10	R 969 Q / G 591 D
17	Male	10	Q 1142 Amb / Q 1142 Amb
18	Male	15	845 Del T / 845 Del T
19	Female	20	Q 680 X (Amb) / Q 680 X (Amb)
20	Female	19	Q 1142 Amb / Q 1142 Amb
21	Male	13	L 939 Amb / L 939 Amb
22	Female	3	3649-3645 Del gttctg /3649-3645 Del gtt
23	Male	10	- / -
24	Male	8	L 1299 R / L 1299 R
25	Male	8	N 1270 S / -
26	Male	8	H 1069 Q / H 1069 Q
27	Female	16	- / -
28	Male	15	G 1089 E / G 1089 E
29	Male	9	I 857 T / I 857 T
30	Female	33	D642 H / D642 H

Moreover, low ceruloplasmin level in 26 (87%) patients, low serum copper level in 23 (76%) patients and increased copper in 24 hour urine in 28 (93%) patients were detected. Assessment of copper in dry

weight liver was only performed on five cases that were 5-40 times of normal range.
Mutations detected in ATP7B gene of our patients had a wide range of variety (Table 3) and they were completely different from the mutations found in European patients. In this study we found some new mutations such as Del C 3696(1232) and S1369L that were not reported up to the time we did our study.

Discussion

Wilson's disease is a rare autosomal recessive disorder. Wilson's disease usually presents with inflammation and chronic liver failure, brain basal ganglia degeneration and Kayser-Fleischer ring in cornea (2,7) The symptoms are due to impaired bile exertion and copper deposition in organs like liver, central nervous system, kidney and cornea. These abnormalities result from mutations in ATP7B, which is located on chromosome 13. ATP7B produces an adenosine three phosphatase (ATPase) that consists of 1443 amino acids (2-9).This membrane protein has 6 binding sites for copper and several sites for phosphorylation and ATPase (2,12). There is no gold standard for diagnosis of Wilson's disease. Diagnosis requires a combination of clinical and biochemical tests. None of these parameters alone allows a certain diagnosis of WD.
Although DNA sequencing made breakthrough in identifying pathophysiology of the disease, this strategy is limited since currently over 320 mutations and 80 polymorphisms have been identified. But in certain populations, a high prevalence of particular mutations allows rapid screening and diagnosis of the disease and in this field many studies were conducted through the world.
Importance of diagnosis is for its role in prevention of morbidity and mortality, early diagnosis result in early treatment. Some variants are associated with ATP7B dysfunction and cause its mislocalization so reduce ATP7B stability; it shows genotype and phenotype correlation and mechanism of disease pathogenesis (13). Two microsatellites, D13S301 and D13S314, are introduced for analysis and detection carriers and affected patient without symptoms (14).
In 2006 Margarit et al, (15), identified ATP7B alterations in 60 Spanish patients with WD from 40 different families, by PCR amplification, single-strand conformation polymorphism (SSCP) analysis and sequencing and 21 different ATP7B gene mutations were identified, eight of which were novel.
WU ZY et al, in 2006 (16), demonstrated that there is no correlation between MURR1 gene (that was thought, it may influence human copper metabolism and the clinical presentations of the disease) and WD in their 218 unrelated Chinese patients.
Zali et al clarified a biomarker, H1069Q, as the most frequent mutation in north and central region of Iran which is useful for rapid detection assay and early diagnosis of WD patients (17). Galehdari and Tangestani found a homozygous pathogenic misssense mutation at codon 778 (R778W) in Iranian patients that previously reported in American and European population (11). Dastsooz et al studied mutations in exon 8 and 14 of ATP7B gene and identified a novel mutation, c.2335T>G, in Iranian population, this mutation demonstrates severe neuropsychiatric condition in patients (18).
In another study by Wan et al in 2006, ten different mutations were identified among 29 Taiwanese WD patients; four of them were novel (Ala1168Pro, Thr1178Ala, Ala1193Pro, and Pro1273Gln) (19), while in India, three mutations, Q1256R, A1003T and I1102T, were characterized in WD patients, using SSCP and DNA sequencing (20).
In our experience; although, because of some financial concerns, we did not entered many patients in our study, like other parts of the world, spectrum of mutations had a large variety; and the most prevalent of them, N1270S/D642H, was only detected in 10% of our patients. And prevalent mutations like H1069Q in European WD patients or R778L in WD patients from the Far East were rare in our patients. Nonetheless Zali et al observed H1069Q mutation which is located in exon 14 in Iranian patients with hepatic presentation, exon 14 is known as hot spot for its several mutations (21).
Moreover, (1232)3696 Del C and S1369L mutations were reported for the first time in WD patients. Interestingly, in 17 (57%) patients, the same mutation occurred in the two alleles, which is probably due to familial and local marriages. In assessment of probable correlation between types of mutations and hepatic or neurological presentations of the disease (genotype-phenotype correlation), we could not reach a result because of small number of our patients. It seems that more studies should be done on large number of patients and in different countries (16, 19).

Conclusion

By performing this study, for the first time we identified mutations in ATP7B gene of Wilson's disease in Iranian patients and some new mutations in ATP7B gene (Del C 3696(1232) and S1369L), were reported for the first time in WD patients.

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