Molecular and Cellular Probes 26 (2012) 147–150

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Probes

journal homepage: www.elsevier.com/locate/ymcpr

Mutation analysis of the ATP7B gene in a new group of Wilson's disease patients: Contribution to diagnosis

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ARTICLE INFO

Article history:
Received 10 February 2012
Received in revised form
5 March 2012
Accepted 14 March 2012
Available online 29 March 2012

Keywords:
Wilson's disease
ATP7B
Mutation
Missense
Diagnosis
Prevention

ABSTRACT

Wilson's disease (WD), an autosomal recessive disorder of copper transport with a broad range of genotypic and phenotypic characteristics, results from mutations in the ATP7B gene. Herein we report the results of mutation analysis of the ATP7B gene in a group of 118 Wilson disease families (236 chromosomes) prevalently of Italian origin. Using DNA sequencing we identified 83 disease-causing mutations. Eleven were novel, while twenty one already described mutations were identified in new populations in this study. In particular, mutation analysis of 13 families of Romanian origin showed a high prevalence of the p.H1069Q mutation (50%). Detection of new mutations in the ATP7B gene in new populations increases our capability of molecular analysis that is essential for early diagnosis and treatment of WD.

1. Introduction

Wilson disease (WD) is a hereditary disease due to mutations of the copper transporting P-type ATPase, ATP7B [1]. WD is associated with copper accumulation, resulting in liver damage and/or neurologic symptoms. The manifestations of liver disease vary from clinically asymptomatic with only biochemical abnormalities to acute liver failure [1]. Similarly, the spectrum of neurologic manifestations ranges from normal or mild disturbances to a rapid and severe progression of neurologic disability [1]. Biochemical parameters typically include a low level of ceruloplasmin, and increased urinary and hepatic copper. However, none of these parameters are specific to WND, and some may not be abnormal [1]. This high degree of variability makes diagnosis by clinical and biochemical features challenging. Furthermore, there may be overlap in results with those of heterozygotes. Prompt diagnosis is essential before tissue damage occurs, since treatment is reasonably simple and effective, using chelation or oral zinc.

The ATP7B gene, cloned in 1993, encodes a P-type ATPase, with six copper binding domains, eight putative transmembrane

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doi:10.1016/j.mcp.2012.03.007

domains, and typical features of P-type ATPases [2,3]. To date, more than 500 different mutations associated with WD are known (http://www.wilsondisease.med.ualberta.ca/database.asp). A large fraction of mutations are missense mutations producing a single amino acid change in ATP7B. In this paper we report the results of molecular analysis of a new group of WD families with the identification of 11 new WD-causing mutations and document their features.

2. Patients and methods

2.1. Patients

Mutation analysis was carried out in 118 WD families of various origin (56 Italian, 37 Sardinian, 13 Romanian, 2 Swiss), 1 family from each of the following countries: the Philippines, Morocco, Ecuador, Former Yugoslav Republic of Macedonia (FYROM), India, Albania, and the USA, but of Chinese origin, for a total of 138 patients. The large majority of patients were clinically characterized in pediatric departments. The diagnosis of WD was based on low ceruloplasmin and copper serum concentrations, increased urinary copper excretion, and high liver copper concentration. Two groups of controls (each of 50 subjects) originating from Italy, Sardinia, were also screened for all the novel missense mutations identified in these populations.

2.2. Methods

DNA was extracted from peripheral blood using salt extraction [4]. Mutation analysis strategy was different according to each analyzed population. Patients originating from Sardinia were first analyzed for the six most common mutations accounting for 85% of cases. The remaining patients and the Sardinian DNA samples not completely characterized with the first step of analysis were analyzed for 12 exons (5, 6, 8, 10, 12, 13, 14, 15, 16, 17, 18, 19), where most mutations reside, according to our previous studies [4] and the Wilson's disease HUGO database (http://www.wilsondisease.med.ualberta.ca/database.asp). DNA samples not completely characterized after the second step of analysis or those found to have a new missense mutation, were further analyzed for the remaining exons of the ATP7B.

Mutation detection was performed by DNA sequencing and analysis of the amplified 21 exons and the known 5′ UTR region of the ATP7B gene as previously described [5]. Sequencing was performed using dGTP technology and the ABI 3100 analyzer (Applied Biosystems—Perkin-Elmer, Norwalk, CT) according to the manufacturer's recommendations. The sequence software package (version 4.2; GeneCodes, Ann Arbor, MI) was used for sequence analysis. BLAST search (http://www.ncbi.nih.gov/BLAST) was used to examine the conservation of the involved residues in homolog proteins. Polyphen, an automatic tool for predicting the impact of an amino acid substitution on a protein structure and function was used to analyze whether the novel missense mutations were disease-causing mutations. The research procedures for both DNA and RNA analyses were in accordance with the institutional guidelines and the Declaration of Helsinki.

3. Results and discussion

Mutation analysis allowed the detection of 83 mutations. In 45 families mutations were detected in homozygosity while in 68 the compound heterozygous state was identified. There are 11 new mutations detected (Table 1) and 21 more mutations already described in other populations that were detected for the first time in the analyzed populations (Table 2). Of the novel mutations, 2 are nonsense, 2 are frameshift, 2 are splice site and 5 are missense.

The deletion mutations c.1739delA and c.1849delG both occurring in exon 5, create a frameshift and an STOP codon in 582 and 647 aa position respectively resulting most likely in a truncated and non-functional protein. The nonsense mutations p.W939X and p.E1082X occurring in exon 12 and 15 respectively are expected to result in truncated non-functional protein products. The c.1544-2A>C and c.3244-2A>G substitution occur in the acceptor splice sites of introns 3 and 14 respectively. The c.1544-2A>C occurs in a position where an another c.1544-2A>G substitution has been described [5]. According to other gene systems, these mutations should lead to exon skipping with the removal of both exons 4 and 15 which most likely results in premature termination of translation [6]. Missense mutations occur in the N-terminal metal binding domain 2 (MBD2), A-domain, and ATP loop (Table 1). Using BLAST search (http://www.ncbi.nih.gov/BLAST) and Polyphen tool we confirmed their disease-causing nature. Furthermore, two groups of controls (each of 50 subjects) originating from Sardinia, Italy were also screened for the novel missense mutations identified in these populations to exclude their presence in the analyzed samples. The mutation p.G170V, occurring in the large second exon leads to the substitution of Glycine, a neutral polar amino acid with a hydrophobic Valine residue. Amino acid sequence alignment of the six metal binding domains (MBDs) of ATP7B showed that Glycine in the 170 aa position of MBD2 corresponds to the same residue in 85th position in MBD1 and in 591st position in MBD6 (Fig. 1). Both residues have been previously reported to be mutated G85V and G851D in Wilson's disease patients and have been well characterized by functional studies [7,8].

Copper doesn't bind to the N-terminal portion of ATP7B as a free ion but is transferred from the Atox1 metallochaperone. The transfer of copper from Atox1 to the N-terminal domain of ATP7B occurs by a selective labeling firstly of MBD2, that results in changes

Table 1
Novel mutations identified in WD chromosomes.

Nucleotide change	Amino acid change	Exon	Type	Region of protein	Ethic origin	Polyphen	Chr
c.509G>T	p.G170V	2	Missense	Cu2	Italy	Deleterious	1
c.1544-2A>C	Intron	4	Splice site	Cu5	Italy	NA	1
c.1739delA	p. H580Pfs	5	Deletion	Cu6	Italy	NA	1
c.1849delG	p.D617Ifs	5	Deletion	Cu6	Italy	NA	1
c.2293G>C	p.D765H	8	Missense	Tm4	Italy	Deleterious	1
c.2507G>A	p.G836E	10	Missense	A-domain	Morocco	Deleterious	1
c.2191T->G	p.V864G	11	Missense	A-domain	Sardinia	Deleterious	1
c.2816G>A	p.W939X	12	Nonsense	Tm5	Switzerland	NA	1
c.3244G>T	p.E1082X	15	Nonsense	ATP-binding	Italy	NA	1
c.3244-2A>G	Intron	15	Splice site	ATP-binding	Italy	NA	1
c.3877G>A	p.E1293K	18	Missense	ATP Hinge	China	Deleterious	1

NA: not applicable.