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Original Article

Gene mutations in Wilson disease in Egyptian children: Report on two novel mutations

Fatma A. El-Mougy [a], Sahar A.A. Sharaf [a], Marwa M. Elsharkawy [a,*], Iman A. Mandour [a], Riham A. El-Essawy [a], Abeer M. Eldin [a], Heba M. Helmy [b], Dina H. Soliman [a], Lamia H. Selim [a], Heba M. Sharafeldin [a], Engy A. Mogahed [b], Hanaa M. El-Karaksy [b]

[a] Department of Clinical and Chemical Pathology, Kasr Al-Aini Hospital, Cairo University, Cairo, Egypt
[b] Department of Pediatrics, Cairo University Pediatric Hospital, Cairo, Egypt

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ABSTRACT

Background and study aims: Wilson disease (WD) is an autosomal recessive disorder, caused by defects in copper-transporting P-type adenosine triphosphatase (ATPase) encoded by the ATP7B gene, resulting in the deposition of copper in the liver and brain with significant disability or death if left untreated. An available regimen of treatment gives hope to those predisposed to the disease if diagnosed early. The objective of this study was to determine the frequency of the most common European mutation (p.H1069Q) in Egyptian children with WD, in addition to screening for previously reported mutations in the Egyptian patients in our selected group.

Patients and methods: Direct DNA sequencing was applied to exons (13, 14, 18, and 19) of the ATP7B gene for 19 patients previously diagnosed with WD. Then DNA sequencing and pedigree analysis were performed in the families of the patients showing variations in their results for the purpose of family screening and carrier detection. Six out of 19 patients were studied with their families (three families).

Results: We identified five variants of which two were novel among the studied patients. One of the novel variants was synonymous substitution (p.A1074A) in 16% of patients and the other was predicted to be missense disease-causing mutations (p.T1076I) in 16% of patients, and three previously published mutations p.H1069Q were detected in 5% of patients, p.P1273Q in 10% of patients, and a silent variant p.A1003A in 26% of patients.

Conclusion: Screening for the two exons 14 and 18 of the ATP7B gene is important in Egyptian patients especially in suspected patients without hepatic manifestations.

Introduction

Wilson disease (WD) is an autosomal recessive disorder of copper metabolism with a worldwide prevalence of one per 30,000 [1], caused by a mutation in the ATP7B gene, which encodes a copper-transporting protein called P-type adenosine triphosphatase (ATPase) ATP7B. This protein has the main role in biliary copper excretion [2]. Mutations in the ATP7B gene alter the function of the protein in its role as a transporter, resulting in toxic accumulation of copper in various tissues, mainly the liver, brain, and cornea [2].

The WD disease gene, ATP7B (MIM#606882) cloned in 1993, mapped to chromosome 13q14.1 [3,4], encodes a P-type ATPase that contains 1465 amino acids with a structure similar to other P-type metal transporters [5]. More than 515 variants (379 probable disease-causing) have been reported in patients with WD from populations worldwide [6]. The mutations were found to have geographical distributions and ethnic relation with the most common European mutation in p.H1069Q, which was found in exon 14 [7].

In Egypt, the most common hepatic disease is viral hepatitis (hepatitis C virus (HCV)) with the highest prevalence worldwide [8]. Accordingly, it masks other liver diseases; therefore, WD can be easily missed during diagnosis in addition to limitations of clinical data on large cohorts [9]. Scant data from Egypt are available on WD mutations [9,10]. Exons 13, 14, 18, and 19 were found to have a high frequency of mutations in the Egyptian population [10].

Abbreviations: WD, Wilson disease; KF, Kayser–Fleischer.

* Corresponding author at: Kasr Al-Aini Hospital, Faculty of Medicine, Cairo University, Cairo 11562, Egypt. Tel.: +20 223640501; fax: +20 223644383.
E-mail address: marwaelsharkawy@yahoo.com (M.M. Elsharkawy).

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In Egyptian children with WD, there is an urgent need for early diagnosis of suspected children and screening of their families for early treatment and prevention of toxic complications. To the best of our knowledge, this is the first report of genetic mutational analysis for WD carried out in an Egyptian laboratory. The establishment of a molecular diagnostic system with a clear algorithm and availability of that work in our area facilitate the follow-up of the patients and reduce the time between shipping the samples and receiving the results.

The aim of this work was to study the frequency of the most common mutation p.H1069Q in the ATP7B gene within exon 14, and also to find genetic variations in exons 13, 18, and 19 due to the high frequency of mutations in these exons in Egyptian children with WD [10], with assessment of the phenotype–genotype relation.

Patients and methods

In this report, we investigated genetic variations in the ATP7B gene in exons 13, 14, 18, and 19 and the intronic boundaries in 19 Egyptian patients with WD. The 19 patients with WD were chosen from 15 families; six patients whose results show genetic variations were studied with their families (three families) for the purpose of family screening and carrier detection. Seven out of the 15 parent pairs were consanguineous. Screening of exon 14 in 50 healthy chromosomes was performed for novel mutations.

Children with WD presenting to the Pediatric Hepatology Unit, Cairo University Pediatric Hospital, were enrolled in the present study after informed consent was obtained from their parents. The study protocol was approved by the institutional ethics committee.

A diagnosis of WD was made if the patient had hepatic and/or neurologic disease in addition to at least two of the following six criteria: [11] a positive family history of WD, low ceruloplasmin level (<20 mg%), elevated baseline urinary copper excretion (>60 μg/24 h), elevated urinary copper excretion following two doses of 500 mg penicillamine (>1600 μg/24 h), Coombs' negative haemolytic anaemia, and the presence of Kayser–Fleischer (KF) rings.

Mutational analysis of ATP7B gene

Genomic DNA was extracted from whole venous blood collected in ethylenediaminetetraacetic acid (EDTA) using the DNA extraction kit QIAamp (QIAGEN GmbH, Hilden, Germany). We used the direct exon sequencing strategy where exons 13, 14, 18, and 19 of the ATP7B gene were selected to be studied as they have the highest probability of having mutations, based on a review of different studies performed in Mediterranean or Arab countries [10,12,13]. The selected exons were amplified in a thermal cycler (Hybaid thermal cycler, Promega Corporation, Madison, WI, USA) using previously described primers [14]. The detection of polymerase chain reaction (PCR) amplification products was performed using 1.5% gel electrophoresis. DNA purification was performed using a purification kit supplied by Qiagen. BigDye terminator cycle sequencing of the purified product was carried out using fluorescent dye terminators followed by dye terminator removal using Centri-Sep columns. The cleaned-up products were injected in an ABI 3500 genetic analyser (Applied Biosystems, Foster City, CA, USA). When mutations were detected, confirmation was carried out by reversed sequence on Applied Biosystems 310 Genetic Analyser and with family screening as it has a role in confirmation.

The mutations were quoted according to the guidelines from www.HGVS.org/mutnomen [15]. The sequences were compared to the published Refseq NM_000053.2; analysis of the selected exons was conducted using BLAST (Basic Local Alignment Search Tool) [16].

Results

Direct sequencing of both the forward and reverse directions of the four exons in all 19 patients with WD and the three families was performed.

Among the 19 patients, variants were detected in 12 patients and seven patients had no changes in the sequencing of the four selected exons (Table 1). The total number of variants detected were five; two of them have not been reported previously in exon 14 (p.T1076I and p.A1074A). Another known mutation was detected in the same exon (p.H1069Q). The remaining two known variants were detected in exons 13 and 18, respectively. No variants were detected in exon 19 in our study (Table 2).

Both novel variants were detected in exon 14 in three patients (Fig. 1). One of the variants is a synonymous (silent) substitution c.3227C>T (p.A1074A) with no amino acid change. The other is a nonsynonymous substitution c.3231A>G (p.T1076 I), where the amino acid threonine is changed to isoleucine. These two variants were associated with neurologic manifestations in the three patients. They were detected in 10 family members (Family 1).

One patient was heterozygous to a previously published missense mutation c.3207C>A (p.H1069Q) in exon 14 (Fig. 2). Family 2 had one diseased child and two normal sibs. The sequence analysis revealed that the proband was heterozygous for the mutation p.H1069Q in exon 14; this child died during the study. The parents and one of the normal sibs were carriers for the same mutation, and the other child was normal as shown in Fig. 2.

Another previously published missense mutation c.3818C>A (p.P1273Q) was detected in exon 18 in homozygous form in two patients (Family 3) (Fig. 3). In Family 3, the two patients were homozygous for the mutation p.P1273Q; the parents were heterozygous. A previously reported silent variant c.3009G>A (p.A1003A) was found in exon 13 in five patients in homozygous form.

Discussion

Genetic testing is important in early WD diagnosis. It can sometimes establish diagnosis, even in the presymptomatic period of the disease [17]. Sequencing of the WD gene is important in molecular diagnosis to focus on investigating the genetic features of the study group, as the disease is population specific [18]. It was suggested that many factors such as gene–environment interactions may cause these variations to be population specific [19].

Although sequencing of the exons in Mendelian disease genetics is very beneficial [20], ATP7B is a large gene, with 21 exons, spanning over 80 kb of genomic sequence [4]. Sequencing of the whole gene is expensive and difficult to conduct in routine clinical practice especially in developing countries such as Egypt; thus, in this study, we focused on the analysis of WD in specific exons of the gene, in order to identify common mutations.

The current study is a single-centre report that addresses the clinical/laboratory findings and genetic analysis of 19 patients chosen from 15 families. Consanguinity was encountered in 7/19 cases (47%) of the studied families. Affected family members shared the same genotype. Abdel Ghaffar et al. [10] reported that consanguinity was present in 75% of their Egyptian WD studied families. In our community where consanguineous marriage forms an integral and respected part of our cultural tradition, expansion of genetic counselling to screen autosomal recessive diseases is mandatory.

In this report, we describe the variants found in the ATP7B gene within four exons (13, 14, 18, and 19). Variants were detected in 63% of the studied WD cases. This makes screening of these exons as hot spots for mutational analysis in Egyptian patients with WD