

## SHORT REPORT

## 4193delC, a common mutation causing Wilson's disease in Saudi Arabia: rapid molecular screening of patients and carriers

R Majumdar, M Al Jumah, M Fraser

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**Background:** In patients with Wilson's disease (WD), an autosomal recessive disorder, toxic accumulation of copper results in fatal liver disease and irreversible neuronal degeneration. ATP7B, the gene mutated in WD, contains 21 exons and encodes a copper transporting ATPase. A novel disease causing mutation (4193delC) in exon 21 of the ATP7B gene has previously been detected by heteroduplex analysis and DNA sequencing.

**Aims:** To screen for the above mutation in patients with WD and carriers using an amplification refractory mutation system (ARMS).

**Methods:** ARMS was used to screen for the 4193delC mutation in 30 patients with WD and their relatives.

**Results:** A homozygous mutation was detected in 16 of 30 patients with WD.

**Conclusions:** This polymerase chain reaction based method, which has been known for years, is a simple, inexpensive, and rapid method for screening common and specific mutations in patients with WD and carriers.

Wilson's disease (WD), an autosomal recessive disorder of copper transport, is characterised by impaired biliary excretion and deficient incorporation of copper into ceruloplasmin.<sup>1</sup> This leads to toxic accumulation of copper in the liver and subsequent overflow and accumulation in the brain, kidney, and cornea. Thus, excess accumulation of copper can cause tissue damage leading to hepatic, neurological, and psychiatric disturbances, or combinations of the three.<sup>1,2</sup> Patients with liver disease generally present in childhood or adolescence. Neurological and psychiatric symptoms begin at the age of 12 years or later.<sup>2</sup> WD occurs in populations of every geographical and ethnic origin. The worldwide prevalence of WD is estimated to be one in 30 000, with a corresponding gene frequency of 0.56% and a carrier frequency of about 1/90.<sup>1–3</sup>

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ATP7B, the gene mutated in WD, has 21 exons and encodes a protein of 1465 amino acids.<sup>1</sup> The protein is a copper transporting P-type ATPase. To date, more than 100 mutations have been detected in the ATP7B gene, few of which are common to several populations, with most being population specific.<sup>1,3,4</sup> We detected a novel deletion mutation, 4193delC, in exon 21 of the ATP7B gene in Saudi patients with WD by means of heteroduplex analysis followed by DNA sequencing.<sup>5</sup> This deletion mutation appears to be unique to Saudi patients and is found frequently in this ethnic group.

The aim of our study was to develop a single amplification refractory mutation system (ARMS) test specific for screening the common 4193delC mutation in Saudi patients with WD and carriers.

## MATERIALS AND METHODS

## Patients

Our study included 30 patients with WD (selected from different regions of Saudi Arabia) and their relatives (20 parents and 40 siblings of the patients). The diagnosis of WD was based on clinical features, liver biopsy results, low ceruloplasmin, low copper serum concentrations, and high urinary copper elimination.<sup>1,2</sup> Our study was reviewed by the local ethics committee, and informed consent was obtained from all patients and their relatives (in some cases parents of the patients) before their inclusion in the study.

For each individual, genomic DNA was isolated from 2 ml of peripheral blood using the QIAamp™ DNA blood midi kit, according to manufacturer's instructions (Qiagen, Hilden, Germany).

## ARMS test

A typical ARMS test consists of two complimentary reactions.<sup>6,7</sup> The first reaction contains an ARMS primer that is specific for the normal DNA sequence and cannot amplify the mutant DNA at a specific locus. Similarly, the second reaction contains a mutant specific primer and does not amplify normal DNA. Thus, normal individuals generate a polymerase chain reaction (PCR) product only in the normal reaction, heterozygotes generate products in both reactions, and homozygous mutant individuals do so only in the mutant reaction.

Normal and mutant specific ARMS primers were designed to amplify a portion of exon 21 of ATP7B that harbours the 4193delC mutation (a cytosine residue is deleted at the 4193 base), as shown in fig 1. Figure 1A shows the normal (control) and mutated sequences in exon 21.<sup>5</sup> Figure 1B shows the primer sequences. In both cases, a cytosine residue was changed to a thymine residue indicated by a bold T at the penultimate base. The choice of mismatched base was determined experimentally. The same common primer is used in both reactions.

PCR amplification was performed in a final volume of 25 µl containing 100 ng of genomic DNA, 0.04 units of Taq DNA polymerase (Pharmacia), 1× PCR buffer, 0.2mM dNTPs, and 200 nM of each primer. All reactions were carried out with a hot start for three minutes at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 64°C, and 45 seconds at 72°C, with a final extension at 72°C for five minutes. Each tube

**Abbreviations:** ARMS, amplification refractory mutation system; PCR, polymerase chain reaction; WD, Wilson's disease



patients, six presented with liver disease (three died), two had only neurological symptoms, and eight of the remaining patients presented with both neurological and liver complications (three died). Thus this disease causing mutation is not specific for a particular phenotype. These eight WD families (from which the 16 patients harbouring the 4193delC mutation derived) are not related. However, they originate from two different tribes scattered all around Saudi Arabia. Therefore, it is possible that this mutation was caused by a founder effect or was the result of a new mutation that arose in an ancestor common to these individuals. In addition, we have also demonstrated that this inexpensive and reliable ARMS test is useful for rapidly screening carriers for 4193delC in the Saudi population. Finally, this test is applicable only in geographical areas where WD is caused predominantly by a single mutation, as is the case in Saudi Arabia. However, this method might not be applicable to other parts of the world with a more heterogeneous population.

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It is clear from our study that the remaining 14 patients with WD do not harbour the common 4193delC mutation because ARMS tests were negative in these cases. As mentioned earlier, the ATP7B is a large gene, with 21 exons, and to date more than 100 mutations have been detected.<sup>3,4</sup> It is possible that the mutation has occurred in other areas of the ATP7B gene in these patients. Further studies are under way to detect mutations in the ATP7B gene of those patients with WD in whom no mutation was detected by the above ARMS test.

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#### Take home messages

- The amplification refractory mutation system (ARMS) reliably detected the 4193delC mutation in the ATP7B gene in Saudi patients with Wilson's disease (WD) and their relatives
- The ARMS test is a simple, inexpensive, and rapid method for screening common and specific mutations in patients with WD and carriers

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