SHORT REPORT

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

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J Clin Pathol: Mol Pathol 2003;**56**:302–304

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.[1] 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.[1 2] Patients with liver disease generally present in childhood or adolescence. Neurological and psychiatric symptoms begin at the age of 12 years or later.[2] 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.[1–3]

> "Excess accumulation of copper can cause tissue damage leading to hepatic, neurological, and psychiatric disturbances, or combinations of the three"

ATP7B, the gene mutated in WD, has 21 exons and encodes a protein of 1465 amino acids.[1] 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.[1 3 4] 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.[5] 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.[1 2] 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.[6 7] 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.[5] 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

A

4168 4193

5′–––//–GGCCACATGAAGCCCCTGACGGCATCCCA––//––3′ control sequence
5′–––//–GGCCACATGAAGCCCCTGACGGCATCCA––//––3′ mutated sequence (419delC)

B

4174

Normal primer (forward): 5′–ATGAAGCCCCTGACGGCATC**T**C–3′
Mutant primer (forward): 5′–ATGAAGCCCCTGACGGCATC**T**A–3′

Common primer (reverse): 5′–GCTTGTGGTGAGTGGAGGCAAGTCCCTG–3′

Figure 1 (A) A partial sequence of exon 21 of ATP7B from controls and patients with Wilson's disease[5]; 4168 and 4193 indicate the nucleotide position in the sequence. The DNA sequence of the patient with WD shows a deletion of a cytosine residue at position 4193 of the sequence derived from the NCBI DNA data bank (accession Gi U03464). (B) Amplification refractory mutation system (ARMS) primers. The forward primer sequence of the normal sequence (sense strand) is indicated by "normal primer" and the mutated sequence is indicated by "mutant primer". The first base of these primers starts at position 4174 of the above sequence (A). The deliberate introduction of a mismatch (C → T) in both primers is indicated by a bold T at the 3′ end. The reverse primer is common to the above primers and is indicated by "common reverse primer".

contained an additional set of primers that amplifies the entire exon 21 of ATP7B (361 bp). This amplification product served as a control for each test. The PCR amplicons were separated by electrophoresis on a 2% agarose gel. Ethidium staining of the agarose gel was used to detect the amplified products.

RESULTS

We performed ARMS tests to screen for the specific point mutation in exon 21 of ATP7B in Saudi control subjects, patients, and the patient's relatives. With the creation of a deliberate mismatch in the forward primer (T instead of C at the penultimate position of the "normal" forward primer), the reaction amplified the control and heterozygous (carrier) DNA, giving rise to a 277 bp product (fig 2). Under similar PCR conditions, the "mutant" forward primer (with an altered sequence (C → T), causing a deliberate mismatch) was able to amplify only patient and heterozygous DNA (fig 2). We further analysed the DNA of 50 control subjects using both sets of primers. Each of the control DNA samples amplified a 277 bp product with the "normal" forward primer and none of the DNA samples was amplified with the "mutant" forward primer, indicating that none of the control DNA contained the mutated sequence. The genomic DNA from 16 patients was amplified only with the "mutant" forward primer, indicating that these patients harboured the homozygous 4193delC mutation in exon 21. The sensitivity of the ARMS test was further validated by screening the DNA of these patients for the 4193delC mutation by DNA sequencing. In addition, the ARMS test also detected the heterozygous status of several relatives (15 siblings and all parents) of the patients who had the confirmed homozygous 4193delC mutation (fig 2A,B).

DISCUSSION

Since its first description,[6] the ARMS test has been widely used for the detection of point mutations because the method is easy to perform and does not need specific PCR materials or detection equipment.[7 8] We have adopted this simple and highly specific method as a first choice to screen for a known disease causing mutation (4193delC) in Saudi patients.

ARMS tests convincingly detected the 4193delC mutation in 16 of 30 patients with WD from Saudi Arabia. Of the 16

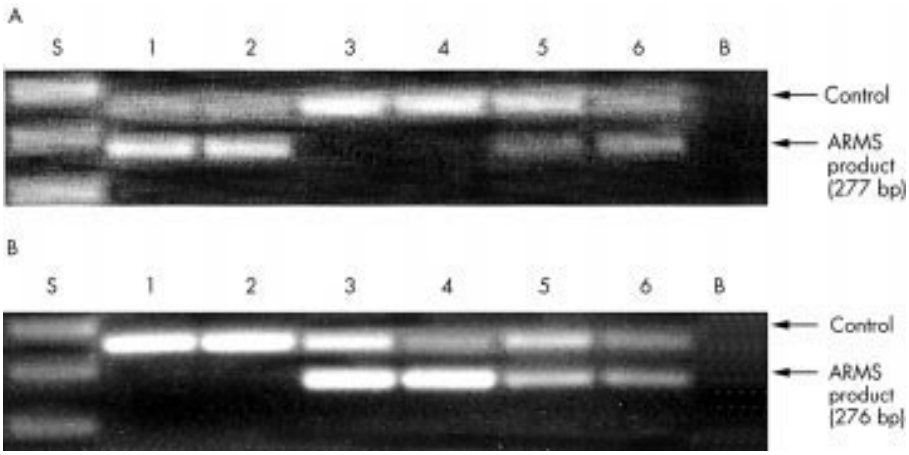

Figure 2 Polymerase chain reaction (PCR) amplification of genomic DNA. (A) Amplification refractory mutation system (ARMS) products using the "normal forward primer" and (B) ARMS products using the "mutant forward primer". Lane S, size marker (corresponding to 200, 300, and 400 bp); lanes 1 and 2, DNA from two normal control subjects; lanes 3 and 4, DNA from two patients with Wilson's disease; lanes 5 and 6, DNA from the parents of one of the patients (lane 4); lane B, blank containing all PCR ingredients except genomic DNA. Control indicates the PCR product of the entire exon 21 (361 bp) using separate PCR primers.

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.

> "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"

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.[3 4] 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.

ACKNOWLEDGEMENTS

This study was supported by King Fahad National Guard Hospital and a grant from the King Abdulaziz City for Science and Technology (KACST project AT-18–03), Saudi Arabia.

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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Accepted for publication 20 June 2003

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