The Same Haplotype for Two Unrelated Wilson Disease Patients with New ATP7B Mutation

Hassan Dastsooz MSc, Seyed Mohsen Dehghani MD, Majid Fardaei PhD

Abstract

Background: Wilson disease is a rare autosomal recessive disorder of copper metabolism caused by mutation in the ATP7B gene. The combination of markers (such as SNPs) on a single chromosome can be used to understand the structure of haplotype in the human genome, in which provide notable information on the origin of the mutation in human genetic disorders. The purpose of this study was to determine a haplotype analysis of two unrelated Wilson disease patients with the same missense mutation, c.2335T>G (g.58164 T>G) in exon 8.

Methods: DNA was prepared from two patients with the c.2335T>G mutation, their first-degree relatives, and 50 selected homozygous individuals from consanguineous marriage for eight SNPs around this particular ATP7B mutation. PCR was performed for SNPs of exons 4 (g.47964 C>T), 5 (g.51482G>A), 6 (g.54622A>G), 7 (g.56255G>A), 9 (g.59042G>T), 11 (g.66363G>A), 13 (g.70004 G>C), and 14 (g.72244 A>G), which are located in upstream and downstream of this mutation. Then, restriction fragment length polymorphism (RFLP) for these eight SNPs was designed and performed using eight different restriction enzymes.

Results: Eight different haplotypes were found in the present study and the patients with the same missense mutation had the same haplotype. The most prevalent haplotype in 100 normal studied ATP7B alleles was the same as reference haplotype (C G A G T G G G A) for ATP7B gene (NG_008806.1).

Conclusion: As these two geographically separated families with the same mutation had the same haplotype, we concluded that this mutation possibly had the same origin in this population.

Keywords: ATP7B, c.2335T>G, haplotype, Wilson disease


Introduction

Wilson disease (WD) (OMIM 277900) is a rare autosomal recessive disorder of copper metabolism caused by different mutations in ATP7B gene. This gene codes a copper transporting p-type ATPase that is essential for biliary copper excretion. So far, more than 500 mutations and several single nucleotide polymorphisms (SNPs) have been reported in the ATP7B gene (Wilson disease database mentioned at the university of Alberta).3–7 The combination of markers (such as SNPs) on a single chromosome can be used to understand the structure of haplotype in the human genome, in which provide notable information on the origin of the mutation in human genetic disorders. In addition, SNPs markers have been used in linkage analysis, recombination, and disease associations.8–12

One of the most approaches implemented for DNA typing is restriction fragment length polymorphism (RFLP) analysis. Using restriction endonucleases, polymorphic DNA digests and separates by gel electrophoresis, which can give different banding patterns from different individuals. This pattern is heritable and results from the presence or absence of the enzyme restriction site at the certain loci.

Several SNPs located at ATP7B gene have been reported as common SNPs in different populations. Several studies in Iranian population had been shown that rare disorder such as Wilson disease was due to the high rate of consanguinity.13,14 Haplotype analysis of the mutations will provide necessary information for convenient screening of carrier individuals in a particular population. In our previous study, we identified a novel mutation in two unrelated WD patients. In order to determine whether these two WD patients with the same mutation, c.2335T>G (g.58164 T>G),13 have the same origin; this study aims to investigate a RFLP haplotype analysis of these patients and 50 unrelated individuals. The present study is the first investigation towards haplotype analysis of a particular mutation in ATP7B gene using RFLP analysis.

Material and Methods

In the present study, eight single nucleotide polymorphisms (SNPs) located at exons 4 (g.47964 C>T, MAF: < 0.01), 5 (g.51482G>A, MAF: 0.01), 6 (g.54622A>G, MAF: < 0.01), 7 (g.56255G>A, MAF:NA), 9 (g.59042G>T, MAF: < 0.01), 11 (g.66363G>A, MAF: < 0.01), 13 (g.70004 G>C, MAF: 0.10), and 14 (g.72244 A>G, MAF:<0.01) (Figure.1) were selected at upstream and downstream of the c.2335T>G (g.58164 T>G) missense mutation which is located in exon 8 of the ATP7B gene. This haplotype track was around 24kb in size. In addition, this investigation was performed for 50 selected homozygous individu-
homozygous individual for these particular SNPs, we investigate families with consanguineous marriage, who are homozygous for these SNPs. These SNPs were obtained from NCBI dbSNP and Wilson disease database at the University of Alberta.

All normal individuals and the patients gave informed consent before undergoing DNA test for haplotype analysis based on the requirements of the ethics committee in Shiraz University of Medical Sciences. Whole blood samples were collected in EDTA tube and stored at -20°C until use. Genomic DNA was extracted from the whole blood samples using a Cinnapure DNA kit (Cinnagen, Iran). Nine primer pairs were used to amplify these nine SNPs using a Cinnagen Taq polymerase protocol. The PCR product was then purified by ethanol precipitation followed by enzyme digestion according to the manufacturer’s instructions (all information about RFLP haplotype are given in Table 1). The 15 μl enzyme digestion reaction was consisted of 10 μl of the purified PCR product, 2 μl diluted enzyme (Vivantis, Malaysia), 1.5 μl buffer V (Vivantis), and 1.5 distilled water. The reaction was then visualized on 2% agarose gel containing ethidium bromide.

Results

We have used single nucleotide polymorphism (SNP) genotyping around a novel mutation to investigate the possibility of the same origin for this mutation of ATP7B gene in two separated families.

In 100 chromosomes from the 50 unrelated homozygous individuals for the studied SNPs, eight different haplotypes were identified. RFLP haplotype analysis of the unrelated patients with c.2335 T>G missense mutation showed the same RFLP haplotype (CGAGGGGGA) (Table 2). In order to detect the presence of the mutation in carrier family members of the patients 1 and 2, NciI restriction enzyme was used. Following enzyme digestion, 360bp, 271bp, and 89bp bands were separated from heterozygous carrier individuals. The 271bp and 89bp bands were also separated from affected homozygous individuals. A 360bp band was detected in wild type individuals following enzyme digestion. The most prevalent haplotype (number 1 in Table 2) was as same as reference haplotype (C G A G T G G G A) for ATP7B gene (NG 008806.1). This common haplotype was identified in 58 chromosomes (Table 2).

Three different haplotypes were identified each in two chromosomes from three different individuals. These three haplotypes had the lowest frequency in normal population. Minor allele frequency (MAF) for each SNP in our population were as follow: g.47964 C>T (MAF: 0), g.51482G>A (MAF: 0.02), g.54622A>G (MAF: 0), g.56255G>A (MAF: 0.02), g.59042G>T (MAF: 0.04), g.66363G>A (MAF: 0), g.70004G>C (MAF: 0.06), and g.72244 A>G, (MAF: 0.02).

Discussion

ATP7B gene c.2335T>G (p.W779Q) mutation was first reported by our group as a new mutation in two far separated families with Wilson disease in Southern Iranian population. Due to the presence of this novel mutation in the separated families, we sought to investigate whether c.2335T>G was originated from a single mutational event. Therefore, we applied RFLP haplotype around this mutation to determine whether there were any segments shared between patients with c.2335T>G mutation that are not present in those without the mutation or controls. We reasoned that if c.2335T>G mutation had a single ancestor origin; patients would have the same chromosomal segments. In order to support our hypothesis, haplotype analysis was performed by the PCR-RFLP method using eight SNPs around this mutation.

The estimated prevalence of Wilson disease is the range of 1 in 30000 to 1 in 100000. To date, more than 500 mutation in ATP7B gene are reported. So far, this is the first report of the C G A G T G G G A haplotype in the Wilson disease patients from Southern Iran.
While in the Iranian population prevalence of Wilson disease has not been reported, several mutations in the ATP7B gene have been identified (Wilson disease database mentioned at the University of Alberta). While in the Iranian population prevalence of Wilson disease has not been reported, several mutations and SNPs have been identified in ATP7B gene. Some genotype-phenotype correlation has been established for different mutations, such as hepatic manifestations with R778L mutation and neurological presentations with H1069Q mutation. Patients with the novel c.2335T>G mutation were characterized by neurological manifestation.

Here, we performed haplotype analysis in 100 alleles from normal homozygous individuals in our population and also two patients with their parents. RFLP haplotype analysis indicated a prevalent haplotype in our population, which is the same segment as patients except mutation. The results of the haplotype analysis on the 100 alleles suggest that this mutant allele has a single origin. Haplotyping of 50 normal unrelated homozygous individuals using a total of the eight SNPs at up and downstream of this mutation, revealed that 58 out of 100 alleles carried the common haplotype [g.4796 C, g.5148 G, g.5462 T, g.5816 T, g.5904 G, g.6636 A, g.7004 C, g.7224 A], and two patients with the same novel mutation had the same haplotype.

Using this data if we accept that these two separated families with the same mutation have the same origin, therefore it can be concluded that this mutation would be common in the south of Iran and should be considered in mutation analysis of ATP7B gene.

### Table 2. Haplotype analysis results

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<th>G</th>
<th>T</th>
<th>G</th>
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(-): mutated RFLP, (+): wild type RFLP
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Conflicts of interest: The authors report no conflicts of interest.

References