Association of a c.1084A>G (p.Thr362Ala) Variant in the DCTN4 Gene with Wilson Disease

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Purpose: Wilson disease is an autosomal recessive disorder which causes excessive copper accumulation in the hepatic region. So far, ATP7B gene is the only disease-causing gene of Wilson disease known to date. However, ATP7B mutations have not been found in ~15% of the patients. This study was performed to identify any causative gene in Wilson disease patients without an ATP7B mutation in either allele.

Materials and Methods: The sequence of the coding regions and exon–intron boundaries of the five ATP7B–interacting genes, ATOX1, COMMD1, GLRX, DCTN4, and ZBTB16, were analyzed in the 12 patients with Wilson disease.

Results: Three nonsynonymous variants including c.1084A>G (p.Thr362Ala) in the exon 12 of the DCTN4 gene were identified in the patients examined. Among these, only p.Thr362Ala was predicted as possibly damaging protein function by in silico analysis. Examination of allele frequency of c.1084A>G (p.Thr362Ala) variant in the 176 patients with Wilson disease and in the 414 normal subjects revealed that the variant was more prevalent in the Wilson disease patients (odds ratio [OR]=3.14, 95% confidence interval=1.36–7.22, P =0.0094).

Conclusion: Our result suggests that c.1084A>G (p.Thr362Ala) in the ATP7B–interacting DCTN4 gene may be associated with the pathogenesis of Wilson disease.

Key Words: Wilson disease, DCTN4, ATP7B–interacting genes, Polymorphism, Association

Introduction

Wilson disease is an autosomal recessive disorder caused by the defect in copper transportation, resulting in hepatic copper accumulation. Generally, copper is metabolized in the enterocyte and transported into the hepatocyte. The defect in hepatic exportation of copper causes Wilson disease. ATP7B mutation is the single cause of Wilson disease known to date1. ATP7B plays a major role in transporting copper to apoceruloplasmin so that it binds copper and changes its form to a holo-ceruloplasmin. This ceruloplasmin is secreted into the bloodstream with copper. Furthermore, ATP7B transfers copper to bile canaliculi via trans–Golgi network, eventually excreting into bile. ATP7B has been suggested to interact with five proteins such as ATOX1, COMMD1,
GLRX1, ZBTB16, and DCTN4. ATOX1 is a cystolic protein that serves as an intracellular donor of copper. This protein also forms a complex with ATP7B. COMMD1, also previously known as Murr1, is involved in the pathway of hepatic biliary copper excretion. GLRX1 is known to interact with the N-terminal of ATP7B. GLRX1 also catalyzes the reduction of intra-molecular disulphide bonds and deglutathionylation of the cystine residues of the CxxC motifs of ATP7B, which results in facilitation of copper binding on ATP7B. ZBTB16 is known to colocalize with ATP7B on the trans–Golgi network. ZBTB16 plays a significant role in the ERK signaling pathway of the hepatocyte. DCTN4 is involved in vesicle transportation of copper. The interaction of DCTN4 with ATP7B suggests that DCTN4 facilitates copper–induced trafficking of ATP7B. The existence of these five ATP7B–interacting proteins suggests that ATP7B interacts with various types of proteins that are critical for supporting the copper transportation by ATP7B.

Approximately, 72–90% of patients with Wilson disease have a mutation in the ATP7B gene. Our main focus is on the remaining 10–28% of Wilson disease patients who do not have an ATP7B mutation. Although there is a possibility that mutation might reside in promoter or deep intron region of the ATP7B, we hypothesized that a mutation in the ATP7B–interacting proteins will cause defects in the transportation of copper in the liver and ultimately manifesting a same phenotype as Wilson disease. Thus, in this study, we screened five ATP7B–interacting genes to identify new disease-causing genes for Wilson disease.

Materials and Methods

1. Subjects

This study consisted of a total of 176 unrelated patients with Wilson disease. Patients were diagnosed based on decreased serum ceruloplasmin level (<15 mg/dL) and increased urinary excretion of copper (>100 ug/day) as well as clinical symptoms, majority of them showing hepatic or neurological manifestations. A total of 414 healthy individuals were also recruited for this study as control group. The study was approved by the Institutional Review Board of Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea. All patients or their parents provided a written informed consent.

2. Mutation Screening

To identify any causal mutations in the ATP7B–interacting genes, genomic DNA was isolated from peripheral blood, all the exons and their respective flanking regions of the 5 ATP7B–interacting genes were analyzed in 12 patients with Wilson disease without an ATP7B mutation in either allele. Genetic variation was identified by comparing the individual’s sequence to the reference sequence. The PCR products were sequenced and analyzed with an ABI Prism 3730 sequencer (Applied Biosystems, Foster City, CA, USA) and Polyphred program (http://www.droog.gs.washington.edu/PolyPhred.html). In silico prediction of functional alterations by the novel genetic variants were performed using Polyphen (http://genetics.bwh.harvard.edu/pph/). Multiple alignments of amino acid sequences from different species were done by clustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html).

3. TaqMan genotyping

To validate the association of c.1084A>G (p.Thr362 Ala) variant of DCTN4 with Wilson disease, Wilson disease samples (N=176) and normal control samples (N=414) were investigated using TaqMan genotyping method with a HT7900 real time PCR machine (Applied Biosystems, Foster City, CA, USA).

4. Statistical Analysis

Statistical analyses were performed using the HapAnalyzer program (http://hap.ngri.go.kr/) and SPSS programs (version 18) (SPSS Inc., Chicago, IL, USA).
To test the association with Wilson disease, $\chi^2$ test was used to compare allele frequencies between cases and controls, and Fisher’s exact test was used when an expected cell count was less than five.

Results

1. Mutation screening of ATP7B–interacting genes in patients with Wilson disease

Among the five ATP7B–interacting genes (ATOX1, COMMD1, DCTN4, GLRX1, and ZBTB16), a total of 11 variants were found in the 12 patients with Wilson disease without an ATP7B mutation in either allele (Table 1). Among these, three variants were nonsynonymous, which were exclusively found in the DCTN4 gene. All of them are known single nucleotide polymorphisms (SNP), rs11954652, rs3733923, and rs117873033. In silico prediction of functional effects of three nonsynonymous variants of DCTN4, using PolyPhen, showed only c.1084A>G (p.Thr362Ala) variant to be “possibly damaging”. Two patients are heterozygotes for this variant. (Table 1). In addition, this site, p.Thr362 of the DCTN4 gene, is highly conserved among various species. These results predict that c.1084A>G (p.Thr362Ala) might alter the biological function of DCTN4 significantly.

2. Association of c.1084A>G (p.Thr362Ala) in the DCTN4 gene with Wilson disease

To determine whether c.1084A>G (p.Thr362Ala) in the DCTN4 gene is associated with Wilson disease, we performed TaqMan genotyping in large control samples (N=414) and patients samples (N=176), including the samples of the 12 patients with Wilson disease without an ATP7B mutation in either allele. The c.1084A>G (p.Thr362Ala) was found in 9 patients of Wilson disease patient group (5 AG heterozygotes and 4 GG homozygotes), whereas 7 individuals in control group harbor c.1084A>G (p.Thr362Ala) (4 AG heterozygotes and 3 GG homozygotes) (Table 2). The c.1084A>G (p.Thr362Ala) in the DCTN4 gene was significantly more prevalent in patients with Wilson disease than in normal population (odds ratio [OR]=3.14, 95% confidence interval=1.36–7.22, $P$-value=0.0094).

Discussion

Our purpose of the mutation screening of five ATP7B–interacting genes in patients with Wilson disease was to discover any novel causative variants that develop a Wilson disease–mimicking phenotype. No causal mutation in the ATP7B–interacting genes has been identified.
reported in humans to date. Among ATP7B–interacting genes, the COMMD1 gene can cause early onset of Wilson–like disease in dogs\(^9\). However, no mutation of the COMMD1 gene was found in Wilson disease patients who were negative for ATP7B mutations\(^10\). Only intronic SNPs and synonymous SNPs were detected in the COMMD1 gene\(^11\). Most variants in the five ATP7B–interacting genes, identified in our patients, were also previously known synonymous SNPs and nonsynonymous SNPs with prediction of none functional alternation. Only one variant in DCTN4 gene was predicted to alter the function of DCTN4 protein significantly. The importance of DCTN4 as an ATP7B–interacting gene is not completely clarified. DCTN4 is expected to play an important role in the vesicle exportation of copper from the liver\(^7\). In addition, DCTN4 interacts with the N–terminal of ATP7B, which might contribute to the development of Wilson disease. Particularly, it has been reported that the amino acid residues 200–460 region of DCTN4 protein interact with the N–terminal of ATP7B protein\(^7\). As the c.1084A>G (p.Thr362Ala) variant of DCTN4 is located in the center of DCTN4–ATP7B interaction, c.1084A>G (p.Thr432Ala) might affect the interaction of DCTN4 with ATP7B and subsequently may have a critical or additionally detrimental effect on copper transportation. In addition, we demonstrated that the c.1084A>G (p.Thr362Ala) variant of DCTN4 is significantly associated with Wilson disease. These data indicate that c.1084A>G (p.Thr362Ala) variant of DCTN4 might modify the clinical outcome of Wilson disease, although the variant is not a direct cause of Wilson disease. Further study is necessary to determine whether the c.1084A>G (p.Thr362Ala) variant of DCTN4 can directly affect the interaction of copper with ATP7B protein and subsequently the copper transport.

In the pedigree analysis of the DCTN4 variant in the two patients, the variant was inherited from respective parent (data not shown), indicating that the c.1084A>G (p.Thr362Ala) is not a de novo variant. We also analyzed some clinical characteristics of the 9 patients, who carry c.1084A>G (p.Thr362Ala) variant of DCTN4, either heterozygotes of Thr/Ala or homozygotes of Ala allele, to find specific combination with ATP7B mutation types and any correlations with clinical features of Wilson disease. However, we could not observe any specific patterns or combinations with ATP7B mutation types, indicating that the c.1084A>G (p.Thr362Ala) variant of DCTN4 does not directly influence the phenotype of Wilson disease as a modifier of ATP7B mutation. Although modifier gene mutation of Wilson disease such as Murr1 in dog was identified, no cases were reported in the human patients with Wilson disease. Therefore, it assumed to be less likely that multiple minor loci may play a role for the occurrence of Wilson disease by influencing the ATP7B–mediated copper transport. However, c.1084A>G (p.Thr362Ala) variant of DCTN4 still has a genetic epidemiological significance.

In summary, we identified a novel variant, c.1084A>G (p.Thr362Ala) in the ATP7B–interacting DCTN4 gene which is highly conserved among species. This variant was significantly associated with Wilson disease. These results indicate that this variant might affect the ATP7B–mediated copper transport in liver. These finding will provide a new insight into the understanding of Wilson disease pathogenesis.

Table 2. Genetic Association of DCTN4 Polymorphism (c.1084A>G) in Wilson Disease

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Wilson disease</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>167</td>
<td>407</td>
</tr>
<tr>
<td>AG</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>GG</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

Abbreviations: MAF, minor allele frequency; OR, odds ratio; CI, confidence interval

Allelic test was performed using HapAnalyzer program for calculating OR (95% CI) and corresponding P–values.

\(\text{OR (95\% CI)} \) = odds ratio (95% confidence interval).

\(\text{P–value} \) = significance level.
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References