Identification of mutations in the ATP7B gene in 14 Wilson disease children

Case series

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Abstract

Introduction: Wilson Disease (WD) is an autosomal recessive inherited metabolic disease caused by mutations in the ATPase copper transporting beta gene (ATP7B). WD can cause fatal neurological and hepatic disorders if not diagnosed and treated.

Objective: To analyze the disease-causing mutations of 14 Chinese WD children, 11 of whom are diagnosed with hepatic disorders, 2 with neurological degeneration and 1 with both hepatic and neurological disorders.

Methods: All ATP7B coding regions were analyzed by Sanger sequencing. Single nucleotide polymorphisms (SNPs) functional impacts were assessed by combining the results of four bioinformatics tools (Poly-phen-2, SIFT, PANTHER-PSEP and PhD-SNPs) in an index that reflects the combined probability (cPdel) of an amino acid change to be deleterious to the protein function.

Results: Two novel variants involved in WD development, c.1448_1455del (p.Arg483SerfsX19) and c.4144G>T (p.Glu1382Stop), and 11 previously reported mutations were detected. Both new variants result in shortened and dysfunctional ATP7B proteins. cPdel score suggests that SNPs may be deleterious to the ATP7B functionality.

Conclusions: This study enriches the library of the ATP7B mutations that lead to WD and can be used as a basis for genetic counseling, for WD prevention and clinical and prenatal diagnosis. Those SNPs that are believed to be harmless to ATP7B protein may be involved in the pathogenesis of WD.

Abbreviations: ATP7B = ATPase copper transporting beta, KF = Kayser–Fleischer, NDVs = non-disease-causing variants, SNPs = single nucleotide polymorphisms, TM = transmembrane domains, WD = Wilson disease.

Keywords: ATPase copper transporting beta, bioinformatics, mutation, single nucleotide polymorphism, Wilson disease.

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1. Introduction

Wilson’s disease (WD; MIM 277900) is an autosomal recessive copper metabolism disorder, including inadequate incorporation of copper into apoceruloplasmin and impaired biliary copper excretion. This metabolic disorder leads to copper accumulation mainly in the liver and brain and concomitantly, which causes hepatic, neurological or psychiatric impairment, Kayser-Fleischer (KF) rings and other complex clinical manifestations. Although copper metabolism disorder begins at birth, symptoms do not usually develop until the age of 3. In most patients, symptoms appear between 5 and 35 years of age.[1] Pediatric WD patients mainly show liver symptoms, whereas neuropsychiatric symptoms predominate in adults.[2]

ATPase copper transporting beta (ATP7B) is the know WD causative gene, located on chromosome 13q14.3 and encodes a copper-transporting P-type ATPase that contains 1465 residues. This protein is formed by 6 mental binding units, 8 transmembrane domains (TM), an actuator domain (A-domain), a phosphorylation domain (P-domain) and a nucleotide binding domain (N-domain).[3] More than 700 mutations and about 800 single-nucleotide polymorphisms (SNPs) have been identified in ATP7B, but only a few have been studied experimentally.[4] The study of function, stability and traffic of ATP7B is difficult for it is a large transmembrane protein. Combined probability (cPdel) can be a useful algorithm to estimate an amino acidic change in proteins. This algorithm is able to combine the results of the four most used bioinformatics tools (Polyphen-2, SIFT, PANTHER, and PhD-SNPs) to evaluate the SNPs effect on the protein function.[5–8] The combined probability of an amino acid changes deleterious to protein function was predicted as Polimanti et al. described with slight modification.[3] We obtained information from four software programs to calculate cPdel: Polyphen-2,[3] SIFT,[6] PhD-SNP[8] PANTHER-PSEP.[7] PANTHER-PSEP estimates the likelihood of an amino acid change to impact the structure or function of a protein by calculating the time period preserved in the evolutionary lineage leading to the protein. We assigned a score of 0 for a likely benign change (time < 200 million years, my), a score of 0.5 for a possibly damaging change (450my > time > 200my) and a score of 1 for a probably damaging change (time > 450my). Hence, we calculate cPdel according to following equation:

\[
cPdel = \text{mean} \left( \frac{\text{Score Polyphen-2} + \text{Score SIFT} + \text{PANTHER-PSEP} (\text{probably damaging}) + \text{PANTHER-PSEP (neutral})}{4} \right)
\]

The cPdel score ranges from 0 (with no impact on function) to 1 (complete loss of function).

3. Results

3.1. Case reports

Fourteen children underwent a careful physical examination and were diagnosed with WD (Table 1). Eleven cases presented mainly liver dysfunction and were diagnosed before 10 years of age, while cases 12 and 13 presented neurological symptoms and case 14 showed both hepatic and neurological disorders.

Cases 1, 3, 4, 8, and 9 were discovered after physical examination of school children without obviously symptoms. Case 12 presented dribbling, involuntary tremor and KF rings, and magnetic resonance imaging showed a low symmetrical signal from the bilateral basal ganglia and the substantia nigra. Case 13 showed map-like brain waves, KF rings, low ceruloplasmin when he had fever and cough at age of 12. His parents reported he experienced Henoch-Schonlein purpura. Case 14 visited our hospital due to a sudden loss of consciousness, limbs convulsion and involuntary staring when he was 12 years old. Physical examination revealed KF rings, abnormal intermittent epileptic discharge and defective electroencephalogram. His parents reported that the development of his intelligence and language functions was retarded at 2 years of age.

3.2. Analysis of mutations in the ATP7B gene

Thirteen mutations were detected in our present analysis (Table 2), including two unprecedented mutations (c.1448_1455del and c.4144G>T) (Fig. 1) and eleven previously reported. Of those, eleven were found to be compound heterozygotes, and c.2333G>T found to be homozygous (Table 2). The c.2333 G>T (p.Arg778Gln) variant is a hotspot
mutation in Chinese people and was identified in patients 1, 3, 4, 11 and 12 (Table 2). In contrast to other patients, only one missense mutation (c.2621C>T) in ATP7B was identified in case 14. The other variants (c.2495A>G, c.2855G>T, c.3419C>T, c.3903+6 C>T) identified in this case were previously reported as NDVs (Table 3).

ATP7B is a transmembrane enzyme that contains 1465 amino acids. The new ATP7B deletion variant c.1448_1455del was identified in case 7, which had liver disorders. This mutation changes the reading frame and introduces an early termination codon in the N-terminal region (Fig. 1 A and C). Consequently, variant c.1448_1455del results in a shortened and functionless protein that lacks 5 vertebrate species, including Pan troglodytes, Macaca mulatta, Mus musculus and Rattus norvegicus (Fig. 1E).

### Table 1
Clinical data of pediatric patients with WD at diagnosis.

| Patients No | Sex  | Age at diagnosis | ALT (U/L) | AST (U/L) | Ceruloplasmin (mg/dl) | K-F ring | Symptoms                        |
|------------|------|------------------|-----------|-----------|-----------------------|----------|---------------------------------|
| 1          | Male | 7                | ND        | ND        | ND                    | –        | No apparent symptoms            |
| 2          | Female | 4             | 248.3     | 168.3     | 0.64                   | –        | Abdominal pain                  |
| 3          | Male  | 3                | 61        | 42        | 0.096                 | ND       | No apparent symptoms            |
| 4          | Male  | 8                | 30.4      | 62.4      | 1                     | +        | No apparent symptoms            |
| 5          | Male  | 4                | 175       | 131       | ND                    | –        | –                               |
| 6          | Female | 8               | ND        | ND        | ND                    | ND       | No apparent symptoms            |
| 7          | Male  | 6                | 109       | 143       | ND                    | –        | –                               |
| 8          | Female | 7               | 105       | 72        | 0.03                  | –        | –                               |
| 9          | Female | 4               | 105       | 72        | 0.03                  | –        | –                               |
| 10         | Male  | 6                | 149       | 86        | ND                    | ND       | ND                              |
| 11         | Female | 6               | 81        | 62        | ND                    | ND       | ND                              |
| 12         | Female | 11              | 16        | 22        | +                     | A        | –                               |
| 13         | Male  | 12               | 63        | 54        | +                     | cough and fever | – |
| 14         | Male  | 12               | 194       | 73.8      | 0.03                  | ND       | B                               |

ND = not defined; a. salivation, unclear speech, slow writing, fonts askew; b. Loss of consciousness, limb convulsions involuntary staring.

### Table 2
Distribution of mutations detected in the ATP7B gene.

| Patients no | Mutation | Amino Acid | Area of Protein | Exon | Cellular Localization |
|-------------|----------|------------|-----------------|------|----------------------|
| 1           | c.2333G>T | p.Arg778Gln | TM4             | 8    | Transmembrane        |
| 2           | c.2809A>G | p.Asn1270ser | TM6             | 13   | Cytoplasm            |
| 3           | c.2975C>T | p.Pro992Leu | TM6             | 13   | Transmembrane        |
| 4           | c.2668G>A | p.Val890Met | TM4             | 11   | Cytoplasm            |
| 5           | c.2333G>T | p.Arg778Gln | TM4             | 11   | Transmembrane        |
| 6           | c.2304dupC | p.Met769HisfsX26 | TM4         | 8    | Transmembrane        |
| 7           | c.1448_1455del | p.Arg483Serfs X19 | Mbu4/Mbu5 | 3    | Cytoplasm            |
| 8           | c.2975C>T | p.Pro992Leu | TM6             | 13   | transmembrane        |
| 9           | c.4144G>T | p.Glu1382Stop | After TM6     | 21   | Cytoplasm            |
| 10          | c.3517G>A | p.Glu1173lys | ATP bind       | 16   | Cytoplasm            |
| 11          | c.3955C>T | p.Asp1319Glu | TM7             | 19   | transmembrane        |
| 12          | c.2294A>G | p.Asp765Gly | TM4             | 4    | Transmembrane        |
| 13          | c.2333G>T | p.Arg778Gln | TM4             | 8    | Transmembrane        |
| 14          | c.2975C>T | p.Pro992Leu | TM6             | 13   | Transmembrane        |
| 15          | c.2662A>C | p.Thr888Pro | TM4/A-domain/TM5 | 11   | Cytoplasm            |
| 16          | c.3316G>A | p.Val1106Ile | ATP loop       | 15   | Cytoplasm            |

ATP7B = ATPase copper transporting beta, Mbu = mental binding units, TM = transmembrane domains.

* new mutations in this reporter.
3.3. cPdel revealed that some SNPs may be deleterious to the ATP7B function

As expected, WD-causing variants obtained high cPdel values (0.746; Table 4), which predict undesirable effects on the ATP7B function. This result indicates that cPdel is a reliable bioinformatics predictor of the impact of SNPs on the ATP7B function. The previously reported c.2621C>T (p.Ala874Val) mutation and 4 NDVs were detected in case 14. cPdel score of c.2621C>T (p.Ala874Val) variant is 0.999. Considering that SNPs contribute to the pathogenesis of WD, we estimated the cPdel of the three SNPs using data obtained by Polyphen-2, SIFT, PANTHER-PSEP and PhD-SNP softwares (Table 4). The score of variant c.2495A>G

Table 3

| Exon | Variant name | Amino acid change | Genotype | Area of protein | Cellular localization |
|------|--------------|-------------------|----------|-----------------|----------------------|
| 10   | c.2495A>G    | p.Lys832Arg       | Heterozygotes | TM4/A-domain/TM5 | Cytoplasm            |
| 11   | c.2621C>T    | p.Ala874Val       | Heterozygotes | TM4/A-domain/TM5 | Cytoplasm            |
| 12   | c.2855G>T    | p.Arg952Lys       | Homozygotes | TM5/TM6         | Lumen                |
| 16   | c.3419C>T    | p.Val1140Ala      | Homozygotes | N-domain: ATP bind | Cytoplasm            |
| 18   | c.3903+6 C>T | Splice            | Heterozygotes | Bet ATP hinge/TM7 | Transmembrane        |

WD = Wilson disease, TM = transmembrane domains.
(p.Lys832Arg) was 0.423 in Polyphen-2 and 0.087 in SIFT1. PANTHER-PSEP estimated that Lys832 has been preserved for 750 million years and that Lys832Arg could be a damaging mutation that obtained a score of 1.000. PhD-SNP predicted that Lys832Arg is a neutral causative variant that obtained 0 score. c.2855G>T (p.Arg952Lys) variant obtained a cPdel value of 0.584. The SNP c.2855G>T (p.Arg952Lys) obtained 0.000 and 1.000 scores in Polyphen-2 and SIFT softwares, respectively. According to the PhD-SNP software, this variant would be a disease causing SNP, but probably a benign change (6 million years) according to PANTHER-PSEP. At last, the cPdel value of variant c.2855G>T (p.Arg952Lys) is 0.25 (Table 4). The SNP c.3419C>T (p.Val1140Ala) obtained 0.000, 0.914, neutral and probably benign (6 million years) as the results of the 4 predictors, resulting in a cPdel score of 0.022 (Table 4). These results showed that each SNP has different effects on protein function and that SNP with moderate cPdel scores can be deleterious to ATP7B function.

4. Discussion and conclusion

WD may present at any age with variable symptoms of liver disease. Copper accumulates in liver tissue during childhood, so that abnormal liver function test results may occur long before symptom onset. 12 WD individuals were showed elevated serum aminotransferases concentrations due to healthy test or WD dis-associated lab examinations. Diagnostic testing for should be taken once increased aminotransferases are observed in WD pediatric cohor.

In this study, we analyzed ATP7B mutations in 14 WD children and identified two, c.1448_1455del (p.Arg483-Ser56X19) and c.4144G>T (p.Glu1382stop), which had not been previously reported. The mutation c.1448_1455del was found in case 7 and occurred as a compound heterozygote with mutation c.2621C>T. The other new mutation was found in case 8 as a compound heterozygote with c.2975C>T (p.Pro992Leu). c.2621C>T is one of the most frequent mutations in Chinese people and was found in cases 3, 5 and 14. Case 5 is a c.2621C>T homozygote with hepatic symptoms, case 3 showed hepatic symptoms, while case 14 showed severe neurological degneration than liver disorders. In case 14, only c.2621C>T mutation was identified, indicating that other mutations may have occurred in ATP7B or other genes. Nevertheless, these results suggested that the phenotype of the c.2621C>T mutation presented a hepatic WD with a relatively early onset and that the WD symptoms are a consequence of each mutation-specific phenotype.

SNPs are the largest source of variation in the human genome. Approximately 800 SNPs have been identified in ATP7B gene, some of which can modulate the cellular and biochemical properties of the ATP7B protein.[4,13] cPdel is an index that combines the outcomes of four popular bioinformatics tools (Polyphen-2, SIFT, PhD-SNP and PANTHER-PSEP) widely used to predict the functional impact of SNPs on gene function.[3] In case 14, 1 mutation c.2621C>T (p.Ala874Val) and 3 SNPs were identified in ATP7B gene; however, there may be other mutations, such as splicing mutation that impair protein function. We observed that cPdel scores varied between SNPs. Val1140Ala is an experimentally validated mutation that is deleterious to the ATP7B function. This mutation obtained a cPdel score of 0.022. The variant Lys832Arg obtained a cPdel score of 0.548, which is more than twice as high as that obtained by the Arg952Lys variant (0.25). The Lys832 residue is located in the actuator domain between TM segments 4 and 5, constitutes the antiparallel β3-strand and affects the conformational dynamics of the A-domain.[14,15] Moreover, R832 has been identified as a loss-of-function SNP in Drosophila melanogaster ATP7.[15,16] These findings suggest that SNPs may contribute to WD progressing, and that cPdel score could be an easy and useful tool for quickly assessing the effect of SNPs on the ATP7B function.

In summary, genetic analysis can be a very useful tool for diagnosis WD, as biochemical tests may be insufficiently sensitive in very young children. Our study enriched the library of ATP7B mutations involved in WD development. ATP7B is a large copper-transporting ATPase that plays a key role in regulating copper homeostasis. Given that it is difficult to trace new genetic variants in ATP7B experimentally, the cPdel bioinformatic method can be a useful and simple tool for the first screening of mutations in the ATP7B gene and to valuate the SNPs effect on ATP7B function.

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