Letter to the Editor

Novel mutations of wolframin: a report with a look at the protein structure

To the Editor:

Wolfram syndrome (WS), also known as DIDMOAD, is a rare genetic disorder related to the WFS1 gene, located on chromosome 4p16.1. Diagnosed by the presence of non-immunogenic diabetes mellitus and a progressive atrophy of the optic nerve, patients with WS may also show central diabetes insipidus, sensory neural hearing loss (1), neurodegenerative signs (2, 3), endocrine problems (3, 4), and psychological complications (5). Detecting mutations is important with regard to genetic counseling (6), and early treatment of WS (7). Here, we present a report of the first molecular-based study on WS in the Iranian population.

Seven WS patients (three females and four males), aged 16–35, entered the study based on the presence of diabetes mellitus in childhood and adolescence with optic nerve atrophy. In Table 1, a summary of their clinical and laboratory findings is shown.

Blood samples were collected from patients and all available family members after obtaining informed consents. In the extracted DNA from cases and several normal control subjects, WFS1 gene exon 8 [location of most causative changes (8)] was screened for mutations. Polymerase chain reaction (PCR) amplification followed by direct sequencing was used and both DNA strands were included. The gene product (wolframin) sequence was used to translate the detected mutation to protein level, with the help of the ExPASy Proteomics Server (http://www.expasy.ch/tools/dna.html). Mutations found believed to be possibly related with the patient’s condition are shown in Table 1.

Seven common polymorphisms were also detected. These included c.1185 C>T, c.2433 G>A, c.2565 A>G (neutral), c.997 A>G (I332V), c.1762 T>G (W588G), c.1832 G>A (H611R), and c.1963 G>A (E655K). Search in literature showed that some of these have been previously reported (Table 2) (9–15).

Topology prediction of the HMMTOP server (http://www.enzim.hu/hmmtop) (16) was used as a model of wolframin. Nine transmembrane (TM) segments were predicted, which are in accordance with experimental data (17) (Fig. 1).

c.2149 G>A resulting in missense mutation E717K was seen in all patients. Patient 1, bearing only this mutation, showed an early onset of diabetes mellitus, and appearance of four cardinal signs of WS until age 15. E717 is located in the endoplasmic reticulum lumen (Fig. 1), where it is suggested to interact with the β1 subunit of sodium–potassium ATPase (SPA) as part of the C-terminal (amino acids 652–890). A glutamate to lysine mutation resulting in a charge reversal may affect this interaction. SPA insufficiency (e.g. in W700X mutants of wolframin) is related to neurodegenerative disease (18).

Based on the rarity of its incidence in other populations (19, 20), it could be suggested that E717K would be a potential founder mutation of WS in the Iranian population, but this assumption needs to be tested on a higher number of patients.

The siblings (patients 2–5) were found to show a quite similar clinical presentation, with light variations in the onset age of symptoms. All of them were also found to possess the same mutations. The principal one was c.1456 C>T resulting in the non-sense mutation Q486X, which causes truncation of the protein after the fifth TM segment (Fig. 1), leading to a non-functional protein. This makes the mutation c.2149 G>A only observable on the gene level.

Patient 6 is the only one showing hypogonadism; in this patient, manifestations of WS had started at the age of 10, and all the DIDMOAD signs had appeared at age 15. Mutations c.1763 G>A leading to the non-sense mutation W588X and c.2051 C>G, which could potentially cause a missense mutation A684G, are both novel. W588X would cause a truncation in the protein just after the seventh TM segment, which is a possible cause of the severity of observed symptoms. It is also
interesting to note that c.2051 seems to be a position that would be particularly prone to mutation (Lesperance database, and polymorphism W588G of the present study).

Patient 7 is the only one that does not suffer from diabetes insipidus. That would make his symptoms slightly less significant than the other cases, but his lower age should also be noted. Mutations found in this patient include the novel mutation E752K, which is comparable to E717K. Non-sense mutation E752X has been previously reported (3, 8).

Correlating the WS phenotype and genotype is still difficult, and needs more data analysis, both on the genetics/proteomics and clinical levels:

- Patients may lack one of the DIMOAD symptoms (3, 21), which could be related to the patients’ onset age, whose condition could worsen afterwards.

Fig. 1. Hypothetical model of wolframin. Nine transmembrane segments are shown, alongside with the position of found mutations. Residues 314-333, 342-361, 405-423, 432-451, 464-481, 496-515, 528-549 564-583, and 635-652 compose the membranes. Mutations found in the patients are indicated with square symbols, and novel mutations are underlined. ER, endoplasmic reticulum. The figure was drawn with the use of TOPO2 (Johns S. J., TOPO2, Transmembrane protein display software, http://www.sacs.ucsf.edu/TOPO2/).
Table 1. Patients characteristics, clinical findings, and genetic experiments results

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age</th>
<th>Sex</th>
<th>DI</th>
<th>DM</th>
<th>OA</th>
<th>D</th>
<th>Urinary tract dilatation</th>
<th>Primary hypogonadism</th>
<th>Neuropsychiatric problems</th>
<th>Growth hormone deficiency</th>
<th>Consanguinity</th>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
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<tr>
<td>1</td>
<td>20</td>
<td>M</td>
<td>6</td>
<td>2</td>
<td>13</td>
<td>15</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>8 e</td>
<td>c.2149 G&gt; A</td>
<td>E717K</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>F</td>
<td>11</td>
<td>8</td>
<td>15</td>
<td>20</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>8 e</td>
<td>c.2149 G&gt; A</td>
<td>E717K</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>F</td>
<td>12</td>
<td>9</td>
<td>17</td>
<td>22</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>8 e</td>
<td>c.1456 C&gt;T</td>
<td>Q486X</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>M</td>
<td>11</td>
<td>9</td>
<td>13</td>
<td>18</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>8 e</td>
<td>c.1456 C&gt;T</td>
<td>Q486X</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>F</td>
<td>10</td>
<td>8</td>
<td>20</td>
<td>20</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>8 e</td>
<td>c.1456 C&gt;T</td>
<td>Q486X</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>M</td>
<td>15</td>
<td>10</td>
<td>15</td>
<td>14</td>
<td>−</td>
<td>+</td>
<td>Seizure (grand mal)</td>
<td>−</td>
<td>+</td>
<td>8 e</td>
<td>c.2149 G&gt; A</td>
<td>E717K</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>M</td>
<td>3</td>
<td>9</td>
<td>12</td>
<td>16</td>
<td>−</td>
<td>Obsessive compulsive disorder</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>8 e</td>
<td>c.2149 G&gt; A</td>
<td>E717K</td>
</tr>
</tbody>
</table>

D, deafness; DI, diabetes insipidus; DM, diabetes mellitus; OA, optical atrophy.

Numbers in these columns indicate the starting age of each of the DIDMOAD symptoms. Patients 2–5 are siblings and have undergone operations for neurogenic bladder. Maternal uncle of patient 1, mother and maternal grandmother of patients 2–5, and mother of patient 6 suffered from type 2 diabetes. Nucleotide changes and corresponding base substitutions are also indicated.

Table 2. Detected polymorphism, their effects on the protein sequence and indication of whether these were found in a literature search or not

<table>
<thead>
<tr>
<th>Detected polymorphism</th>
<th>Type of polymorphism</th>
<th>Comments and references</th>
</tr>
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<tbody>
<tr>
<td>c.997 A&gt;G</td>
<td>Non-synonymous: I332V</td>
<td>I333V has been previously reported (rs1801212) (3, 9, 10); no report was found for I332V</td>
</tr>
<tr>
<td>c.1185 C&gt;T</td>
<td>Synonymous: V395V</td>
<td>rs1801206 (3, 8–14)</td>
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<tr>
<td>c.1762 T&gt;G</td>
<td>Non-synonymous: W588G</td>
<td>No report was found</td>
</tr>
<tr>
<td>c.1832 G&gt;A</td>
<td>Non-synonymous: H611R</td>
<td>rs734312 (3, 8–10, 12–15)</td>
</tr>
<tr>
<td>c.1963 G&gt;A</td>
<td>Non-synonymous: E655K</td>
<td>No report was found</td>
</tr>
<tr>
<td>c.2433 G&gt;A</td>
<td>Synonymous: K811K</td>
<td>rs1046314 (3, 8–14)</td>
</tr>
<tr>
<td>c.2565 A&gt;G</td>
<td>Synonymous: S855S</td>
<td>rs1046316 (3, 8–14)</td>
</tr>
<tr>
<td>c.2966 A&gt;G</td>
<td>Synonymous: S855S</td>
<td>rs1046316 (3, 8–14)</td>
</tr>
<tr>
<td>c.1185 C&gt;T</td>
<td>Synonymous: V395V</td>
<td>rs1801206 (3, 8–14)</td>
</tr>
<tr>
<td>c.1762 T&gt;G</td>
<td>Synonymous: W588X</td>
<td>(novel)</td>
</tr>
<tr>
<td>c.2433 G&gt;A</td>
<td>Synonymous: K811K</td>
<td>(novel)</td>
</tr>
<tr>
<td>c.2565 A&gt;G</td>
<td>Synonymous: S855S</td>
<td>(novel)</td>
</tr>
</tbody>
</table>

Some genotypes would result in milder manifestations of WS (22).

In conclusion, this study has added to the known mutational spectrum of the WFS1 gene, and will hopefully be a contribution to a better understanding of the disease pathophysiology.

References
References


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