Wilson’s disease: a patient undiagnosed for 18 years

Wilson’s disease, an autosomal recessive disorder of copper metabolism, is the most common inherited hepatic disease in Hong Kong. Diagnosis is based on the presence of Kayser-Fleischer rings, typical neurological symptoms, and/or a low serum ceruloplasmin concentration (<0.20 g/L). Early detection and treatment protect patients and their presymptomatic siblings from devastating organ damage. The diagnosis of Wilson’s disease may nonetheless be overlooked if only established clinical and laboratory tests are used as diagnostic criteria. We report diagnosis of the disorder using genetic analysis of ATP7B in a presymptomatic sibling who escaped diagnosis during family screening 18 years previously. The patient was 11 months old when family screening was performed following diagnosis of Wilson’s disease in an elder sister. The boy was considered to be unaffected on the basis of laboratory results in the expected range: serum copper level, 4.6 µmol/L; serum ceruloplasmin level, 0.16 g/L; and 24-hour urinary copper excretion, 0.14 µmol/day. Molecular analysis of ATP7B was performed; it revealed that the two siblings shared the same compound heterozygous mutations (G943D and 2299delC). We recommend that molecular diagnosis is the only definitive means of diagnosing Wilson’s disease in children younger than 1 year.

Introduction

Wilson’s disease (WD) is an autosomal recessive disorder of hepatic copper metabolism, first described as progressive hepatolenticular degeneration by Wilson in 1912.¹ A detailed pathogenesis of the disorder remains obscure despite knowledge of its existence for almost a century. Fortunately, clinical phenotypes, diagnostic biochemical markers, and effective treatment are well established. Defective copper excretion leads to systemic accumulation of copper that gives rise to typical phenotypes that include progressive liver damage, neurological deficits, psychiatric illness, presence of Kayser-Fleischer (KF) rings, renal tubular disorders, arthropathy, cardiomyopathy, and hypoparathyroidism.² The worldwide prevalence has been reported to be approximately 1 in 30 000 with a carrier rate of 1 in 90. Wilson’s disease is the most common inherited hepatic disease in Hong Kong. Diagnosis is based on at least two of the following: detection of KF rings on slit-lamp examination, typical neurological symptoms, and/or a low serum ceruloplasmin (Cp) concentration (<0.20 g/L). Early detection and treatment protect patients from devastating organ damage. Timely diagnosis of WD benefits the patient as well as presymptomatic but affected family

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members, who may be missed if only established clinical and laboratory tests are used as diagnostic criteria. We report on a patient in whom the diagnosis was missed for 18 years. Family screening had been previously performed when the patient was 11 months old. Wilson’s disease was ultimately diagnosed by genetic testing.

Case report

In 1986, a 4-year-old female (II-1) first presented with liver impairment associated with generalised malaise and hepatomegaly. Laboratory investigations revealed a deranged liver function profile: alanine transaminase (ALT), 347 U/L; alkaline phosphatase (ALP), 230 U/L; and total bilirubin, 13 µmol/L. Hepatitis markers were all negative. Further results demonstrated serum copper was 8.3 µmol/L (reference range, 12-25 µmol/L), serum Cp 0.16 g/L (0.18-0.38 g/L), and 24-hour urinary copper excretion 2.9 µmol/day (<1.0 µmol/day). Wilson’s disease was diagnosed and she was prescribed penicillamine. Family screening for WD was performed in her father (I-3), mother (I-4), two paternal aunts (I-1 and I-5), an elder paternal uncle (I-2), and her 11-month-old brother (II-2). None of them was thought to be affected. The patient (II-1) defaulted from further follow-up until 12 years later in 1998 when she presented in acute hepatic failure with abdominal distension, jaundice, and hepatomegaly. No neurological deficit was noted at the time. She was referred to the Queen Mary Hospital for living-related liver transplantation at the age of 18 years with her paternal aunt...
Peripheral blood samples were collected from I-1, I-2, I-3, I-4, I-5, II-1, and II-2 after informed consent was obtained. Genomic DNA was extracted using a QIAamp Blood Kit (Qiagen, Hilden, Germany). The coding exons and the flanking introns of the ATP7B gene were amplified by polymerase chain reaction (PCR). The PCR products were sequenced directly by BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, US).

Two known disease-causing mutations of ATP7B were found in the proband II-1, namely glycine-to-aspartate substitution at codon 943 (G943D) and a deletion of cytosine at nucleotide 2299 (2299delC) [Fig b]. The younger brother (II-2), now aged 18 years, was also found to be a compound heterozygote. Serum copper and Cp were analysed again in view of these results (Fig a).

**Discussion**

We confirmed the diagnosis of WD using genetic analysis of ATP7B in an 18-year-old boy, whose diagnosis was initially missed during previous family screening based solely on biochemical investigations. Copper homeostasis is mainly regulated by biliary excretion, and only about 10% of the absorbed copper is incorporated into Cp that is secreted into the peripheral circulation. Ceruloplasmin binds more than 95% of plasma copper and thus protects peripheral cells from free copper toxicity. It is synthesised in hepatocytes and secreted into the circulation, with copper incorporated during transit through the late secretory pathway. In WD, the defective ATP7B protein fails in biliary copper excretion and copper incorporation into apoceruloplasmin (apoCp) which is devoid of copper. Most apoCp is degraded intracellularly, but moderate amounts are released into the circulation where apoCp has a very short half life of a few hours compared with several days for holoceruloplasmin (holoCp). This explains the significantly reduced serum Cp concentration in WD. Intriguingly, despite WD being a disorder of copper overload, total serum copper is reduced as a result of holoCp deficiency, even though elevated free copper concentrations lead to unrelenting systemic damage. In our experience, Cp is the most sensitive biochemical marker for the diagnosis of WD. Most patients with WD have a serum Cp level of less than 0.10 g/L. Nevertheless, it is important to realise that several other factors, including acute hepatic failure of any cause, nephrotic syndrome and protein-losing enteropathy, malnutrition, and hereditary hypo-aceruloplasminaemia, can influence serum levels. We should also bear in mind that about 10% of homozygotes may show normal Cp at the time of diagnosis, especially during the acute phase of reaction, whereas a similar percentage of heterozygotes may have reduced levels.

In WD, the serum free copper concentration is elevated. This fraction is indirectly measured by the non-Cp–bound serum copper. It is calculated using the formula: total serum copper (µmol/L) – 47 × serum Cp (g/L). An important assumption of this formula is that all of the serum Cp measured are holoCp replete of copper. Serum Cp can be measured by immunochemical or enzymatic activity methods, expressed in mass unit g/L and in activity unit µmol/L/min, respectively. Because the former measures both holoCp and apoCp, immunochemical methods may give higher results. Therefore, a negative value can be commonly obtained when serum free copper concentration is calculated using the immunochemically determined Cp concentration. However, the validity of this equation has been seriously challenged and we recommend the direct measurement of serum-free copper instead. On the other hand, some homozygotes with normal Cp concentration were reported to have negative Cp oxidase activity revealing the circulating apoCp. Since each Cp carries six copper atoms, their concentrations are positively correlated provided that holoCp is measured. Multiplying the Cp result (g/L) by 47 gives its contribution to serum copper in µmol/L. It is helpful to check that serum copper and Cp results are compatible with one another, especially when spurious results are observed, for example, due to copper contamination and laboratory error.

Age- and sex-specific reference ranges should be provided to enable accurate interpretation of laboratory results when the analyte is known to be age- and/or sex-dependent. Serum Cp concentration is lower in neonates of 25% to 40% of the normal adult level and usually reaches adult levels by the age of 6 months. It further increases and reaches its maximum at 2 to 3 years of age, then falls slowly until the teenage years when adult levels are finally reached. For patient II-2, the reference ranges quoted in the previous laboratory report were those for an adult. If the paediatric reference ranges are applied to the early results for II-2 (ie serum copper, 3.8–23.8 µmol/L; serum Cp, 0.15–0.48 g/L in 1 to 12 months old), the results (serum copper, 4.6 µmol/L; serum Cp, 0.16 g/L) would have been within the normal ranges. It is noteworthy that since the original assay methods are unknown, the reference ranges quoted here may not apply and are thus just for general reference. Reference ranges are usually method-dependent and advice from the manufacturer should be sought if local data are not available. It should be the responsibility of the laboratory to provide evidence-based and valid information on the report to ensure proper interpretation. A chemical pathologist should be consulted when there is any doubt. Age-specific reference ranges of serum copper and Cp are listed in the Table.

Shimizu et al have reported the youngest child (8 months old) to be diagnosed with WD detected through a mass screening system using serum Cp. In contrast to his greatly decreased serum Cp (0.01 g/L), our case illustrates that none of the conventional biochemical markers is reliable in diagnosing WD in paediatric patients, especially in those under 1 year of age. We speculate that the discrep-
The diagnostic challenges of WD cannot be overemphasised and the limitations of current clinical and biochemical tests, especially when performed on the very young, should be borne in mind. Tests should be repeated at a later stage if doubt remains. Nevertheless, diagnosis should be made as early as possible so that an optimal clinical outcome can be ensured with prophylactic treatment. Since the total body copper load is expected to be low in young patients, decoppering agents such as penicillamine are less preferable because of their adverse effects. Zinc is safer and lacks the adverse effects on growth. Marcellini et al.\textsuperscript{12} have demonstrated the excellent effectiveness of zinc in disease control in a cohort study of 22 paediatric patients with WD spanning 10 years.

Hepatic dysfunction usually precedes neurological abnormalities in WD. Disease phenotypes and the age of onset are known to be almost identical among sibling patients.\textsuperscript{13} Interestingly, these two siblings who shared the same mutations presented with two markedly different phenotypes. Patient II-1 presented with hepatic dysfunction in her early childhood while II-2 remained fairly asymptomatic until the teenage years. Mild hand tremors were noticed only recently. His ALT level was 131 U/L and ALP level was 134 U/L with normal total bilirubin. It is postulated that other factors may modulate the clinical manifestations, for example ApoE\textsuperscript{14} and MURR1,\textsuperscript{15} and varying dietary copper contents. This possible marked intra-familial phenotypic variation attests to the importance of screening by DNA-based testing for family members at all ages once a proband is diagnosed.

With the advent of molecular biology, it should no longer be reserved as a research tool. The diagnostic approach is robust and should be deployed more liberally in clinical diagnosis, especially where there is already an established case within the family. The culprit \textit{ATP7B} gene consists of 21 exons that span a genomic region of about 80 kb and encode a protein of 1465 amino acids. Mutant \textit{ATP7B} results in defective copper incorporation into Cp and a reduction in biliary copper. To date, 287 mutations have been reported worldwide.\textsuperscript{16} Most are missense mutations and small deletions/insertions. The spectrum of mutations is population-specific: the most common European mutation is H1069Q with a frequency of 26\% to 70\%.\textsuperscript{17} and

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**Table. Age-specific reference ranges\textsuperscript{*} for (a) serum copper and (b) serum ceruloplasmin\textsuperscript{5,9}

### (a)

<table>
<thead>
<tr>
<th>Age-group</th>
<th>Serum copper ((\mu)mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td></td>
</tr>
<tr>
<td>0 to &lt;3 m</td>
<td>5.9-16.3</td>
</tr>
<tr>
<td>6 m to &lt;1 y</td>
<td>3.8-23.8</td>
</tr>
<tr>
<td>1 to &lt;2 y</td>
<td>11.9-30.3</td>
</tr>
<tr>
<td>2 to &lt;4 y</td>
<td>13.7-29.3</td>
</tr>
<tr>
<td>4 to &lt;6 y</td>
<td>8.8-30.0</td>
</tr>
<tr>
<td>6 to &lt;10 y</td>
<td>18.4-28.4</td>
</tr>
<tr>
<td>10 to &lt;14 y</td>
<td>13.7-28.5</td>
</tr>
<tr>
<td>14 to &lt;18 y</td>
<td>11.7-29.3</td>
</tr>
<tr>
<td>Adults (218 y)</td>
<td>11.0-25.0</td>
</tr>
</tbody>
</table>

### (b)

<table>
<thead>
<tr>
<th>Age-group</th>
<th>Serum ceruloplasmin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Children</td>
<td></td>
</tr>
<tr>
<td>1-30 d</td>
<td>0.07-0.25</td>
</tr>
<tr>
<td>31-365 d</td>
<td>0.15-0.48</td>
</tr>
<tr>
<td>1-3 y</td>
<td>0.25-0.56</td>
</tr>
<tr>
<td>4-6 y</td>
<td>0.29-0.56</td>
</tr>
<tr>
<td>7-9 y</td>
<td>0.25-0.52</td>
</tr>
<tr>
<td>10-12 y</td>
<td>0.21-0.51</td>
</tr>
<tr>
<td>13-15 y</td>
<td>0.20-0.50</td>
</tr>
<tr>
<td>16-18 y</td>
<td>0.20-0.45</td>
</tr>
<tr>
<td>Adults (&gt;18 y)</td>
<td>0.20-0.40</td>
</tr>
<tr>
<td>No oral contraceptives</td>
<td>-</td>
</tr>
<tr>
<td>With oral contraceptives</td>
<td>-</td>
</tr>
<tr>
<td>Pregnant women\textsuperscript{1}</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{*}Reference ranges, which may vary between different methods, are quoted here for general reference only

\textsuperscript{1}Second and third trimesters; levels increase with gestational age
in Asians R778L with a frequency of 28% to 44%. Genetic diagnosis confers superior diagnostic specificity and sensitivity over conventional biochemical tests, especially for family screening. With proper genetic counselling, similar tragedies can be prevented or reduced in subsequent generations with timely diagnosis and therapeutic intervention.

It has been argued that genetic screening is impractical for WD in view of the many different mutations and the technical expertise required. While it is generally true that molecular testing should not be used as a screening test where there is low clinical suspicion, it remains the most reliable method of determining the genetic status of siblings or other relatives when an index case has been identified.

In conclusion, we have reported on two siblings who presented with totally different phenotypes despite harbouring the same mutations (G943D and 2299delC). In the boy, diagnosis was confirmed after 18 years using genetic analysis of ATP7B.

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References