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Wilson's disease: a patient undiagnosed for 18 years

潛伏十八年的威爾遜氏病個案

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.

威爾遜氏病是一種銅代謝常染色體隱性失調，是香港最普遍的遺傳肝病，診斷徵狀為病人眼角膜邊出現棕色的凱 - 弗氏環、典型神經病徵和血清銅藍蛋白濃度過低（少於 0.20 g/L）。及早診斷和治療能避免病人和未顯現病徵的兄弟姐妹出現嚴重器官損害。然而，如果只進行常規的臨床及化驗檢測，很可能會診斷不到隱藏的威爾遜氏病。我們報告一宗利用酵素基因*ATP7B*分析診斷出威爾遜氏病的個案，患者未出現病徵，十八年前家庭檢查時亦沒有被診斷出來。當時他十一個月大，由於姐姐被診斷有威爾遜氏病而接受家庭檢查。當時化驗結果屬正常範圍：血清銅水平為4.6 μmol/L；血清銅藍蛋白水平為0.16 g/L；廿四小時尿銅水平為0.14 μmol/D。然而，現在以酵素基因*ATP7B*分子分析，顯示他們兩姐弟都有同樣的複合性異質性之突變（G943D 和 2299delC）。我們認為對一歲以下兒童進行檢查時，只有基因分析能確切診斷是否患有威爾遜氏病。

Key words:
Adenosinetriphosphatase/genetics;
Ceruloplasmin;
Copper/metabolism;
Hepatolenticular degeneration;
Liver diseases

關鍵詞：
腺嘌吟核苷三磷酸 / 基因；
血清銅藍蛋白；
銅 / 代謝；
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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

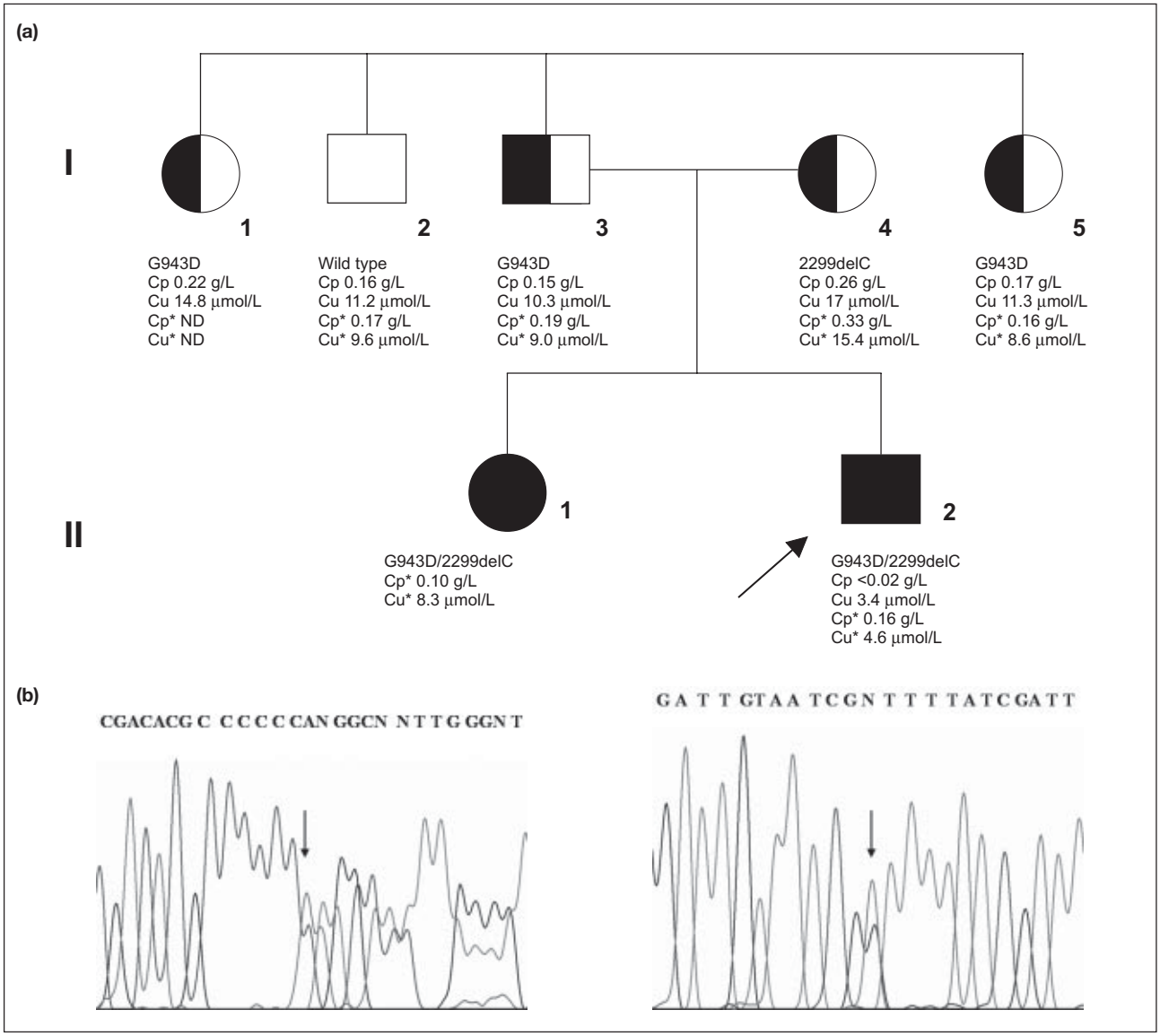

Fig. (a) Family pedigree of the reported case. (b) The DNA sequence (in sense direction) of II-2 shows 2299delC (left) and G943D (right) [arrows]

Cu* and Cp* denote serum copper and ceruloplasmin results reported at the time of II-1 being diagnosed; Cu and Cp serum copper and ceruloplasmin results performed during this study; and ND not done

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 0.10 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) [Fig a]. 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

(I-1) as donor and made a good recovery. The family was recruited for genetic analysis of *ATP7B* in 2002.

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[3]) and a deletion of cytosine at nucleotide 2299 (2299delC[4]) [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).[5] 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).[6] 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.[7] On the other hand, some homozygotes with normal Cp concentration were reported to have negative Cp oxidase activity revealing the circulating apoCp.[8] 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[9]), 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[10] 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-

Table. Age-specific reference ranges* for (a) serum copper and (b) serum ceruloplasmin[5,9]

(a)

Age-group	Serum copper (μmol/L)
Children	
0 to <6 m	5.9-16.3
6 m to <1 y	3.8-23.8
1 to <2 y	11.9-30.3
2 to <4 y	13.7-29.3
4 to <6 y	8.8-30.0
6 to <10 y	18.4-28.4
10 to <14 y	13.7-28.5
14 to <18 y	11.7-29.3
Adults (≥18 y)	11.0-25.0

(b)

Age-group	Serum ceruloplasmin (g/L)	
	Male	Female
Children		
1-30 d	0.07-0.25	0.03-0.28
31-365 d	0.15-0.48	0.15-0.43
1-3 y	0.25-0.56	0.29-0.54
4-6 y	0.29-0.56	0.26-0.54
7-9 y	0.25-0.52	0.23-0.48
10-12 y	0.21-0.51	0.21-0.48
13-15 y	0.20-0.50	0.21-0.46
16-18 y	0.20-0.45	0.22-0.50
Adults (>18 y)		
No oral contraceptives	0.20-0.40	0.25-0.60
With oral contraceptives or oestrogens	-	0.27-0.66
Pregnant women†	-	0.30-1.20

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

† Second and third trimesters; levels increase with gestational age

ancy between the Cp level at 11 months old and at 18 years old of II-2 may be attributed to the higher production of apoCp during infancy. Ceruloplasmin oxidase activity assay may have been able to confirm this reason if it has been done.

In addition, one should be extremely cautious in applying the usual diagnostic cut-offs for WD in infancy when liver function may not be sufficiently mature and dietary copper content is conceivably much lower than in a normal adult diet. The latter may also complicate the interpretation of urinary copper excretion and result in false-negative results. The results of two 24-hour urinary copper excretion calculations for II-2 performed at 11 months old were 0.19 μmol/day and 0.14 μmol/day. Care should be exercised to ensure accurate urine collection, although this is difficult to achieve in paediatric patients without a urinary catheter. Furthermore, the moderately elevated urinary copper may have been due to copper contamination if ordinary urine bags, which are not copper-free, were used, or if extraneous copper in tap water was accidentally added. Presymptomatic patients or heterozygotes may also demonstrate borderline results; further investigation is indicated in such cases. A penicillamine challenge test to increase urinary copper excretion would have been of little diagnostic value in this case, since the infant body copper is expected to be low and conceivably it would not give a definitive answer here. We do not recommend measuring urinary copper excretion in paediatric patients in whom accurate urine collection cannot be ensured. Measurement of liver copper content was once advocated as the definitive diagnosis for WD, especially in young patients with conflicting biochemical patterns. Apart from being an invasive procedure with inherent risk, up to 500 fold of copper content differences can occur due to sampling error and the heterogeneous distribution of liver copper.[11] We recommend that such an investigative procedure should be discouraged when molecular analysis is available.

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[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.[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 examples ApoE[14] and MURR1,[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 *ATP7B* gene consists of 21 exons that span a genomic region of about 80 kb and encode a protein of 1465 amino acids. Mutant *ATP7B* results in defective copper incorporation into Cp and a reduction in biliary copper. To date, 287 mutations have been reported worldwide.[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%[17] and

in Asians R778L with a frequency of 28% to 44%.[18] 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

1. Wilson SA. Progressive lenticular degeneration. A familial nervous disease associated with cirrhosis of the liver. Brain 1912;34:295-509.
2. Yarze JC, Munoz SJ, Friedman LS. Diagnosing Wilson disease. Ann Intern Med 1992;117:91.
3. Tsai CH, Tsai FJ, Wu JY, et al. Mutation analysis of Wilson disease in Taiwan and description of six new mutations. Hum Mutat 1998; 12:370-6.
4. Kim EK, Yoo OJ, Song KY, et al. Identification of three novel mutations and a high frequency of the Arg778Leu mutation in Korean patients with Wilson disease. Hum Mutat 1998;11:275-8.
5. Burtis CA, Ashwood ER, editors. Tietz textbook of clinical chemistry. Pennsylvania: WB Saunders; 1999.
6. O'Donnell JG, Watson ID, Fell GS, Allison ME, Russell RI, Mills PR. Wilson's disease presenting as acute fulminant hepatic failure. Scott Med J 1990;35:118-9.
7. Twomey PJ, Viljoen A, House IM, Reynolds TM, Wierzbicki AS. Relationship between serum copper, ceruloplasmin, and non-ceruloplasmin-bound copper in routine clinical practice. Clin Chem 2005;51: 1558-9.
8. Steindl P, Ferenci P, Dienes HP, et al. Wilson's disease in patients presenting with liver disease: a diagnostic challenge. Gastroenterology 1997;113:212-8.
9. Soldin SJ, Brugnara C, Wong EC, editors. Pediatric reference ranges. Washington DC: AACC press; 2003.
10. Shimizu N, Nakazono H, Takeshita Y, et al. Molecular analysis and diagnosis in Japanese patients with Wilson's disease. Pediatr Int 1999;41:409-13.
11. McDonald JA, Snitch P, Painter D, Hensley W, Gallagher ND, McCaughan GW. Striking variability of hepatic copper levels in fulminant hepatic failure. J Gastroenterol Hepatol 1992;7: 396-8.
12. Marcellini M, Di Ciommo V, Callea F, et al. Treatment of Wilson's disease with zinc from the time of diagnosis in pediatric patients: a single-hospital, 10-year follow-up study. J Lab Clin Med 2005;145: 139-43.
13. Ferenci P, Caca K, Loudianos G, et al. Diagnosis and phenotypic classification of Wilson disease. Liver Int 2003;23:139-42.
14. Wang XP, Wang XH, Bao YC, Zhou JN. Apolipoprotein E genotypes in Chinese patients with Wilson's disease. QJM 2003;96:541-2.
15. Stuehler B, Reichert J, Stremmel W, Schaefer M. Analysis of the human homologue of the canine copper toxicosis gene MURR1 in Wilson disease patients. J Mol Med 2004;82:629-34.
16. ATPase, copper transporting, beta polypeptide. The Human Gene Mutation Database website: http://uwcmml1s.uwcm.ac.uk/uwcm/mg/search/120494.html. Accessed 15 Nov 2005.
17. Hawkes ND, Mutimer D, Thomas GA. Generalised oedema, lethargy, personality disturbance, and recurring nightmares in a young girl. Postgrad Med J 2001;77:529,537-9.
18. Liu XQ, Zhang YF, Liu TT, et al. Correlation of *ATP7B* genotype with phenotype in Chinese patients with Wilson disease. World J Gastroenterol 2004;10:590-3.