

## ORIGINAL ARTICLE

# Identification of a novel Wilson disease gene mutation frequent in Upper Austria: a genetic and clinical study

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Wilson disease (WD), a disorder of copper metabolism is caused by mutations in the *ATP7B* gene, a copper transporting ATPase. In the present study we describe a novel mutation in exon 9 of the *ATP7B* gene. The *ATP7B* gene was analyzed for mutations by denaturing HPLC and direct sequencing. DNA from 100 healthy blood donors from the same geographic area was examined as control. Sixteen (7.4%) out of the 216 patients diagnosed with WD in Austria carried the newly identified 2447+1G>T(c.2448G>T) point mutation in exon 9 (4 male, age: 19 (6–30) years, median (range)). One patient was homozygous for 2447+1G>T(c.2448G>T). Thirteen patients were compound heterozygotes (p.H1069Q(c.3207C>A)/2447+1G>T(c.2448G>T) (N=6), P539L/2447+1G>T(c.2448G>T) (N=3), each one G710S/2447+1G>T(c.2448G>T), P767P(2299insC)/2447+1G>T(c.2448G>T), W779G/2447+1G>T(c.2448G>T), T1220M/2447+1G>T(c.2448G>T)). In two patients nose condensation was identified. Interestingly, all but three of the patients originated within a distinct geographical area in Austria. Eleven patients presented with hepatic disease, 3 patients with neurological disease and 2 were asymptomatic sisters of an index case. A liver biopsy was available in 14 patients. Three patients showed advanced liver disease with liver transplantation for acute hepatic failure in two. The remaining patients had only mild histological changes, most commonly steatosis. Chronic hepatitis was described in five patients. Kayser–Fleischer ring was present in five patients. None of the 100 healthy controls carried the mutation. We described a novel mutation in the *ATP7B* gene, occurring in patients originated from a distinct geographical area in Austria associated with a variable course of the disease.

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## INTRODUCTION

The hallmarks of Wilson disease (WD), an autosomal recessive inherited disorder of biliary excretion of copper, are the presence of liver disease and neurological symptoms due to accumulation of copper in liver and the brain.<sup>1</sup> Diagnosis of WD can be challenging in patients presenting with liver diseases, as serum ceruloplasmin may be in the low normal range, Kayser–Fleischer ring may be absent in up to half of the patients and hepatic copper content can be found below 250 µg g<sup>-1</sup> dry weight in up to 20% of patients.<sup>2,3</sup> Thus diagnosis of WD requires a combination of a variety of clinical and biochemical tests including genetic testing.<sup>4</sup>

The molecular basis of WD is the presence of mutations in the *ATP7B* gene located on chromosome 13. *ATP7b*—a copper transporting P-type ATPase—is the gene product of the *WD* gene.<sup>5–7</sup> *ATP7b* resides mainly in hepatocytes in the trans-Golgi network, transporting copper for incorporation into apoceruloplasmin and excretion into the bile.<sup>8</sup> More than 500 distinct mutations in the *ATP7B* gene,

including missense and nonsense mutations, insertions and deletions, have been described. Only a few have been found with high frequency in WD patients such as the p.H1069Q(c.3207C>A) amino-acid substitution, the most common mutation in patients of Middle or East-European ancestry.<sup>9,10</sup> In other regions, other mutations are more frequent, like in Sardinia (c.-441\_-427del), in Spain (p.Met645Arg(c.1934T>G), exon 6) or in the Canary Islands (p.Leu708(Proc.2123T>C), exon 8).<sup>11–13</sup> Most of the other mutations appear at low frequency and are associated with specific ethnic groups, or occur within single families.<sup>14–16</sup>

Knowledge of the regional distribution of mutations of the *WD* gene is important to allow testing for the most common mutations in a population, thereby obtaining a rapid diagnosis in unclear cases, while comprehensive genetic testing will take at least several weeks currently.<sup>17</sup> In addition, the molecular analysis of *ATP7B* is important to facilitate the subsequent screening of family members. In Austria the most common mutation is p.H1069Q(c.3207C>A) in exon 14

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with an allele frequency of 36%. Other common mutations include p.Gly710Ser (c.2128G>A), exon 8), p.P767P-fs (2298-2299insC, exon 8) and p.Arg969Gln(c.2906G>A, exon 13).<sup>9</sup> In the present study, we present clinical features of 16 patients with WD originating from a distinct geographical area in the federal state of Upper Austria carrying a novel mutation in exon 9 (2447+1G>T(c.2448G>T)) of *ATP7B*.

## PATIENTS AND METHODS

### Patients

Two hundred sixteen patients with WD were diagnosed in Austria since 1973, 194 of them are native Austrians (age at diagnosis: 3–61 years, m:91, f: 103, presenting symptom: hepatic: 123; neurological: 45; asymptomatic sibling: 26). Diagnosis was established based on the Leipzig score.<sup>4</sup>

### PCR and sequence analysis

The *ATP7B* gene was analyzed for mutations by denaturing HPLC and direct sequencing as described previously.<sup>3</sup> DNA from 100 healthy blood donors from the same geographic area was examined as control.

### Liver biopsy/hepatic copper determination

Liver biopsies were performed by the Menghini-technique and portal and periportal inflammation and fibrosis were graded/staged on hematoxylin/eosin and Masson trichrome stained sections according to Ludwig. Hepatic copper content was determined in liver biopsy specimens by atomic absorption spectroscopy ( $\text{Cu } \mu\text{g g}^{-1}$  dry weight liver tissue).<sup>3</sup>

### Control population

The control population consisted of 100 healthy blood donors from Upper Austria (where this mutation was found).

## RESULTS

Fifteen of 194 native Austrian WD patients carried the newly identified 2447+1G>T(c.2448G>T) point mutation in exon 9 (4 male/11 female, age: 20(6–30) median(range), 13 index patients, 2 asymptomatic siblings). One additional male patient carrying the mutation originated from Serbia. One patient was a homozygote, thirteen

patients were compound heterozygotes (6 patients: p.H1069Q (c.3207C>A)/ 2447+1G>T(c.2448G>T), 3 patients. P539L(c.1616C>T)/ 2447+1G>T(c.2448G>T), each one patient: p.Gly710Ser(c.2128G>A)/ 2447+1G>T(c.2448G>T), p.P767P-fs(c.2298\_2299insC)/2447+1G>T (c.2448G>T), W779G(c.2335T>G)/2447+1G>T(c.2448G>T) and p.Thr1220Met(c.3659C>T)/2447+1G>T(c.2448G>T). In two patients no second mutation could be identified (Table 1). Thirteen of the 15 Austrian patients originated within a distinct geographical area in the state of Upper Austria (Figure 1). Two patients were born in Tyrol. One female patient born in Tyrol presented with fulminant hepatic failure because of WD and underwent living donor liver transplantation, receiving liver segments II, III and IV from her mother (Table 1, patient no. 16). Her mother originated from Upper Austria and moved to Tyrol after her marriage. Genetic analysis revealed that the mother was a carrier of 2447+1G>T(c.2448G>T) and the father of W779G(c.2335T>G). The parents of the other child with WD born in Tyrol (patient no. 15, Table 1) originated from Ukraine and the federal state of Styria (Austria).

In total 28 cases with WD have been identified in Upper Austria comprising a population of 1.4 million people until now. Assuming a prevalence of 3/100 000 about 42 cases are expected to occur in this state.

Eleven (68.7%) patients presented with hepatic disease (one was the daughter of an index case, see Figure 2), 3 (18.7%) patients with neurological disease and 2 patients were asymptomatic sisters of an index case. Based on pedigree analysis spanning four generations none of the symptomatic patients were related.

A liver biopsy was available in 14 patients. Evaluation of hepatic parenchymal copper concentration ( $N=12$ ) revealed a median hepatic copper content of 603 (40–1360)  $\mu\text{g g}^{-1}$  dry weight. The histological evaluation of the patient with only 40  $\mu\text{g g}^{-1}$  copper content showed presence of liver cirrhosis. Two patients presented with advanced disease and fulminant hepatic failure of whom one underwent living donor liver transplantation from her mother and the other deceased donor liver transplantation. In two additional

**Table 1 Characteristics of patients carrying the 2447+1G>T(c.2448G>T) mutation**

Patient no.	Age <sup>a</sup> /sex	KFR	CPL	ATP7B <sup>b</sup>	Liver histology	HCuC	Presentation
1 <sup>c</sup>	30/M	No	21	H1069Q/2447+1G>T	Minimal changes	258	Hepatic
2 <sup>c</sup>	10/F	No	18.4	H1069Q/2447+1G>T	Advanced fibrosis	1278	FHF
3 <sup>c</sup>	12/M	Yes	11	H1069Q/2447+1G>T	Cirrhosis	40 <sup>d</sup>	Hepatic
4 <sup>c</sup>	15/M	No	2.1	H1069Q/2447+1G>T	Chronic hepatitis	Not available	Hepatic
5 <sup>c</sup>	25/F	Yes	1.0	2447+1G>T/?	Steatosis	540	Neurological
6 <sup>c</sup>	20/F	No	0.0	2447+1G>T/P539L	Minimal changes	960	Sister of no. 8
7 <sup>c</sup>	18/F	No	Not available	2447+1G>T/P539L	Chronic hepatitis	665	Sister of no. 8
8 <sup>c</sup>	21/F	No		2447+1G>T/P539L	Chronic hepatitis	Not available	Hepatic
9 <sup>c</sup>	18/F	No	15	G710S/2447+1G>T	Chronic hepatitis	1360	Hepatic
10	20/F	Yes	21	H1069Q/2447+1G>T	Not available	Not available	Neurological
11 <sup>c</sup>	26/F	Yes	1	2299insC/2447+1G>T	Minimal changes	359	Neurological
12 <sup>c</sup>	20/M	Yes	1	H1069Q/2447+1G>T	Cirrhosis	851	Hepatic
13 <sup>c</sup>	27/F	No	9.0	2447+1G>T/?	Steatosis	294	Hepatic
14 <sup>c</sup>	6/F	No	0.0	2447+1G>T/2447+1G>T	Steatosis	745	Hepatic (daughter of no. 13)
15	10/F	No	5	2447+1G>T/T1220M	Not done	Not done	Hepatic
16 <sup>e</sup>	8/F	No	11.8	2447+1G>T/W779G	Cirrhosis	122 <sup>d</sup>	FHF

Abbreviations: CPL, ceruloplasmin ( $\text{mg dl}^{-1}$ , normal  $\geq 20$ ); F, female; FHF, fulminant hepatic failure; HCuC = hepatic copper content ( $\mu\text{g g}^{-1}$  dry weight); KFR, Kayser–Fleischer ring; M, male.

<sup>a</sup>Age at diagnosis of WD.

<sup>b</sup>P539L: c.1616C>T; 2447+1G>T: c.2448G>T; H1069Q: c.3207C>A; G710S: c.2128G>A, P767P: fs 2298-2299insC; W779G: c.1616C>T; T1220M:c.3659C>T.

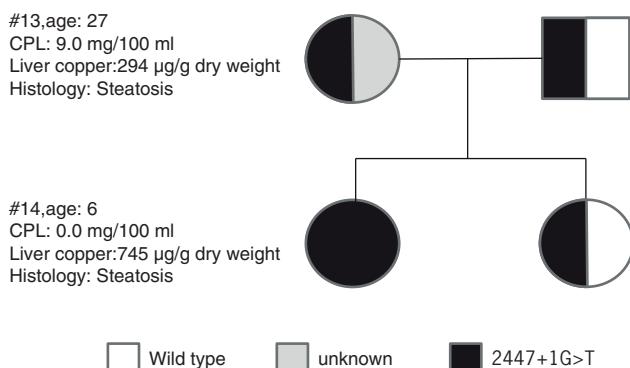
<sup>c</sup>From Upper Austria.

<sup>d</sup> $\mu\text{g g}^{-1}$  wet weight.

<sup>e</sup>Mother from Upper Austria.



**Figure 1** Geographical distribution of Austrian Wilson disease (WD) patients carrying the 2447+1G>T(c.2448G>T) mutation. The gray area marks the federal state of Upper Austria. The black spots indicate the region of origin of the patients with WD. A full color version of this figure is available at the *Journal of Human Genetics* journal online.



**Figure 2** Family tree of patients no. 13 and no. 14. Patients no. 13 was the index case carrying the 2447+1G>T(c.2448G>T) mutation on exon 9 (the other mutation was not identified). Her husband was an asymptomatic 2447+1G>T(c.2448G>T) heterozygote. Her daughter (no. 14) was homozygous for 2447+1G>T(c.2448G>T) and was diagnosed with WD. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

patients liver biopsy revealed presence of liver cirrhosis, which was classified by clinical assessment as compensated (child A) cirrhosis (Table 1). The remaining patients had only mild, unspecific histological changes. Minimal inflammation and microvesicular steatosis was found in six patients. In four patients evaluation of liver tissue specimen showed unspecific mild lobular inflammation (Table 1). Kayser–Fleischer ring was present in five patients of whom two patients presented with liver disease. None of the 100 healthy controls investigated carried the mutation.

## DISCUSSION

Like in many Central European countries, the p.H1069Q (c.3207C>A) mutation is the most common mutation in Austrian WD patients and was found in 104 of 190 (54.7%) patients representing an allele frequency of 36%. The present study indicates that 2447+1G>T(c.2448G>T), a novel mutation, is the second most common one in Austria with an allele frequency of 3.9%. Within the federal state of Upper Austria (about 1.4 million inhabitants, 28 known cases with WD) the allele frequency in WD patients is even 27%. 2447+1G>T(c.2448G>T) was most frequently found in combination with p.H1069Q(c.3207C>A) (37.5%) followed by P539L (18.8%). All other combinations were single cases.

More than two thirds (69%) of the patients carrying the (2447+1G>T (c.2448G>T) mutation presented with hepatic disease including two with fulminant WD. In contrast, 59.6% of 1225 European WD patients in our database (unpublished observation) presented with liver disease. Of the 14 patients with biopsy, only 4 had advanced fibrosis (3 with cirrhosis), 10 (71.4%) had only mild liver disease (most of them only had extensive steatosis). Twelve of 16 patients were female, consistent with the observation of a female preponderance in young patients with hepatic WD.<sup>18</sup> Not all mutations have the same pathophysiological consequence at the cellular level. The most prevalent mutation in European patients is the p.H1069Q (c.3207C>A) point mutation (accounting for about 25% of WD patients). In 303 p.H1069Q(c.3207C>A) homozygotes the mean age at presentation in patients with liver disease was lower ( $18.9 \pm 7.7$  years (s.d.)) than in patients with neurological disease ( $25.1 \pm 7.1$ ).<sup>18</sup> Similar data were reported in an analysis of Dutch patients.<sup>19</sup> Patients carrying the 2447+1G>T(c.2448G>T) mutation had about the same mean age at presentation than p.H1069Q(c.3207C>A) homozygotes (hepatic: 16.3 years; neurological: 23.6 years), with a higher frequency of hepatic presentation. In contrast, patients with truncating<sup>20</sup> or frameshift mutations<sup>21</sup> are associated with early onset of hepatic disease. Thus, like p.H1069Q(c.3207C>A), the 2447+1G>T(c.2448G>T) mutation might be considered as a ‘mild’ mutation, although the number of patients is too low to draw firm conclusions.

Knowledge of the regional distribution of mutations of the WD gene is important (i) to establish a reliable and timely diagnosis, (ii) to design appropriate screening strategies and (iii) to provide better insight into underlying pathophysiological mechanisms in WD through genotype phenotype analysis. Direct molecular-genetic diagnosis is difficult because of the occurrence of more than 500 mutations. Therefore, comprehensive molecular-genetic analysis will take several months currently, which makes this an impractical method in a patient who needs a final diagnosis within a short time frame. Furthermore, most patients are compound heterozygotes, which was also the case in the majority of our patients. Nevertheless it is reasonable to perform molecular analysis of the *ATP7B* gene in any patient who has a provisional diagnosis of WD, both for confirmation purposes and to facilitate the subsequent screening of family members (see Figure 2). Based on the frequency of certain mutations in a given population, mutation analysis can be focused to a few hot-spot exons.

A multiplex PCR for the most frequent mutations makes direct mutation analysis for diagnosis feasible.<sup>22,23</sup> The most common mutation in Northern and Central Europe is the point mutation p.H1069Q(c.3207C>A) in exon 14 of the WD gene. Its frequency is highest in Poland and Eastern Germany and decreases to the west and to the south. Other common mutations in Central and Eastern Europe are located on exon 8 (p.P767P-fs (c.2298-2299insC), p.Gly710Ser(c.2128G>A)), exon 15 (p.Ala1135GlnfsX13(c.3402delC)) and exon 13 (p.Arg969Gln(c.2906G>A)) with allele frequencies of <10%. Limited mutation analysis of exons 8, 13, 14 and 15 can be carried out within a week. In 151 (78.2%) of Austrian WD patients mutations in exons 8, 13, 14 and 15 were detected at least on one chromosome, respectively. By exon 9 the number can be further increased to 158 (81.8%).

Like in our study, besides the common WD mutations, a high degree of variation may exist in restricted areas within a country. For example, in Apulia one third of WD patients carry the p.Gly591-Gly(c.1773C>G) mutation while most patients in Sardinia have mutations in the 5'-UTR region.<sup>11,15,16</sup> Both mutations are very rare

in the rest of Italy.<sup>24</sup> In the Island of Gran Canaria, an archipelago near the northwest Atlantic coast of Africa, a high frequency of the very rare p.Leu708Pro(c.2123T>C) mutation was described, finding a total of 12 homozygous and 7 heterozygous individuals.<sup>13</sup> All known WD patients in Iceland carry the Y760X (c.2007\_2013del, exon 7) mutation.<sup>25</sup> These findings reflect local founder mutations in geographically restricted areas. However, as migration becomes an increasing phenomenon of our society the knowledge of local mutations is important in establishing the diagnosis also outside the regions where the mutation has been described initially.

This study highlights also the need to screen children of patients presenting with WD (Figure 2). Although this risk is low, analysis of the *ATP7B* gene for mutations in the children of an index patient is justified given the potential devastating course of WD. We have identified so far two families in which both—one parent and the offspring—had WD.<sup>26,27</sup>

In summary, we describe a novel mutation in the *ATP7B* gene, occurring in a distinct geographical area in Austria. It should be considered in patients with hepatic injury of unknown origin and may also serve to screen young infants with a family history of WD, because of the severity of the disease and the favorable response to medication.

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