Copper toxicosis gene *MURR1* is not changed in Wilson disease patients with normal blood ceruloplasmin levels

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**Abstract**

AIM: To analyze our Wilson disease patient cohort (*n* = 106) for alterations in the gene coding for *MURR1*.

METHODS: Patients with an established diagnosis of Wilson disease but normal ceruloplasmin blood levels were chosen for our study (*n* = 14). Patients with two known disease-causing mutations in the *ATP7B* gene were not included. The three exons of the human *MURR1* gene were sequenced after amplification of the genomic DNA by polymerase chain reaction.

RESULTS: Our study did not reveal any mutations leading to an amino acid change in the *MURR1* sequence of Wilson disease patients. A polymorphism at 472 bp of the coding sequence could be confirmed.

CONCLUSION: The *MURR1* gene plays no role in the pathogenesis of Wilson disease patients with normal serum ceruloplasmin levels.

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Key words: Wilson Disease; *ATP7B*; *MURR1*; COMMD1

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INTRODUCTION

In humans, Wilson disease (WD) is an autosomal recessively inherited disorder of copper metabolism\(^1\)\(^,\)\(^2\) characterized by the impaired biliary excretion of copper. Wilson disease leads to toxic copper accumulation predominantly in the liver and brain, causing liver cirrhosis and severe neurological defects. Common clinical findings in WD are low serum ceruloplasmin (CP) levels, elevated hepatic copper contents, elevated urine 24-h copper excretion and Kayser-Fleischer rings\(^3\). Homozygous or compound heterozygous mutations in the copper-transporting P-type ATPase *ATP7B* lead to Wilson disease\(^4\)\(^,\)\(^5\).

The genetic background is highly variable, with more than 300 mutations reported so far\(^6\). But not all cases are unambiguous because no mutations in the *ATP7B* gene have been found in some WD patients. It is unclear why no *ATP7B* mutations are detectable in a subgroup of patients presenting with typical features of Wilson disease. It might be due to an incomplete analysis of the *ATP7B* gene or due to other yet unidentified defects of genes involved in copper metabolism.

The clinical presentation is highly variable even among patients with the same mutation. In Wilson disease a low serum ceruloplasmin level is a typical finding and can be observed in 80%-90% of the patients. Ceruloplasmin is a copper binding ferroxidase in blood\(^1\). Today’s understanding of the underlying molecular mechanism\(^7\) is that *ATP7B* is predominantly localized to the trans Golgi network and transports copper across the membrane to the lumen of the Golgi apparatus where apoceruloplasmin is loaded with copper. In case of a malfunction of *ATP7B*, apoceruloplasmin can not be loaded with copper and is degraded more rapidly, resulting in reduced blood levels of ceruloplasmin. Under elevated copper conditions, *ATP7B* translocates from the trans Golgi network to a vesicular compartment where it may facilitate biliary copper excretion\(^8\)\(^,\)\(^9\).

Recently, the autosomal recessively inherited canine copper toxicosis has been described in Bedlington terriers. Like in Wilson disease these dogs develop copper accumulation in the liver due to impaired biliary copper excretion leading to chronic hepatitis and cirrhosis. Neurological abnormalities have not been reported. The genetic basis of this defect is a deletion of the exon2 of the *Murr1* gene\(^10\)\(^,\)\(^12\) mapped to 10q26 in Bedlington terriers. The human orthologous gene has been identified on chromosome 2p13-16 and is distinct from the *ATP7B* gene locus\(^13\). Furthermore, affected dogs present with normal ceruloplasmin serum levels, suggesting that the defect is beyond the trans-Golgi network. A direct interaction be-
tween MURR1 and ATP7B has been reported\(^{[13]}\). There is biochemical evidence that decreased MURR1 levels lead to intracellular copper accumulation\(^{[14]}\). Based on these observations a role of MURR1 in the biliary copper excretion downstream of ATP7B has been suggested\(^{[15]}\).

It would be interesting to identify a human disorder caused by defects in the human MURR1 gene. Recently, a novel protein family (COMMD proteins) of structural and functional homologues of MURR1 (COMMD1) has been identified\(^{[16]}\). Recently, we reported an association between an MURR1 polymorphism and onset of neurological and hepatic symptoms in WD patients homozygous for the most common ATP7B mutation H1069Q. Onset of disease was significantly earlier in patients with a heterozygous state at codon Asn-164 with an earlier mutation H1069Q. Onset of disease was significantly earlier in patients with a heterozygous state at codon Asn-164 with an earlier mutation H1069Q. In the former study patients with low ceruloplasmin serum levels were included.

To identify possible disease related mutations in the MURR1 gene in the current study we focused on patients with Wilson Disease but normal ceruloplasmin serum levels and at least one unknown mutation of ATP7B.

**MATERIALS AND METHODS**

**Patients**

Data of patients with an established diagnosis of Wilson disease were collected (\(n = 106\)). The diagnosis of Wilson disease was based on the criteria of the 8\(^{th}\) International Conference on Wilson Disease and Menkes disease\(^{[17]}\) (Leipzig, Germany April 16-18, 2001). For this study only patients with at least one undetermined mutation of the ATP7B gene were selected (\(n = 73\)), patients with two or less identified mutated alleles of ATP7B (\(n = 33\)) or more than two identified mutations (\(n = 14\)). Ten Patients (71\%) were homozygous for wild-type GAT, 3 patients (21\%) were heterozygous GAT/GAC and 1 patient was homozygous for GAC. The frequency of these variations was in line with previous reports\(^{[18]}\). We already reported a putative association between the GAT/GAC heterozygous state at codon Asn-164 with an earlier onset of disease in H1069Q ATP7B homozygous patients\(^{[19]}\). However, in the present study no significant genotype/phenotype correlation could be found, which might be due to the small number of patients.

**RESULTS**

The human orthologue of the Murr1 gene encoded a protein of 190 amino acids. The gene spanned nearly 235 kb. Both introns were about 100 kb each in size (Figure 2). Therefore only the three exonic sequences were analyzed. In this study no mutations changing the amino acid sequence were found in the analyzed patients. A polymorphism at 472 bp of the coding sequence was detected (Table 1). Ten Patients (71\%) were homozygous for wild-type GAT, 3 patients (21\%) were heterozygous GAT/GAC and 1 patient was homozygous for GAC. The frequency of these variations was in line with previous reports\(^{[18]}\). We already reported a putative association between the GAT/GAC heterozygous state at codon Asn-164 with an earlier onset of disease in H1069Q ATP7B homozygous patients\(^{[19]}\). However, in the present study no significant genotype/phenotype correlation could be found, which might be due to the small number of patients.

**DISCUSSION**

Some patients with Wilson disease show no disease causing mutation in the ATP7B gene. Therefore other patho-

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**Figure 1** Mean ceruloplasmin blood levels in patients with two or less identified mutated alleles of ATP7B.
genetic factors might be involved. Due to the in part comparable phenotype of canine copper toxicosis and the reported interaction with ATP7B, the MURR1 protein is an interesting candidate.

In this study we focused on Wilson disease patients showing a comparable phenotype to the copper toxicosis in dogs in regard to the normal ceruloplasmin serum level. However, in our group of patients with non-homzygous ATP7B mutation, no mutation in the coding sequence of the MURR1 gene was found. Mutations in other parts of the Murr1 gene were not analyzed but could affect the gene expression and thus affect the phenotype. The finding that copper toxicity gene MURR1 is not changed in Wilson disease patients with normal blood ceruloplasmin levels needs to be evaluated in a larger clinical study.

Although our data do not necessarily rule out the possibility that MURR1 is involved in biliary copper excretion under physiological conditions, there is no direct evidence that it is indeed a disease-causing factor in human Wilson disease. This is in agreement with the findings of other investigators. In fact, a homzygous mutation of ATP7B resulting in the absence of this protein in the trans-Golgi network may be the only cause of Wilson disease. In our study population with detectable heterozygous or absent mutations of ATP7B, the other mutations may have not been identified yet. A low normal ceruloplasmin concentration was also observed in patients with homozygous mutations, suggesting that it may represent an undefined compensatory process by which apoceruloplasmin is loaded with copper by an ATP7B independent route, even outside the trans-Golgi network. This route seems to be less efficient but could result in low normal serum ceruloplasmin levels.

Although the possibility that MURR1 is involved in biliary copper excretion in humans has not been ruled out, MURR1 does not seem to play a role in the pathogenesis of Wilson disease.

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Figure 2 Map of the human MURR1 gene. Localization of the reported polymorphism (492 T>C) in exon 3 is marked.

Table 1 MURR1 gene analysis in WD patients and associated CP blood values listed by WD gene mutation

| Mutation in the ATP7B gene | n | MURR1 gene base changes | CP level |
|---------------------------|---|------------------------|---------|
| 2299insC/ m n d.          | 1 | 1                      | -       | 0.15    |
| 3400DelC/ m n d.          | 1 | 1                      | -       | 0.18    |
| G1030C/ m n d.            | 1 | 1                      | -       | 0.28    |
| H1069Q/ m n d.            | 1 | 1                      | 1       | 0.31    |
| m.n.d./ m n d.            | 7 | 7                      | -       | 0.23 (±0.09) |
| m.n.d./ m n d.            | 3 | 3                      | -       | 0.26 (±0.08) |

m n d: mutation not detected.
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