Influence of Homozygosity for Methionine at Codon 129 of the Human Prion Gene on the Onset of Neurological and Hepatic Symptoms in Wilson Disease

Uta Merle, MD; Wolfgang Stremmel, MD; Reinhard Geßner, PhD

Background: The clinical heterogeneity of Wilson disease expression cannot be fully explained by the various mutations of the Wilson disease gene. The prion-related protein (PrP) has been shown to bind copper in vitro and might therefore influence Wilson disease.

Objective: To examine the effect of the PrP polymorphism at codon 129, resulting in either methionine or valine (M129V), on the clinical phenotype of patients with Wilson disease.

Design and Setting: Retrospective cross-sectional study at a university hospital.

Participants: A total of 134 patients were grouped according to their PrP M129V genotypes and initial clinical symptoms (hepatic vs neurological).

Results: The onset of symptoms was significantly delayed in patients homozygous for the 129M allele as compared with patients with at least 1 V allele (mean±SD age, 20.90±11.9 years vs 15.5±7.6 years; P = .003). No significant correlation was found when analyzing the impact of the PrP M129V genotype on the clinical symptoms at initial manifestation (hepatic vs neurological; P = .44).

Conclusion: This study shows for the first time, to our knowledge, that the human PrP polymorphism M129V influences the onset of symptoms in patients with the copper storage disorder Wilson disease.

Arch Neurol. 2006;63:982-985

Wilson Disease (WD) is an autosomal recessive copper storage disorder characterized by a decreased biliary copper excretion and a defective incorporation of copper ion into ceruloplasmin leading to hepatic and/or neurological symptoms. The wide spectrum of clinical signs and symptoms, as well as the difference in age at onset of clinical manifestations in WD, cannot be fully explained by the type of mutation in the WD disease gene ATP7B. Until yet, no “modifier genes” are known except from 1 study on apolipoprotein E (ApoE) genotype showing a weak correlation for ApoE3 homozygosity with higher age at initial manifestation in the subgroup of patients homozygous for the ATP7B H1069Q mutation. Most likely, variability in manifestation is due to other, yet unidentified genetic factors.

The prion-related protein (PrP) is expressed at high concentrations in the central nervous system and at lower concentrations in other tissues. Although the pathogenic, scrapie-associated isoform PrPSc (where Sc indicates scrapie) has attracted worldwide attention because of its involvement in the pathogenesis of transmissible spongiform encephalopathies, the biological function of cellular PrPC (where C indicates cellular) is not well defined. A natural polymorphism at codon 129 of the human prion gene, resulting in either methionine or valine, has profound influence on susceptibility to variant Creutzfeldt-Jakob disease. The finding that purified, overexpressed PrPC binds copper ion with low micromolar affinity has led to the proposal that the protein may play a role in copper homeostasis. There are also observations suggesting a connection between PrPC, copper ions, and protection of cells from oxidative stress. However, so far no direct link has yet been established between PrPC expression and copper metabolism.

To determine whether the age at clinical onset and the disease course are influenced by the PrP 129 genotype, 134 patients with symptomatic WD were evaluated with regard to (1) a possible dif-
ference in genotype frequency between patients with WD and healthy subjects of central European descent, (2) the impact of PrP M129V genotypes on phenotypic disease expression (hepatic vs neurological), and (3) the influence of the PrP M129V genotype on the onset of initial symptoms in patients with WD.

**METHODS**

**STUDY POPULATION**

Between 1997 and 2005, 167 patients with WD of central European origin were included in this study at the Department of Gastroenterology and Hepatology, University of Heidelberg, Heidelberg, Germany. The study was approved by the local ethics committee and informed consent was obtained from all patients. Wilson disease was diagnosed on the basis of typical clinical symptoms, characteristic biochemical markers (levels of serum ceruloplasmin <20 mg/dL, 24-hour urinary copper >100 µg/dL, and serum “free” copper >10 µg/dL [1.6 µmol/L] and hepatic copper content >250 µg/g dry weight), and the presence of Kayser-Fleischer rings. The diagnosis of WD was reevaluated independently prior to the current study by 2 WD experts. Specifically, the medical history, laboratory findings, and medical records were reassessed and the diagnosis was confirmed in 164 of the 167 initial patients. Three patients with symptoms that could not be attributed unequivocally to WD were excluded, as well as all 30 siblings of index patients.

The remaining 134 patients with WD were grouped according to their symptoms at initial manifestation as either neurological (neuropsychiatric) or hepatic. Ascertainment of neurological symptoms was accomplished by a standard patient questionnaire. The neurological examination resembled the scoring system published by Oder et al with a scoring of the following items: tremor, rigidity, gait disturbances, dysarthria, dysphagia, ataxia, depression, affective instability, irritability, and temper outbursts. The hepatic items analyzed were elevation of transaminases, Child-Pugh score, ultrasonic signs of liver damage, and histological signs of liver disease. The scores ranged from 0 (completely normal) to 3 (severely impaired). The sum scores of the single items were divided by the number of items to get an average neurological and hepatic score.

The first appearance of clinical manifestations (onset of WD) was established by 1 of 2 WD experts on analyzing the standard patient questionnaire, medical history, laboratory tests, and medical records.

**GENOTYPING**

Genomic DNA was isolated from whole blood using the QIAamp Blood Kit (Qiagen, Hilden, Germany). The PrP M129V, ApoE, and ATP7B H1069Q genotypes were determined by polymerase chain reaction amplification of the respective genomic regions and subsequent restriction fragment length polymorphism analysis.

**STATISTICAL ANALYSIS**

Statistical analyses were performed with SPSS for Windows, release 10.05 (SPSS Inc, Chicago, Ill). Because of the limited number of subjects with PrP at codon 129 resulting in homozygous valine (129V/V), they were combined with the PrP 129M/V heterozygous group (called “PrP 129V+”) for statistical analysis. The comparisons of the quantitative variable “age at onset of symptoms” between the 2 sets of patients (and the analysis of subgroups of patients) were performed by the unpaired t test. Contingency tables were used to analyze the correlation of nominal variables (genotype, phenotypic disease expression, and sex) between different patient groups. Data are represented as mean ± SD. A P value <.05 was considered statistically significant.

**RESULTS**

A total of 134 patients with symptomatic WD (78 women, 56 men) were analyzed for a potential correlation between the PrP M129V genotype, the phenotypical disease expression, and age at onset of symptoms. Of those, 46 patients (34%) were initially seen with neurological symptoms and 88 patients (66%), with hepatic symptoms. Patients with neurological symptoms were older at initial manifestation than patients with hepatic dysfunction (mean ± SD age, 19.1 ± 8.3 years vs 15.2 ± 7.6 years; P = .009). The table shows the breakdown of distribution of the PrP M129V genotype, ApoE genotype, and the ATP7B H1069Q mutation status. No sex-specific differences were found with respect to clinical symptoms (neurological vs hepatic) and the PrP 129 geno-
The overall PrP M129V genotype distribution in our patients was similar to that of a cross-sectional control sample (Table) and values published for healthy European subjects. There was no significant difference between the 2 subgroups classified according to the PrP M129V genotype (P = .26) (Figure, A) (Table). However, within the subgroup of ApoE3/3 patients with WD (n = 89), we found a significant delay of age at symptom onset (mean ± SD age, 20.7 ± 11.6 years vs 15.5 ± 7.6 years; P = .02) (Figure, A) (Table).

Our data show no difference in PrP M129V genotype frequency between a cohort of 134 patients with WD and healthy European subjects, indicating that this polymorphism has no influence on the penetrance of WD. No significant correlation was found when analyzing the influence of the PrP M129V genotype on clinical symptoms at initial examination (hepatic vs neurological; P = .44). However, whether the PrP M129V genotype has an influence on severity of symptoms and on long-term outcome during therapy has to be analyzed in future studies.

It is conceivable that PrP may have an important role in cellular copper metabolism, which may be affected by the PrP M129V polymorphism. The identification of the PrP M129V polymorphism modulating onset of the copper storage disorder WD could potentially help to elucidate the role of PrP in copper metabolism and in WD manifestation.

Figure. Mean age at onset of symptoms in patients with Wilson disease (WD), comparing prion-related protein (PrP) at codon 129 resulting in homozygous methionine (129M/M) and PrP at codon 129 resulting in either methionine or valine (129V+). Data are expressed as mean ± SD. A, All patients with WD and subgroups defined by their PrP1298/H1069Q and apolipoprotein E (ApoE) genotype. B, Patients with WD divided in 2 subgroups defined by initial clinical manifestation, hepatic (n = 88) or neurological (n = 46).

Our data show no difference in PrP M129V genotype frequency between a cohort of 134 patients with WD and healthy European subjects, indicating that this polymorphism has no influence on the penetrance of WD. No significant correlation was found when analyzing the influence of the PrP M129V genotype on clinical symptoms at initial examination (hepatic vs neurological; P = .44). However, whether the PrP M129V genotype has an influence on severity of symptoms and on long-term outcome during therapy has to be analyzed in future studies.

Our data could not confirm an association between age at onset of symptoms and phenotypic disease expression with the ATP7B mutation H1069Q in the WD gene, as reported by others. When analyzing this cohort of patients with WD, irrespective of their H1069Q mutational status, there was no significant association of ApoE genotype with age at or type of onset. This is in concordance with the study of Schiefermeier et al, where an association with age was only found in the subgroup of homozygous H1069Q patients, indicating only a minor influence on manifestation of disease.

In our study, the PrP 129M/M genotype was established as an important factor in delaying the onset of neurological and hepatic symptoms. This observation is novel and highly relevant, as there are currently no known genetic and/or environmental disease modifiers of this clinically highly variable disorder. While our study was not designed to test biochemical mechanisms that may explain the later onset of neurological and hepatic symptoms associated with the PrP 129M/M genotype in patients with WD, other investigators demonstrated copper ion binding and antioxidative properties of PrP in vitro. It is conceivable that PrP may have an important role in cellular copper metabolism, which may be affected by the PrP M129V polymorphism. The identification of the PrP M129V polymorphism modulating onset of the copper storage disorder WD could potentially help to elucidate the role of PrP in copper metabolism and in WD manifestation.
Presidential Address: From the Beginning to the Present: Wilson’s Disease Revisited

Correspondence: Uta Merle, MD, Department of Gastroenterology and Hepatology, University Hospital, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany (uta_merle@med.uni-heidelberg.de).

Author Contributions: Prof Dr Stremmel and Dr Geßner contributed equally to this work. Dr Merle had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. 

Study concept and design: Merle, Stremmel, and Geßner. Acquisition of data: Merle and Geßner. Analysis and interpretation of data: Merle and Geßner. Drafting of the manuscript: Merle. Critical revision of the manuscript for important intellectual content: Merle, Stremmel, and Geßner. Statistical analysis: Merle and Geßner. Administrative, technical, and material support: Merle and Stremmel. Study supervision: Merle, Stremmel, and Geßner.

Acknowledgment: We thank Peter Ferenci, MD, for performing the ATP7B H1069Q mutation analysis and Sabine Tuma and Petra Felsch for expert technical support.

REFERENCES