GENETIC POLYMORPHISM AT CODON 129 OF THE PRION PROTEIN GENE IS NOT ASSOCIATED WITH MULTIPLE SCLEROSIS

The endogenous cellular prion protein (PrPC) is an α-helical glycoprophosphatidylinositol–anchored sialoglycoprotein highly expressed in neurons, lymphoid cells, and myeloid cells. In humans, the PrP gene (Prnp) is located on chromosome 20p12. Single-nucleotide polymorphisms (SNPs) in Prnp have been shown to determine the susceptibility to inherited, sporadic, and infectious forms of prion diseases. Interestingly, it was also recently shown that the same SNP plays a role in MS susceptibility. The role of other unlinked polymorphisms or the potential role of Prnp129 SNP has a significant effect on the clinical course of numerous nonprion neurodegenerative disorders of the central nervous system (CNS), including early-onset Alzheimer disease, Down syndrome, and Wilson disease. In these studies, methionine/valine (M/V) heterozygosity was associated with less severe clinical disease. Finally, the Prnp129 SNP was also shown to negatively affect long-term memory in adult and senescent healthy individuals. The exact mechanisms by which genetic polymorphisms of Prnp alter CNS function and disease phenotypes are largely unknown.

In this study, we investigated the effect of the Prnp129 M/V SNP on disease susceptibility to multiple sclerosis (MS), a human inflammatory neurodegenerative disease of the CNS. To determine whether the Prnp129 M/V SNP plays a role in MS susceptibility, we genotyped 973 families with nuclear MS (n = 2998 individuals). Appropriate institutional review boards approved the studies and informed consent was obtained from all participants. The ratio of women to men for affected individuals was 2.9:1, and 91.7% presented with relapsing-remitting (67.6%) or secondary progressive (24.1%) MS at the time of study entry. Genotypes were generated by a TaqMan allelic discrimination assay on an ABI7900HT genotyping platform using the Assay-by-Design service from Applied Biosystems (Foster City, California). All family genotypes were examined for mendelian inconsistencies using PedCheck (University of Pittsburgh, Pennsylvania), and any discrepancies were addressed. No deviations from Hardy-Weinberg equilibrium were observed for genotypes in the patient, parent, or combined group (data not shown).

Family-based association analysis was performed using the reconstructed-combined transmission disequilibrium test as implemented in SAS Genetics (v.9.1.3; SAS Institute, Cary, North Carolina). There was no evidence of transmission distortion in this data set (P = .5). Families were stratified by sex and by the presence of HLA-DRB1*1501 in the affected individuals to identify potential genetic interactions. No difference in allelic transmission was observed in either subgroup. The cases from the 973 families with nuclear MS were combined with 490 additional MS cases (total ratio of women to men, 2.7:1) ascertained using identical inclusion criteria and compared with 1131 controls (ratio of women to men, 1.9:1). There was no statistically significant difference in the frequency of Prnp129 genotypes between patients and controls. Allele frequency distributions were similar in patients with mild (EDSS < 3, 15 years after onset) or severe (EDSS > 6 within 10 years after onset) MS (data not shown) or when using the Multiple Sclerosis Severity Score as a dependent variable (data not shown). Similarly, no effect of Prnp129 on age of onset was detected.

Using the family-based association analysis, we can conclude that an SNP in Prnp129 plays no major role in MS susceptibility. The role of other unlinked polymorphisms or the potential role of Prnp129 in the progression of neuroradiologic or cognitive endpoints of MS cannot be excluded.

Olaf Stüve, MD, PhD
Carsten Korth, MD, PhD
Pablo Gabatto, BSc
Elizabeth M. Cameron, BSc
Wei Hu, MD, PhD
Todd N. Eagar, PhD
Nancy L. Monson, PhD
Elliot M. Frohman, MD, PhD
Michael K. Racke, MD
Cyrus P. Zabetian, MD, MS
Jorge R. Oksenberg, PhD

Correspondence: Dr Stüve, Neurology Section, Veterans Affairs North Texas Health Care System, Medical Service, 4500 S Lancaster Rd, Dallas, TX 75216 (olaf.stuve@utsouthwestern.edu).

Author Contributions: Study concept and design: Stüve, Korth, Hu, Frohman, and Oksenberg. Acquisition of data: Stüve, Gabatto, Cameron, Monson, Frohman, Zabetian, and Oksenberg. Analysis and interpretation of data: Stüve, Korth, Eagar, Monson, Frohman, Racke, and Oksenberg. Drafting of the manuscript: Stüve, Korth, Hu, Eagar, Monson, Frohman, and Oksenberg. Critical revision of the manuscript for important intellectual content: Stüve, Korth, Gabatto, Cameron, Frohman, Racke, Zabetian, and Oksenberg.

©2009 American Medical Association. All rights reserved.
No TARDBP Mutations in a French Canadian Population of Patients With Parkinson Disease

Parkinson disease (PD) is a neurodegenerative disorder characterized by bradykinesia and at least one of the following primary features: truncal and limb muscle rigidity, resting tremor, and postural instability. Protein aggregates known as Lewy bodies found in dopaminergic neurons of the substantia nigra are deemed to be the major pathological hallmark of this disease. Lewy bodies from several patients with PD have been reported to be positively labeled by an antibody specific to the transactive response (TAR) DNA-binding protein 43 (TDP-43), which is encoded by the TAR DNA-binding protein gene (TARDBP). Aggregates positive for TDP-43 were also reported in other neurodegenerative disorders such as frontotemporal dementia, Alzheimer disease, and Lewy body diseases. Interestingly, inclusion bodies in a subset of patients with amyotrophic lateral sclerosis (ALS) were also found to be positive for TDP-43. Recently, independent studies by our group and others described 15 missense

TARDBP mutations in patients with sporadic and familial ALS, further confirming the causative nature of TDP-43 in ALS. In the present study we sequenced TARDBP in patients with PD to test whether mutations of this gene could also be observed in these patients and consequently be implicated in PD pathogenesis.

Methods. French Canadian patients with PD (n=125) were recruited through 2 designated movement disorder clinics in Quebec City (Quebec, Canada). Every index case was seen by a neurologist (N.D. or E.P.) specialized in movement disorders and met the Ward and Gibb criteria for PD. Additionally, they had to be dopa-responsive. A detailed standardized clinical assessment form was completed for each subject. All cases signed a consent form approved by local ethics committees prior to being enrolled in the study. Controls (n=360) consist of a group without disease and of similar age and the same ethnicity as the cases.

Cases were all negative for Parkin gene mutations as well as exons 31 and 41 of the LRRK2 gene, which contains a significant proportion of LRRK2 mutations.

On receipt of informed consent, blood samples were obtained from patients and DNA was prepared using standard methods. For each individual, the entire coding sequence (5 exons) of TARDBP (RefSeq NM_007375) was sequenced at the Genome Quebec Innovation Center using a 3730XL DNA analyzer (Applied Biosystems, Foster City, California). Mutation surveyor (v.3.10) was used for mutation detection analyses (SoftGenetics, State College, Pennsylvania).

Results. A summary of the clinical data are presented in the Table. The sequence analysis of all five TARDBP coding exons did not reveal the presence of previously reported or novel mutations, including the C terminus where 14 of 15 of the previously reported mutations of TARDBP in ALS were found. A variant that encoded for a silent mutation (A66A) of TDP-43 was observed in a single patient with PD. However, this variant was previously reported by us in healthy controls; thus it is likely to be a benign polymorphism.

Comment. In this study, we evaluated the role of TARDBP in PD pathogenesis by undertaking a sequence analysis of a cohort comprising 125 patients with PD and 360 healthy French Canadian controls. We did not observe

Table. Characteristics of the PD Population Recruited

<table>
<thead>
<tr>
<th>Clinical Data</th>
<th>Affected, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>89 (71)</td>
</tr>
<tr>
<td>From Eastern Quebec</td>
<td>125 (100)</td>
</tr>
<tr>
<td>French Canadian</td>
<td>125 (100)</td>
</tr>
<tr>
<td>Juvenile PD (age &lt;40 y)</td>
<td>11 (9)</td>
</tr>
<tr>
<td>Family history (first-degree relative)</td>
<td>13 (10)</td>
</tr>
<tr>
<td>Positive family history overall</td>
<td>28 (22)</td>
</tr>
<tr>
<td>Average age of onset, y (range)</td>
<td>55.3 (30-79)</td>
</tr>
<tr>
<td>Average duration of symptoms, y</td>
<td>8.0</td>
</tr>
<tr>
<td>Average Hoen &amp; Yahur stage at assessment</td>
<td>1.90</td>
</tr>
</tbody>
</table>

Abbreviation: PD, Parkinson disease.