A Rare Truncating Mutation in ADH1C (G78Stop) Shows Significant Association With Parkinson Disease in a Large International Sample

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Background: Alcohol dehydrogenases (ADHs) may be involved in the pathogenesis of neurodegenerative disorders because of their multiple roles in detoxification pathways and retinoic acid synthesis. In a previous study, significant association of an ADH class IV allele with Parkinson disease (PD) was found in a Swedish sample.

Patients: The previously associated single-nucleotide polymorphism plus 12 further polymorphisms in the ADH cluster on human chromosome 4q23 were screened for association in an extension of the original sample that now included 123 Swedish PD patients and 127 geographically matched control subjects. A rare nonsense single-nucleotide polymorphism in ADH1C (G78stop, rs283413) was identified in 3 of these patients but in no controls. To obtain sufficient power to detect a possible association of this rare variant with disease, we screened a large international sample of 1076 PD patients of European ancestry and 940 matched controls.

Results: The previously identified association with an ADH class IV allele remained significant (P<.02) in the extended Swedish study. Furthermore, in the international collaboration, the G78stop mutation in ADH1C was found in 22 (2.0%) of the PD patients but only in 6 controls (0.6%). This association was statistically significant (χ² = 7.5; 2-sided P = .007; odds ratio, 3.25 [95% confidence interval, 1.31-8.05]). In addition, the G78stop mutation was identified in 4 (10.0%) of 40 Caucasian index cases with PD with mainly hereditary forms of the disorder.

Conclusion: Findings presented herein provide further evidence for mutations in genes encoding ADHs as genetic risk factors for PD.

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The etiology of Parkinson disease (PD) remains largely a mystery despite considerable research efforts in the fields of epidemiology, genetics, biochemistry, and cellular biology. Neurodegeneration in PD is most prominent in the dopaminergic cell population, but is by no means confined to this anatomical site, suggesting a more widespread metabolic deficiency and/or toxic event in the pathogenesis of the disorder. Epidemiological data suggest that environmental factors are likely to play a role in PD pathogenesis, although the exact nature of such agents remains to be identified. Among candidate environmental risk substances for PD are substances leading to enhanced oxidative stress and lipid peroxidation products, such as reactive aldehydes. Mesencephalic dopamine neurons may be especially vulnerable to reactive substances because aldehydes may react with dopamine in vivo, forming toxic tetrahydroisoquinolines similar to MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). In addition, proper development and maintenance of the dopaminergic system may be strongly dependent on the supply of retinoic acid, which needs to be synthesized from retinol.

Alcohol dehydrogenase (ADH) enzymes of classes I and IV are mainly expressed in the lining of the digestive tract and in the liver. They are thought to play important roles in the metabolism of ethanol and in the synthesis of retinoic acid, and may also be important in the detoxification of reactive substances such as 4-hydroxynonenal. The dehydrogenases may, therefore, constitute a first line of defense in the gastrointestinal tract against exogenous toxic agents, and genetic defects in these enzymes may lead to increased uptake of potentially harmful substances that may eventually reach the dopamine system of the brain. Proper function of these enzymes may also be required during pe-
periods of reduced access to retinol or, conversely, during dietary overload of retinoids.10 Studying genetic variation in genes encoding ADH enzymes may, thus, offer links between nature and nurture in the pathogenesis of the different manifestations of PD.

In a sample of Swedish patients, association with PD was previously identified for a haplotype at the ADH4 gene (also known as ADH7; the present study uses the more recent class-based nomenclature11), encoding a class IV ADH, μ or σ subunit.12 This finding, however, could not be reproduced in a US sample of unrelated PD patients.13 We selected the previously associated single-nucleotide polymorphism (SNP) plus 12 further SNPs from the literature14 and the public SNP database. These SNPs are located in 2 class I genes, class III ADH, and class IV ADH (Table 1). One of the nucleotide variations (rs283413) led to a predicted early truncation of the protein chain at residue 78 of the ADH1C protein sequence (Figure). Nonsense mutations have been shown to be 9 times more likely to lead to a phenotype than mutations leading to amino acid substitutions,15 and deserve special attention in sequence-based association studies such as this one. Possible association of this variant with PD was, therefore, tested in a further international extension of the study by obtaining DNA from 1076 PD patients and 940 control subjects.

**METHODS**

Approval for this study was obtained from the local ethical committees by each recruiting site, and samples were drawn after informed consent. Seven collections of samples from PD patients and geographically matched controls were combined for case-control analysis (Table 2) provides the diagnostic criteria and demographic details). To establish case-control DNA material for the London Brain Bank collection, tissue samples were supplied by the Queen Square Brain Bank for Neurological Disorders, London, England. DNA was extracted from tissue blocks using a standard kit (Qiagen; VWR International AB, Stockholm). DNA samples from 129 unrelated CEPH (Centre d’Etude du Polymorphisme Humain) founders from the French and Utah pedigrees were purchased from Coriell Cell Repositories, Camden, Pa. An eighth collection sample (not included in the case-control study because of variable ascertainment and lack of controls for this sample) contained DNA from material for the London Brain Bank collection, tissue samples were supplied by the Queen Square Brain Bank for Neurological Disorders, London, England. DNA was extracted from tissue blocks using a standard kit (Qiagen; VWR International AB, Stockholm). DNA samples from 129 unrelated CEPH (Centre d’Etude du Polymorphisme Humain) founders from the French and Utah pedigrees were purchased from Coriell Cell Repositories, Camden, Pa. An eighth collection sample (not included in the case-control study because of variable ascertainment and lack of controls for this sample) contained DNA from 40 Caucasian PD patients with mainly hereditary types of PD. Genotyping was performed by pyrosequencing, as described in detail elsewhere.16 Sequences of primers, magnesium concentrations, and nucleotide dispensation orders are available from us on request. The presence of the ADH1C nonsense mutation was confirmed by automated capillary sequencing or re-sequencing leading to amino acid substitutions,15 and deserve special attention in sequence-based association studies such as this one. Possible association of this variant with PD was, therefore, tested in a further international extension of the study by obtaining DNA from 1076 PD patients and 940 control subjects.

Genotype distributions between patients and controls of the investigated SNPs in the initial screening of the Swedish samples are summarized in Table 3. Except for the association of SNP ADH4-2, previously identified in a sub-set of the material described herein, no significant association of the investigated SNPs with PD was found.

A SNP retrieved from a public database, rs283413, leading to an early truncation of the ADH1C protein chain, was identified in 3 patients, but was absent from control samples. We screened for the presence or absence of this ADH1C nonsense variant in a large collaborative sample of 1076 PD patients of European ancestry and 940 matched controls. There was a significant association with PD in the total sample (Table 4) (χ² = 7.5; 2-sided P = .007; odds ratio, 3.25 [95% confidence interval, 1.31-8.05]). The ADH1C nonsense mutation was also found in an additional 4 of 40 tested Caucasian patients with PD, 3 having a strong family history of the disorder and 1 being a sporadic case. No DNA was available from further affected or unaffected family members of these mutation carriers.

Previous studies performed by other investigators have suggested large regions of linkage disequilibrium (LD) within the ADH cluster.19 Herein, we studied LD in the Swedish case-control material for the 13 SNPs listed in Table 1. The results indicate that there is extensive LD within the ADH cluster in the Swedish population (data available from us on request). In addition, to determine how far LD extended between the identified truncating mutation in ADH1C and other SNPs within the cluster or at its border, we studied 40 SNPs in a 4.8-megabase region around the ADH gene cluster in 3 Swedish and 2 English mutation carriers. We also typed the same 40 SNPs in 5 previously identified homozygous carriers of the ADH4 polymorphism that was associated with PD in a Swedish set of samples. We found that the ADH mutations associated with PD in our material are unlikely to be in strong LD with other mutations located outside the cluster.

### Table 1. SNPs Across the ADH Cluster at 4q23 Selected for This Study

<table>
<thead>
<tr>
<th>Location of SNP</th>
<th>Reference</th>
<th>Polymorphism, Allele 1/Allele 2</th>
<th>Location and Characterization of the Sequence Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH1B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>rs1041969</td>
<td>C/A</td>
<td>exon 3, N57K</td>
</tr>
<tr>
<td>II</td>
<td>rs17033</td>
<td>T/C</td>
<td>exon 9, UTR</td>
</tr>
<tr>
<td>ADH1C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>rs283413</td>
<td>G/T</td>
<td>exon 3, G78Stop</td>
</tr>
<tr>
<td>II</td>
<td>rs1693482</td>
<td>A/G</td>
<td>exon 5, Q227R</td>
</tr>
<tr>
<td>III</td>
<td>rs1042758</td>
<td>G/A</td>
<td>exon 7, V350I</td>
</tr>
<tr>
<td>ADH3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Reference 14</td>
<td>C/T</td>
<td>promoter, C+9/T+9</td>
</tr>
<tr>
<td>II</td>
<td>Reference 14</td>
<td>G/A</td>
<td>promoter, G−79/A−79</td>
</tr>
<tr>
<td>III</td>
<td>Reference 14</td>
<td>GG/AA</td>
<td>exon 1, silent, GG−197, GG−196/AA−197, AA−196</td>
</tr>
<tr>
<td>IV</td>
<td>rs13832</td>
<td>T/G</td>
<td>exon 9, UTR</td>
</tr>
<tr>
<td>ADH4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Reference 12</td>
<td>rs1573496 and G/C</td>
<td>exon 3, G92A, P5</td>
</tr>
<tr>
<td>II</td>
<td>Reference 12</td>
<td>A/G</td>
<td>promoter, P1</td>
</tr>
<tr>
<td>III</td>
<td>Reference 12</td>
<td>T/C</td>
<td>promoter, P2</td>
</tr>
<tr>
<td>IV</td>
<td>rs971074</td>
<td>G/A</td>
<td>exon 6, silent, P7</td>
</tr>
</tbody>
</table>

Abbreviations: ADH, alcohol dehydrogenase; SNP, single-nucleotide polymorphism; UTR, untranslated region.
The major finding of our study is the significant association of a nonsense mutation in the ADH1C gene with PD. The observed statistically significant association of the G78stop variant remains significant when disregarding the original Swedish material in which the mutation was first validated (2-tailed \( P \) value after removal of the Swedish/Stockholm region material from the case-control study, .015). The average age at onset of all mutation carriers was

![Figure](image_url)

**Figure.** Effect of the truncating nonsense mutation on the ADH1C (dimeric) protein. The effect of the nonsense mutation G78stop in ADH1C is illustrated by removal of the deleted amino acid residues from the protein structure database entry 1HT0 for ADH1C and subsequent display of the conformation by a computer program (Protein Explorer). ADH indicates alcohol dehydrogenase; NAD, nicotinamide adenine dinucleotide; and Zn, zinc.
49 years. Remarkably, most mutations were identified in patients with low average age at onset of disease, in accordance with the observation that early-onset PD seems to be most heritable. Matching of cases and controls was mainly based on ethnicity and geographic origin, while there was only an incomplete match in the average age of patients and controls (mean age of patients vs controls, 66 vs 57 years). This might have led to sampling of future PD cases among controls or, if the variant is associated with survival, to an overestimation of the effect of the mutation on PD susceptibility. While almost all mutations among controls were identified in samples from central Europe, the rare nature of the ADH1C nonsense variant makes it impossible to determine whether this is a sampling artifact or not.

We found strong LD between individual SNPs of the ADH cluster, as previously noted in association studies of alcoholism with mutations in the cluster. Likely ancestral recombination events were found between the associated ADH4 SNP and the G78stop mutation in ADH1C and polymorphisms in adjacent genes in 10 mutation carriers from Sweden and the United Kingdom (data not shown), rendering it unlikely that the true risk-conferring mutation is located far outside the ADH cluster.

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Much attention within the PD research field has been aimed directly at the dopamine neurons, degeneration of which leads to the most prominent symptoms of the disorder. Considering epidemiological evidence pointing at environmental toxins in the pathogenesis of the disorder, the idea of studying candidate genes expressed in the gastrointestinal system rather than the dopamine system of the brain may seem less far-fetched than first thought. Defects in enzymes, such as the truncating mutation in ADH1C described herein, may ren-
under an individual more susceptible to environmental toxins. Such genetic knowledge may also lead to identification of environmental agents that are metabolized by ADHs and that may promote development of disease even in individuals with little genetic susceptibility.

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