Genetic association study of the P-type ATPase ATP13A2 in late-onset Parkinson's disease

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Genetic association study of the P-type ATPase

ATP13A2 in late onset Parkinson disease

Aleksandar Rakovic, MSc1,2,*, Barbara Stiller, MSc3,4,5,*, Ana Djarmati, PhD1,2, Antonia Flaquer, MSc7, Jan Freudenberg, MD8, Mohammad-Reza Toliat, PhD9,6, Michael Linnebank, MD9, Vladimir Kostic, MD10, Katja Lohmann, PhD11,2, Sebastian Paus MD9, Peter Nürnberg, PhD4,6,11, Christian Kubisch, MD3,4,5,6,11, Christine Klein, MD1,2, Ullrich Wüllner, MD9 and Alfredo Ramirez, MD1,4,5

1Department of Neurology and 2Department of Human Genetics, University of Lübeck, Lübeck, Germany
3Institute of Human Genetics, 4Institute for Genetics, 5Center for Molecular Medicine Cologne, 6Cologne Center for Genomics, and 11Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECEAD), University of Cologne, Cologne, Germany
7Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany
8Laboratories of Neurogenetics, Department of Neurology, University of California at San Francisco, San Francisco, CA 94143, USA
9Department of Neurology, University of Bonn, Bonn, Germany
10Department of Neurology, University of Belgrade, Belgrade, Serbia
*
These authors contributed equally

Running title: Role of ATP13A2 in Parkinson disease

Corresponding author: Alfredo Ramirez, MD
Institute of Human Genetics
University of Cologne
Kerpener Strasse 34
50931 Cologne, Germany
phone: +49-221-478 86828
fax: +49-221-478 86435
e-mail: alfredo.ramirez@uk-koeln.de

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\textbf{Keywords:} \textit{ATP13A2}, Parkinson disease, association study, parkinsonism
Introduction

Parkinson disease (PD) and parkinsonism are a heterogeneous group of common neurodegenerative disorders with a prevalence of approximately 3% in people over the age of 65 years. Both diseases show several common clinical and pathological features, such as hypokinetic-rigid syndrome, tremor at rest, and nigral degeneration.\textsuperscript{1-3} Regarding the age of disease onset, the term early onset parkinsonism (EOP) describes a group of patients with onset of symptoms before the age of 40 years.\textsuperscript{4}

Several common key factors have been involved in the molecular etiology of PD and parkinsonism, including enhanced oxidative stress, mitochondrial dysfunction, accumulation of aberrant or misfolded proteins, and dysfunction of the ubiquitin-proteasome system (UPS) and the lysosome.\textsuperscript{5-7} In this regard, we have identified loss-of-function mutations in \textit{ATP13A2}, a mainly neuronal P-type ATPase gene, the gene product of which is localized in the lysosome. Initially, mutations were found in two families affected by a rare form of EOP with dementia called Kufor-Rakeb disease (KRD).\textsuperscript{8} However, the clinical characterization of the KRD phenotype showed additional features beyond the spectrum of "pure" PD. Nevertheless, the presence of levodopa-responsive parkinsonism and the increased expression of \textit{ATP13A2} in the brain of idiopathic PD patients raised the question whether variants in this gene could also be associated with idiopathic PD.\textsuperscript{8,10} This issue has attracted further attention by the identification of a homozygous missense mutation and two heterozygous mutations in EOP patients.\textsuperscript{11} However, whether \textit{ATP13A2} also contributes to the pathogenesis of late onset PD is currently unknown. Therefore, we investigated this question in two independent case-control samples of late onset PD by a systematic single nucleotide polymorphism (SNP)-based association study. Here we present the results of 5 SNPs which capture the predicted common haplotype variations of \textit{ATP13A2} locus.
Material and Methods

Patients: After obtaining informed consent, all patients underwent a standardized neurological examination by a movement disorder specialist. The study was approved by the local university ethics committees. The diagnosis of PD was based on the UK Brain Bank diagnostic criteria (with the exception that positive family history was not regarded an exclusion criterion). Patients were collected at movement disorders centres in Bonn, Cologne, Luebeck, and Belgrade and represent consecutive series, not originating in a geographic isolate. The initial case-control sample was collected in Bonn and Cologne.

Patients were included in this study only if the onset of symptoms was over the age of 40 years and they had German background (i.e., both parents were born in Germany). Controls of this sample were included if their age was over 40 years and they had a German background.

A replication sample of German and Serbian origin was collected in Luebeck and Belgrade. Family history was regarded positive if parkinsonism was reported for one first- or second-degree relative. PD was rated using the Unified Parkinson Disease Rating Scale (UPDRS) III.

A more detailed description of both patient and control samples including their ethnic background is found in table 1.

SNP selection: Haplotype-tagging (ht-)SNPs were selected from the HapMap database (http://www.hapmap.org; version July 2006) to discriminate between all predicted common haplotypes (with an estimated frequency >5%) within haplotype blocks in the Central European sample. Haplotype blocks were defined as regions in which >85% of total haplotype diversity is covered by common haplotypes, using the program hapblock.13

Genetic analysis: Initial SNP-genotyping on genomic DNA was performed on a TaqMan™ platform with assays designed by Applied Biosystems. The SNP rs3754511 was genotyped using pyrosequencing. The genotyping of the replication sample was performed by RFLP for
SNPs rs2871775 and rs3754511, using HpaI and MspI restriction enzymes, respectively.

Genotyping of rs3738815 and rs11203280 was carried out on a LightCycler (Roche Diagnostics). All primer sequences and experimental conditions are available from the authors.

Statistics: First, quality control was applied to the dataset. Thus, individuals displaying a low call rate (one missing SNP) were removed from the analysis. SNPs passed the quality control when showing a call rate higher than 95%. The exact test described and implemented by Wigginton and colleagues was used to check for Hardy-Weinberg equilibrium (HWE).\textsuperscript{14} Distribution of genotypes was consistent with HWE in both groups of cases and controls.

Genotypes and allele distributions were compared between cases and controls for all SNPs using the global genotype test and Armitage trend test, genotypic test, dominant test, recessive test. To perform case-control tests on the distribution of a probabilistically inferred set, the haplotypes standard EM algorithm was used. All analyses were performed using the free open source PLINK v0.99r developed by Shaun Purcell et al.\textsuperscript{15}

Power calculation: A power analysis was performed with the Genetic Power calculator.\textsuperscript{16} We estimate that, under the assumption of complete LD between the marker tested and the disease-causing variant, we had 77% power to detect a true difference in allele frequency between the 220 PD patients and 232 controls (i.e., in the first sample) with a single-marker association analysis ($\alpha$=0.05), further assuming a frequency of the disease-associated allele A of 0.25, a relative risk of 1.5 for genotype Aa and of 2.25 for genotype AA, and a prevalence of PD in the general population over the age of 50 years of 1.5%. Using these parameters with both samples together the estimated power increases to 95%.
Results

The initial genotyping involved 7 SNPs covering the entire ATP13A2 locus. The sample used for the first stage of our association analysis included a total of 452 unrelated individuals from Germany (Table 1). Although two SNPs failed the quality control and were omitted from the analysis, the remaining 5 SNPs described >90% of the most common haplotypes. Therefore, genotypic distributions between cases and control subjects were compared for these 5 SNPs. These comparisons were performed using different test models including a recessive model assuming homozygocity, dominant model, genotypic model, and additive model. Regardless which test was performed, the results for the first sample did not support a significant allelic or genotypic association with PD with a minimal p-value for rs11203280 of $P_{UNC}=0.134$ (Table 2). Interestingly, the same SNP showed a significant association in the men subgroup with an uncorrected p-value of $P_{UNC}=0.016$. However, this result was not significant after permutation-based correction for the number of test performed (p>0.05).

This finding prompted us to analyse a second independent sample of cases and controls. Especially since the initial sample had a reduced power to detect a true allelic difference. Thus, we genotyped the same 5 SNPs in a cohort containing 311 individuals of German and Serbian background (Table 1). The analysis revealed no association between this second sample and PD and no gender effect was observed in this second cohort (rs11203280 of $P_{UNC}=0.389$ overall and $P_{UNC}=0.570$ in men; Table 2). As expected, the analysis of both samples combined showed no association with PD (data not shown). To avoid any bias in the analysis due to population stratification in the second sample, we merged the German individuals from both samples to determine whether these initially observed differences were specific to German PD patients (Table 2). However, no positive association emerged from this analysis in either the complete sample and in the German men subgroup, best p-value at rs11203280 of $P_{UNC}=0.514$ and $P_{UNC}=0.084$, respectively.
Discussion

We present here the data of a systematic case-control association study in two independent late onset PD samples. The overall results of our analysis did not support an association between \textit{ATP13A2} and PD. Interestingly, gender stratification of the initial sample showed a significant association for men with PD in the single marker analysis. Unfortunately, this finding did not withstand a correction for multiple testing. Nevertheless, we decided to test the veracity of this finding by increasing the power of detection of our study. Therefore, we analyzed a second independent sample of individuals with German and Serbian background. However, the gender effect could neither be replicated in the second sample nor in both samples combined. Furthermore, no gender effect was observed in the analysis of all German individuals from both samples. To confirm our findings, we analyzed the raw data for the \textit{ATP13A2} locus contained in two different genome wide association studies (GWA)\textsuperscript{17,18}. This analysis revealed that all SNPs from our study were contained in one of the GWA\textsuperscript{17} and that they showed no association between this ATPase gene and PD, either (data not shown). Interestingly, the cohort analyzed in this GWA was composed of white individuals from USA with an age of onset of PD ranging from 55 years to 84 years. Thus, our data combined with the results from Fung and colleagues strongly argue for a lack of association between \textit{ATP13A2} and late onset PD, at least in European and US populations.

This negative association between SNPs within the \textit{ATP13A2} gene and PD is not a unique finding. Likewise, negative association were also reported for idiopathic PD and several common polymorphisms at gene loci involved in other monogenic forms of parkinsonism\textsuperscript{19,20}, with rare exceptions, such as common variants in \textit{α}-synuclein.\textsuperscript{21}

In conclusion, we currently cannot conclude that common variants in the \textit{ATP13A2} locus play a major role in the molecular etiology of late onset PD. Yet, the question whether private mutations could be responsible for the PD in this age group remains open and further studies are warranted.
Acknowledgements

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References

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Peter Nürnberg: Research project, conception and organization
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ATP13A2 in late onset Parkinson disease

Aleksandar Rakovic, MSc\textsuperscript{1,2,*}, Barbara Stiller, MSc\textsuperscript{3,4,5,*}, Ana Djarmati, PhD\textsuperscript{1,2}, Antonia Flaquer, MSc\textsuperscript{7}, Jan Freudenberg, MD\textsuperscript{8}, Mohammad-Reza Toliat, PhD\textsuperscript{4,6}, Michael Linnebank, MD\textsuperscript{9}, Vladimir Kostic, MD\textsuperscript{10}, Katja Lohmann, PhD\textsuperscript{1,2}, Sebastian Paus MD\textsuperscript{9}, Peter Nürnberg, PhD\textsuperscript{4,6,11}, Christian Kubisch, MD\textsuperscript{3,4,5,6,11}, Christine Klein, MD\textsuperscript{1,2}, Ullrich Wüllner, MD\textsuperscript{9} and Alfredo Ramirez, MD\textsuperscript{3,4,5}

\textsuperscript{1}Department of Neurology and \textsuperscript{2}Department of Human Genetics, University of Lübeck, Lübeck, Germany
\textsuperscript{3}Institute of Human Genetics, \textsuperscript{4}Institute for Genetics, \textsuperscript{5}Center for Molecular Medicine Cologne, \textsuperscript{6}Cologne Center for Genomics, and \textsuperscript{11}Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECEAD), University of Cologne, Cologne, Germany
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\textsuperscript{*}These authors contributed equally

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Corresponding author: Alfredo Ramirez, MD
Institute of Human Genetics
University of Cologne
Kerpener Strasse 34
50931 Cologne, Germany
phone: +49-221-478 86828
fax: +49-221-478 86435
email: alfredo.ramirez@uk-koeln.de

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Antonia Flaquer: Statistical analysis
Jan Freudenberg: Statistical analysis, execution, review and critique
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Michael Linnebank: Research project, organization
Vladimir Kostic: Research project, patient recruitment
Katja Lohmann: Research project, organization
Sebastian Paus: Research project, patient recruitment
Peter Nürnberg: Research project, conception and organization
Christian Kubisch: Research project, conception. Manuscript, review and critique
Christine Klein: Research project, conception. Manuscript, review and critique
Ullrich Wuellner: Research project, conception. Manuscript, review and critique
Alfredo Ramirez: Research project, conception and organization. Manuscript, writing of the first draft
### Table 1: General characteristics of the case control dataset.

<table>
<thead>
<tr>
<th>Sample Origin</th>
<th>Inclusion Criteria*</th>
<th>N° of Patients</th>
<th>Ethnic Background</th>
<th>Gender</th>
<th>Age of onset ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonn and Cologne, Germany</td>
<td>UBBCDC</td>
<td>220</td>
<td>German</td>
<td>Male</td>
<td>60.1 ± 9 (41-85)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Serbian</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Luebeck, Germany</td>
<td>UBBCDC</td>
<td>161</td>
<td>123</td>
<td>126</td>
<td>51.7 ± 10.3 (40-80)</td>
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<td></td>
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<td>38</td>
<td>35</td>
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<table>
<thead>
<tr>
<th>Control Data</th>
<th>Inclusion Criteria</th>
<th>N° of Controls</th>
<th>Ethnic Background</th>
<th>Gender</th>
<th>Age ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population from University Hospital Bonn</td>
<td></td>
<td>232</td>
<td>German</td>
<td>Male</td>
<td>69.6 ± 11.7 (41-99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serbian</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Healthy volunteers</td>
<td>150</td>
<td>119</td>
<td>97</td>
<td>53.9 ± 10.8 (40-90)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31</td>
<td>53</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: UBBCDC, UK Brain Bank Clinical Diagnostic Criteria; age and standard deviation (S.D.) are given in years

*Daniel and Lees"
Table 2: Statistical analysis of SNPs with differential allele distribution between men and women

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>allele distribution (%)</em></td>
<td><em>P-value</em> (OR [95% CI])</td>
<td><em>allele distribution (%)</em></td>
</tr>
<tr>
<td></td>
<td>Patients</td>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>1st Sample</td>
<td>(n=220)</td>
<td>(n=232)</td>
<td>0.540</td>
</tr>
<tr>
<td>rs3738815</td>
<td>18.5</td>
<td>81.5</td>
<td>17.0</td>
</tr>
<tr>
<td>rs11203280</td>
<td>30.6</td>
<td>69.4</td>
<td>29.8</td>
</tr>
<tr>
<td>2nd Sample</td>
<td>(n=161)</td>
<td>(n=150)</td>
<td>0.134</td>
</tr>
<tr>
<td>rs3738815</td>
<td>20.2</td>
<td>79.8</td>
<td>21.8</td>
</tr>
<tr>
<td>rs11203280</td>
<td>33.5</td>
<td>66.5</td>
<td>36.1</td>
</tr>
<tr>
<td>1st + 2nd Sample (Germans only)</td>
<td>(n=343)</td>
<td>(n=351)</td>
<td>0.550</td>
</tr>
<tr>
<td>rs3738815</td>
<td>19.0</td>
<td>81.0</td>
<td>18.9</td>
</tr>
<tr>
<td>rs11203280</td>
<td>31.9</td>
<td>68.1</td>
<td>32.9</td>
</tr>
</tbody>
</table>

*n* indicates the number of tested individuals.
P-values were obtained using the Armitage Trend Test. Results shown here are not corrected for multiple testing. CI confidence interval. Positive uncorrected p-value is shown in bold.