A Two-Stage Meta-Analysis Identifies Several New Loci for Parkinson’s Disease

International Parkinson’s Disease Genomics Consortium (IPDGC), Wellcome Trust Case Control Consortium 2 (WTCCC2)

Abstract

A previous genome-wide association (GWA) meta-analysis of 12,386 PD cases and 21,026 controls conducted by the International Parkinson’s Disease Genomics Consortium (IPDGC) discovered or confirmed 11 Parkinson’s disease (PD) loci. This first analysis of the two-stage IPDGC study focused on the set of loci that passed genome-wide significance in the first stage GWA scan. However, the second stage genotyping array, the ImmunoChip, included a larger set of 1,920 SNPs selected on the basis of the GWA analysis. Here, we analyzed this set of 1,920 SNPs, and we identified five additional PD risk loci (combined p < 5 × 10^{-10}, PARK16/1q32, STX1B/16p11, FGF20/8p22, STBD1/4q21, and GPNMB/7p15). Two of these five loci have been suggested by previous association studies (PARK16/1q32, FGF20/8p22), and this study provides further support for these findings. Using a dataset of post-mortem brain samples assayed for gene expression (n = 292) and methylation (n = 399), we identified methylation and expression changes associated with PD risk variants in PARK16/1q32, GPNMB/7p15, and STX1B/16p11 loci, hence suggesting potential molecular mechanisms and candidate genes at these risk loci.

Citation: International Parkinson’s Disease Genomics Consortium (IPDGC), Wellcome Trust Case Control Consortium 2 (WTCCC2) (2011) A Two-Stage Meta-Analysis Identifies Several New Loci for Parkinson’s Disease. PLoS Genet 7(6): e1002142. doi:10.1371/journal.pgen.1002142

Editor: Greg Gibson, Georgia Institute of Technology, United States of America

Received March 1, 2011; Accepted April 8, 2011; Published June 30, 2011

This is an open-access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Funding: This work was supported in part by the Wellcome Trust/MRC Joint Call in Neurodegeneration award (WT089698) to the UK Parkinson’s Disease Consortium (IPDGC), whose members are from the UCL Institute of Neurology, the University of Sheffield, and the MRC Protein Phosphorylation Unit at the University of Dundee. Additionally, part of the study was undertaken at UCLH/UCL using funding through a Department of Health NHIR Biomedical Research Centre. This work was also supported by Parkinson’s UK (Grants B047 and J-0804) and the Medical Research Council (G0700943). Genotyping of UK replication cases on Immunochip was part of the Wellcome Trust Case Control Consortium 2 project which is funded by the Wellcome Trust (085475/B/08/Z and 085475/2/08/2). P Damier is partly supported by a Wolfson-Royal Society Merit award. The UK gene expression work was supported in part by the UK Medical Research Council (G0901254) to researchers based in the UCL Institute of Neurology and King’s College London. J Holton receives support from the Reta Lila Weston Trust for Medical Research. This work was also supported by the Landspitali University Hospital Research Fund (S Sveinbjörnsdóttir), the Icelandic Research Council (Sveinbjörnsdóttir), the European Community Framework Programme 7, People programme, IAPP on novel genetic and phenotypic markers of Parkinson’s disease, and Essential Tremor (MarkMD), contract no PIAP-GA-2008-230596 MarkMD (H Pétersson, J Holton). This US work was supported in part by the Intramural Research Programs of the National Institute on Aging, National Institute of Neurological Disorders and Stroke, National Institute of Environmental Health Sciences, National Human Genome Research Institute, National Institutes of Health, Department of Health and Human Services; project numbers Z01 AG000949-02 and Z01-ES101986. In addition this study was supported by the US Department of Defense, award number W81XWH-09-2-0126. Funding to support collection of a portion of the samples was obtained from the National Institutes of Health (grants NS057105 and RO2/21692); the American Parkinson Disease Association (APDA), Barnes Jewish Hospital Foundation, and the Greater St. Louis Chapter of the APDA. The KORA research platform (KORA: Cooperative Research in the Region of Augsburg; http://www.gsf.de/KORA) was initiated and financed by the Forschungszentrum für Umwelt und Gesundheit (FSF), which is funded by the German Federal Ministry of Education, Science, Research, and Technology and by the State of Bavaria. The study was additionally funded by the German National Genome Network (NGFNplus) (01GS0508314; German Ministry for Education and Research) and by the German Federal Ministry of Education and Research (BMBF) NGFN (01GR0468) and in the frame of ERA-Net NEURON (01GW0908). This work was also supported by the Helmholtz Alliance Mental Health in an Ageing Society (HeIMA, HA-215) funded by the Initiative and Networking Fund of the Helmholtz Association. The French GWA scan work was supported by the French National Agency of Research (http://www.agence-nationale-recherche.fr, ANR-08-MNP-012) and by the National Research Funding Agency (ANR-08-NEUR-004-01) in ERA-Net NEURON (http://www.neuron-eranet.eu). We also want to thank the Hersenstichting Nederland (http://www.hersenstichting.nl), the Neuroscience Campus Amsterdam and the section of Medical genomics, the Prinses Beatrix Fonds (http://www.prinsesbeatrixfonds.nl) for sponsoring this work. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: n.wood@ion.ucl.ac.uk

* A full list of authors and affiliations is provided in the Acknowledgments. A full list of members of the Wellcome Trust Case Control Consortium 2 (WTCCC2) consortium is provided in Text S1.

Introduction

Until the recent developments of high throughput genotyping and genome-wide association (GWA) studies, little was known of the genetics of typical Parkinson’s disease (PD). Studies of the genetic basis of familial forms of PD first identified rare highly penetrant mutations in LRRK2 [1,2], PINK1 [3], SNCA [4], PARK2 [5] and PARK7 [6]. Following these findings, GWA scans for idiopathic PD identified SNCA and MAPT as unequivocal risk loci [7,8,9,10,11] as well as implicated BST1 [8], GAK [12], and HLA-DR [13]. Using sequence based imputation methods [14], the meta-analysis of several GWA scans [7,9,10,11] conducted by the International Parkinson’s Disease Genomics Consortium (IPDGC) identified and replicated five new loci: AGMSD, STX39, MCC11/LAMPS, STT11, and CCDC62/HIP1R [15] and confirmed association at SNCA, LRRK2, MAPT, BST1, GAK and HLA-DR [15].

We conducted a two-stage association study. Combining stage 1 and stage 2, the data consist of 12,386 PD cases and 21,026 controls genotyped using a variety of platforms (Table 1). Stage 1 used genome-wide genotyping arrays and our initial analysis [15] focused on the subset of SNPs that passed genome-wide significance in stage 1. For stage 2 genotyping, we used a custom content Illumina iSelect array, the ImmunoChip and additional
GWAS typing as previously described [15]. The primary content of the ImmunoChip data focuses on autoimmune disorders but, as part of a collaborative agreement with the Wellcome Trust Case Control Consortium 2, we included 1,920 ImmunoChip SNPs on the basis of the stage 1 GWAS results.

Here, we report the combined analysis for this full set of 1,920 SNPs. This step1+2 analysis identified seven new loci that passed genome-wide significance in the meta-analysis. During the process of analyzing these data and preparing for publication, we became aware that another group was also preparing a large independent GWAS scan in PD for publication (Do et al, submitted). Following discussion with this group we agreed to cross validate the top hits from each study by exchanging summary statistics for this small number of loci.

To provide further insights into the molecular function of these associated variants, we tested risk alleles at these loci for correlation with the expression of physically close gene (expression quantitative trait locus, eQTL) and the methylation status (methQTL) of proximal DNA CpG sites in a dataset of 399 control frontal cortex and cerebellar tissue samples extracted post-mortem from individuals without a history of neurological disorders.

Results

In addition to eleven loci that passed genome-wide significance in stage 1 [15], we identified over 100 regions of interest defined as 10 kb windows containing at least one SNP associated at \( p < 10^{-8} \). We submitted the most associated SNP in each region for probe design and follow-up genotyping using the ImmunoChip platform. For each region of interest, we also added four SNPs in high level of linkage disequilibrium (LD) to provide redundancy where the most associated SNP would not pass the Illumina probe design step or the assay for that SNP would fail. To complete the array design we also added all non-synonymous dbSNPs located in known PD associated regions [1,2,3,4,5,6]. Out of these 2,400 submitted SNPs, 1,920 passed QC and were included in the final array design. For these 1,920 SNPs we combined stage 1 and stage 2 associated data in a meta-analysis of 12,836 cases and 21,026 controls (Table 1) from the IPDGC. We exchanged summary array design. For these 1,920 SNPs we combined stage 1 and stage 2 results, seven new SNPs passed our defined genome-wide significance threshold \( p < 5 \times 10^{-8} \), Table 2 and Figure 1. These loci are either novel or the previous evidence of association was not entirely convincing in individuals of European descent. We combined these results with the independent replication. Five of these seven loci replicated and showed strong combined evidence of PD association \( p < 10^{-10} \) overall. Taking either the nearest gene (or the strongest candidate when available) to designate these regions, these five loci are 4q21/STBD1, 7p15/GPNMB, 8p22/FGF20 [16] and 16p11/STX1B. rs700723/1q32 has been previously reported as PD associated (PARK16 [7,8]) but this SNP lacked the unequivocal evidence of association in European samples \( p = 9.47 \times 10^{-10} \) in stage 2 only. To understand the potential biological consequences of risk variation at this locus we tested whether rs700723 was correlated with either gene expression or DNA methylation status of proximal transcripts or CpG sites respectively (Table 3). We found correlations with the expression of \( NUCKS1 \left( p = 1.8 \times 10^{-7} \right) \) and \( RAB7L1 \left( p = 7.2 \times 10^{-5} \right) \). We also found correlations with the methylation state of CpG sites located in the \( FLJ3269 \) gene \( p = 3.9 \times 10^{-22} \).

In the case of 16p11/STX1B, the proximal gene to the most associated SNP rs4889603 is \( SETD1A \). However, \( STX1B \) is located 18 kb upstream of rs4889603 and is a more plausible PD candidate gene [17] owing to its synaptic receptor function. We therefore used this gene to designate this region. Our methQTL/eQTL dataset identified a correlation between the rs4889603 risk allele and increased methylation of a CpG dinucleotide in \( STX1B \) (Table 3).

The SNP rs591323 in the 8p22 region is located ~150 kb downstream of the \( FGF20 \) gene (NCBI build 36.3), for which association with PD has been suggested previously in familial PD samples [16,18] but which remained controversial [19]. Our findings provide further support for a PD association at this locus, but again, whether the functionally affected transcript is \( FGF20 \) or not remains unclear.

The regions 4q21/STBD1 and 7p15/GPNMB have not been previously implicated in PD etiology. We found that the risk allele of rs156429, the most associated SNP in the 7p15 region, is associated in our eQTL dataset with decreased expression of the proximal transcript encoded by \( NUP22 \) (Table 3). The same risk allele is also associated with increased methylation of multiple CpG sites proximal to \( GPNMB \) itself (Table 3). Neither of these regions contains an obvious candidate gene.

Two additional loci (3q26/NAD3 and 8q21/AMMP1F6) showed strong evidence of association in stage 1 and 2 but were not disease associated in the Do et al dataset. Further replication is required to clarify the role of variation at these loci in risk for PD.

The strongly associated G2019S variant in the \( LRRK2 \) gene [20] was included in the Immunochip design and we replicated the published association: control frequency: 0.045% case frequency 0.61%, estimated odds ratio: 13.5 with 95% confidence interval: 5.5–43. However, the case collections have been partially screened for this variant therefore its frequency in cases and the odds ratio is likely to be underestimated.

The ImmunoChip array design provides some power to detect whether multiple distinct association signals exist at individual loci. Indeed, if a SNP showed an independent and sufficiently strong association in stage 1, it would have been included in stage 2 provided that it was not located in the same 10 kb window as the primary SNP in the region. There is precedent for this in PD, with the previous identification of independent risk signals at the \( SNCA \) locus [11]. We therefore used the Immunochip data to test whether any of the seven loci in Table 2 showed some evidence of more than one independent signal. None of these seven loci showed any association \( p > 0.01 \) after conditioning on the main SNP in the region. In contrast, after conditioning on the most associated SNPs rs356182 in the \( SNCA \) region, several SNPs...
remained convincingly associated ($p = 9.7 \times 10^{-8}$ for rs2245801 being the most significant).

Lastly, we performed a risk profile analysis to investigate the power to discriminate cases and controls on the basis of the 16 confirmed common associated variants (Table 4). For each locus, we estimated the odds ratio on the basis of stage 1 data and we applied these estimates to compute for each individual in the ImmunoChip cohort a combined risk score. Solely based on these 16 common variants, and therefore not considering rare highly penetrant variants such as G2019S in LRRK2 [20], we found that individuals in the top quintile of the risk score have an estimated three-fold increase in PD risk compared to individuals in the bottom quintile (Table 4). We note however that the effect size of several of these associated variants could be over-estimated (an effect known as winner’s curse, see [21]) but given the consistent estimates of odds ratio across studies (Table 4) we expect this bias to be minimal.

**Discussion**

The combination of GWA scans and imputation methods in large cohorts of PD cases and controls has enabled us to identify five PD associated loci in addition to the 11 previously reported by us. Two of these loci (1q32/PARK16, 8p22/FGF20) implicate regions that had been previously associated with PD risk [8,16]. The 1q32/PARK16 showed convincing evidence of association in the Japanese population [8] but until now the association P-value had not passed a stringent genome-wide significance threshold in samples of European descent [7]. The 8p22/FGF20 locus had been previously reported in a study of familial PD [16] and we provide the first evidence of association in a case-control study. The remaining three loci (STX1B/16p11, STBD1/4q21 and GPNMB/7p15) are new.

Adding the eleven previously reported common variants [15] to the five convincingly associated loci identified in this study, common variants at 16 loci have now been associated with PD. Controlling for the risk score based on the 11 SNPs previously identified [15] in the risk profile analysis (Table 4), the addition of these five new loci provides a modest but significant ($p = 2.2 \times 10^{-5}$) improvement of our ability to discriminate PD cases from controls.

Combining eQTL/methylation and case-control data implicates potential mechanisms which could explain the increased PD risk associated some of these variants. In particular, the strong eQTL in the 1q32/PARK16 region with the RAB7L1 and NUCKS1 genes (Table 3) suggests that either one of these genes could be the biological effector of this risk locus. However, existing data show that eQTLs are widespread and this co-localization could be the result of chance alone [22]. Additional fine-mapping work will be required to assess whether the expression and case-control data are indeed fully consistent.

While we are unable to unequivocally pinpoint the causative genes underlying these associations, their known biological function can suggest likely candidates. At the 1q32/PARK16 loci our association and eQTL data indicate that RAB7L1 and NUCKS1 are the best candidates. The former is a GTP-binding protein that plays an important role in the regulation of exocytotic and endocytotic pathways [23]. Exocytosis is relevant for PD for two main reasons: firstly, since dopaminergic neurotransmission is mediated by the vesicular release of dopamine, i.e. dopamine exocytosis [24], and secondly because it has been shown that alpha-synuclein knock-out mice develop vesicle abnormalities [25], thus providing a potential direct link between genetic variability in the gene and a biological pathway involved in the disease. Less is known regarding NUCKS1; it has been described to be a nuclear protein, containing casein kinase II and cyclin-dependant kinases phosphor-

| Table 1. Sample size and genotyping platform for the cohorts included in stage 1 (top set of rows), stage 2 (middle set of rows), and independent replication (bottom row). |
|-----------------|-------|---------|------------------|
| **Cohort**      | **Controls** | **Cases** | **Genotyping platform** |
| United Kingdom  | 5,200 | 1,705   | Illumina 660W-Quad  |
| USA-NIA         | 3,034 | 971     | Illumina HumanHap 550 |
| USA-dbGAP       | 857   | 876     | Illumina 370 K     |
| German          | 944   | 742     | Illumina HumanHap550 |
| French          | 1,984 | 1,039   | Illumina 610-Quad  |
| **Total Stage 1** | **12,019** | **5,333** |                     |
| Icelandic       | 1,427 | 479     | Illumina HumanHap 300 |
| Dutch           | 2,024 | 772     | Illumina 610-Quad  |
| USA             | 2,215 | 2,807   | ImmunoChip         |
| United Kingdom  | 1,864 | 1,271   | ImmunoChip         |
| Dutch           | 402   | 304     | ImmunoChip         |
| French          | 363   | 267     | ImmunoChip         |
| German          | 712   | 1,153   | ImmunoChip         |
| **Total Stage 2** | **9,007** | **7,053** |                     |
| **Stage 1+Stage 2** | **21,026** | **12,386** |                     |
| **Do et al- USA** | **29,624** | **3,426** |                     |

DOI:10.1371/journal.pgen.1002142.t001
PD that have a complex genetic component. We therefore expect that further and larger association analyses, perhaps using dedicated high-throughput genotyping arrays like the ImmunoChip, will continue to yield new insights into PD etiology.

Material and Methods

Genotyping and case control cohorts

Participating studies were either genotyped using the ImmunoChip as part of a collaborative agreement with the ImmunoChip Consortium, or as part of previous GWA studies provided by members of the IPDGC or freely available from dbGaP [7,9,10,11]. Genotyping of the UK cases using the Immunochip was undertaken by the WTCCC2 at the Wellcome Trust Sanger Institute which also genotyped the UK control samples. The constituent studies comprising the IPDGC have been described in detail elsewhere [15], although a summary of individual study quality control is available as part of Table S1. In brief all studies followed relatively uniform quality control procedures such as: minimum call rate per sample of 95%, mandatory concordance between self-reported and X-chromosome-heterozygosity estimated sex, exclusion of SNPs with greater than 5% missingness, Hardy Weinberg equilibrium p-values at a minimum of 10^{-7}, minor allele frequencies at a minimum of 1%, exclusion of first degree relatives, and the exclusion of ancestry outliers based on either principal components or multidimensional scaling analyses using either PLINK [33] or EIGENSTRAT [34] to remove non-European ancestry samples. All GWAS studies utilized in this analysis (and in the QTL analyses) were imputed using MACH1.0.16 [14] to conduct a two-stage imputation based on the August 2009 haplotypes from initial low coverage sequencing of 112 European ancestry samples in the 1000 Genomes Project [35], filtering the data for a minimum imputation quality of (RSQR >0.3) [14]. Logistic regression models were utilized to quantify associations with PD incorporating allele dosages as the primary predictor of disease. Imputed data was analyzed using MACH2DAT, and genotyped SNPs were analyzed using PLINK. All models were adjusted for covariates of components 1 and 2 from either principal components or multidimensional scaling analyses to account for population substructure and stochastic genotypic variation (except in the UK-GWAS data which were not adjusted for population substructure).

Association test statistics

Single SNP test statistics were combined across datasets using a score test methodology, essentially assuming equal odds ratio across cohorts. In addition, fixed and random effects meta-analyses were implemented in R (version 2.11) to confirm that the score test approximation does not affect the interpretation of the results. We also tested the relevant SNPs heterogeneity across cohorts and no significant heterogeneity was detected (Table S2).

Data exchange

We communicated to our colleagues in charge of the independent study (Do et al) the seven SNPs listed in Table 2. For this subset of SNPs they selected the marker with the highest r^2 value on their genotyping platform and provided us with the following summary statistics: odds ratio, direction of effect, standard error for the estimated odds ratio and one degree-of-freedom trend test P-value.

eQTL analysis and methylation analysis

Quantitative trait analyses were conducted to infer effects of risk SNPs on proximal CpG methylation and gene expression. For the five replicated SNP associations (Table 2), all available CpG probes and expression probes within +/-1 MB of the target SNP were

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chrom</th>
<th>Gene(s)</th>
<th>Alleles</th>
<th>MAF</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs708723</td>
<td>1q32</td>
<td>RAB7L1/PARK16</td>
<td>T&gt;C</td>
<td>0.439</td>
<td>0.905 (0.862–0.95)</td>
<td>6.68 × 10^{-5}</td>
<td>0.863 (0.824–0.905)</td>
<td>9.47 × 10^{-10}</td>
<td>1.00 × 10^{-12}</td>
<td>0.758 (0.65–0.88)</td>
<td>2.12 × 10^{-6}</td>
<td>8.82 × 10^{-15}</td>
<td></td>
</tr>
<tr>
<td>rs34016896</td>
<td>3q26</td>
<td>NMD3</td>
<td>C&gt;T</td>
<td>0.305</td>
<td>1.14 (1.09–1.2)</td>
<td>3.00 × 10^{-7}</td>
<td>1.08 (1.02–1.14)</td>
<td>0.00399</td>
<td>1.81 × 10^{-8}</td>
<td>1.002 (0.95–1.06)</td>
<td>0.954</td>
<td>1.31 × 10^{-6}</td>
<td></td>
</tr>
<tr>
<td>rs6812193</td>
<td>4q21</td>
<td>STBD1</td>
<td>C&gt;T</td>
<td>0.36</td>
<td>0.886 (0.843–0.932)</td>
<td>2.52 × 10^{-6}</td>
<td>0.906 (0.864–0.95)</td>
<td>5.29 × 10^{-5}</td>
<td>7.46 × 10^{-10}</td>
<td>0.839 (0.79–0.89)</td>
<td>7.55 × 10^{-10}</td>
<td>1.17 × 10^{-17}</td>
<td></td>
</tr>
<tr>
<td>rs156429</td>
<td>7p15</td>
<td>GPNMB</td>
<td>A&gt;G</td>
<td>0.403</td>
<td>0.894 (0.849–0.942)</td>
<td>2.15 × 10^{-5}</td>
<td>0.893 (0.852–0.917)</td>
<td>3.86 × 10^{-10}</td>
<td>3.27 × 10^{-10}</td>
<td>0.901 (0.85–0.95)</td>
<td>0.000193</td>
<td>3.05 × 10^{-13}</td>
<td></td>
</tr>
<tr>
<td>rs591323</td>
<td>8p22</td>
<td>FG20</td>
<td>G&gt;A</td>
<td>0.271</td>
<td>0.884 (0.836–0.935)</td>
<td>1.59 × 10^{-5}</td>
<td>0.875 (0.83–0.923)</td>
<td>8.49 × 10^{-7}</td>
<td>7.45 × 10^{-11}</td>
<td>0.932 (0.88–0.99)</td>
<td>0.023</td>
<td>1.92 × 10^{-11}</td>
<td></td>
</tr>
<tr>
<td>chr8:89442157</td>
<td>8q21</td>
<td>MMP16</td>
<td>C&gt;T</td>
<td>0.0247</td>
<td>1.38 (1.21–1.57)</td>
<td>1.10 × 10^{-6}</td>
<td>1.29 (1.12–1.49)</td>
<td>0.000451</td>
<td>2.26 × 10^{-9}</td>
<td>0.969 (0.86–1.09)</td>
<td>0.589</td>
<td>2.36 × 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>rs4889603</td>
<td>16p11</td>
<td>STX1B</td>
<td>A&gt;G</td>
<td>0.413</td>
<td>1.12 (1.06–1.18)</td>
<td>4.13 × 10^{-5}</td>
<td>1.15 (1.11–1.21)</td>
<td>8.21 × 10^{-9}</td>
<td>2.66 × 10^{-12}</td>
<td>1.070 (1.01–1.13)</td>
<td>0.014</td>
<td>6.98 × 10^{-13}</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chrom</th>
<th>Gene(s)</th>
<th>Alleles</th>
<th>MAF</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1027730</td>
<td>1q32</td>
<td>RAB7L1/PARK16</td>
<td>T&gt;C</td>
<td>0.906</td>
<td>0.905 (0.862–0.95)</td>
<td>6.68 × 10^{-5}</td>
</tr>
<tr>
<td>rs2012407</td>
<td>9p21</td>
<td>GRIN1</td>
<td>G&gt;A</td>
<td>0.906</td>
<td>0.905 (0.862–0.95)</td>
<td>6.68 × 10^{-5}</td>
</tr>
<tr>
<td>rs1277921</td>
<td>9p21</td>
<td>GRIN1</td>
<td>G&gt;A</td>
<td>0.906</td>
<td>0.905 (0.862–0.95)</td>
<td>6.68 × 10^{-5}</td>
</tr>
<tr>
<td>rs708723</td>
<td>1q32</td>
<td>RAB7L1/PARK16</td>
<td>T&gt;C</td>
<td>0.906</td>
<td>0.905 (0.862–0.95)</td>
<td>6.68 × 10^{-5}</td>
</tr>
<tr>
<td>rs34016896</td>
<td>3q26</td>
<td>NMD3</td>
<td>C&gt;T</td>
<td>0.906</td>
<td>0.905 (0.862–0.95)</td>
<td>6.68 × 10^{-5}</td>
</tr>
<tr>
<td>rs6812193</td>
<td>4q21</td>
<td>STBD1</td>
<td>C&gt;T</td>
<td>0.906</td>
<td>0.905 (0.862–0.95)</td>
<td>6.68 × 10^{-5}</td>
</tr>
<tr>
<td>rs156429</td>
<td>7p15</td>
<td>GPNMB</td>
<td>A&gt;G</td>
<td>0.906</td>
<td>0.905 (0.862–0.95)</td>
<td>6.68 × 10^{-5}</td>
</tr>
<tr>
<td>rs591323</td>
<td>8p22</td>
<td>FG20</td>
<td>G&gt;A</td>
<td>0.906</td>
<td>0.905 (0.862–0.95)</td>
<td>6.68 × 10^{-5}</td>
</tr>
<tr>
<td>chr8:89442157</td>
<td>8q21</td>
<td>MMP16</td>
<td>C&gt;T</td>
<td>0.906</td>
<td>0.905 (0.862–0.95)</td>
<td>6.68 × 10^{-5}</td>
</tr>
<tr>
<td>rs4889603</td>
<td>16p11</td>
<td>STX1B</td>
<td>A&gt;G</td>
<td>0.906</td>
<td>0.905 (0.862–0.95)</td>
<td>6.68 × 10^{-5}</td>
</tr>
</tbody>
</table>
investigated as candidate QTL associations in frontal cortex and cerebellar tissue samples. 399 samples were assayed for genome-wide gene expression on Illumina HumanHT-12 v3 Expression Beadchips and 292 samples were assayed using Infinium HumanMethylation27 Beadchips, both per manufacturer’s protocols in each brain region. A more in depth description of the sample series comprising the QTL analyses, relevant laboratory procedures and quality requirements may be found in [15]. The QTL analysis utilized multivariate linear regression models to estimate effects of allele dosages per SNP on expression and methylation levels adjusted for covariates of age at death, gender, the first 2 component vectors from multi-dimensional scaling, post mortem interval (PMI), brain bank from where the death, gender, the first 2 component vectors from multi-dimensional scaling, post mortem interval (PMI), brain bank from where the death, gender, the first 2 component vectors from multi-dimensional scaling, post mortem interval (PMI), brain bank from where the

Table 3. Significant eQTL associations (p<0.01) between the five SNPs with positive replication data (Table 2) and proximal (cis) changes in gene expression/methylation in frontal cortex and cerebellar tissue.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Region</th>
<th>SNP</th>
<th>Gene Tagged by Probe</th>
<th>Illumina Probe</th>
<th>Alleles</th>
<th>Effect Estimate</th>
<th>Standard Error</th>
<th>Unadjusted P</th>
<th>Adjusted P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression</td>
<td>Frontal Cortex</td>
<td>rs156429</td>
<td>NUP1L2</td>
<td>ILMN_1789616</td>
<td>A&gt;G</td>
<td>0.083</td>
<td>0.018</td>
<td>3.6E-06</td>
<td>1.0E-04</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>rs156429</td>
<td>7p15/GPNMB</td>
<td>NUP1L2</td>
<td>ILMN_1789616</td>
<td>A&gt;G</td>
<td>0.078</td>
<td>0.017</td>
<td>3.1E-06</td>
<td>1.0E-04</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>rs156429</td>
<td>1q32/PARK16</td>
<td>NUCK51</td>
<td>ILMN_1680692</td>
<td>T&gt;C</td>
<td>0.155</td>
<td>0.033</td>
<td>1.8E-07</td>
<td>1.5E-05</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>rs156429</td>
<td>1q32/PARK16</td>
<td>RA87L1</td>
<td>ILMN_1813685</td>
<td>T&gt;C</td>
<td>-0.062</td>
<td>0.018</td>
<td>7.2E-04</td>
<td>1.2E-02</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>rs156429</td>
<td>16p11/STX1B</td>
<td>ZNF668</td>
<td>ILMN_1793236</td>
<td>A&gt;G</td>
<td>0.062</td>
<td>0.015</td>
<td>4.1E-05</td>
<td>8.7E-04</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>rs156429</td>
<td>16p11/STX1B</td>
<td>MYST1</td>
<td>ILMN_1804679</td>
<td>A&gt;G</td>
<td>-0.053</td>
<td>0.018</td>
<td>3.4E-03</td>
<td>4.8E-02</td>
</tr>
<tr>
<td>Methylation</td>
<td>Frontal Cortex</td>
<td>rs156429</td>
<td>GPNMB</td>
<td>ILMN_1789616</td>
<td>A&gt;G</td>
<td>0.133</td>
<td>0.025</td>
<td>1.0E-07</td>
<td>3.7E-06</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>rs156429</td>
<td>7p15/GPNMB</td>
<td>NUP1L2</td>
<td>ILMN_1789616</td>
<td>A&gt;G</td>
<td>0.131</td>
<td>0.023</td>
<td>1.2E-08</td>
<td>1.0E-06</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>rs156429</td>
<td>1q32/PARK16</td>
<td>NUCK51</td>
<td>ILMN_1680692</td>
<td>T&gt;C</td>
<td>0.133</td>
<td>0.029</td>
<td>5.3E-06</td>
<td>1.1E-04</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>rs156429</td>
<td>1q32/PARK16</td>
<td>RA87L1</td>
<td>ILMN_1813685</td>
<td>T&gt;C</td>
<td>-0.106</td>
<td>0.022</td>
<td>1.3E-07</td>
<td>3.7E-06</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>rs156429</td>
<td>16p11/STX1B</td>
<td>ZNF668</td>
<td>ILMN_1793236</td>
<td>A&gt;G</td>
<td>0.075</td>
<td>0.019</td>
<td>5.0E-05</td>
<td>1.3E-03</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>rs156429</td>
<td>16p11/STX1B</td>
<td>MYST1</td>
<td>ILMN_2371147</td>
<td>A&gt;G</td>
<td>0.069</td>
<td>0.018</td>
<td>2.6E-03</td>
<td>3.8E-02</td>
</tr>
</tbody>
</table>

Table 4. Estimated PD risk profile for the five cohorts genotyped using the Immunochip.

<table>
<thead>
<tr>
<th>Study</th>
<th>Trend P-value</th>
<th>AUC</th>
<th>1st quintile</th>
<th>2nd quintile</th>
<th>3rd quintile</th>
<th>4th quintile</th>
<th>5th quintile</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>&lt;2E-16</td>
<td>0.614</td>
<td>1.54</td>
<td>1.29-1.84</td>
<td>1.92</td>
<td>1.61-2.29</td>
<td>2.21</td>
</tr>
<tr>
<td>UK</td>
<td>&lt;2E-16</td>
<td>0.636</td>
<td>1</td>
<td>1.34</td>
<td>1.05-1.71</td>
<td>1.79</td>
<td>1.41-2.28</td>
</tr>
<tr>
<td>Germany</td>
<td>1.29E-11</td>
<td>0.692</td>
<td>1</td>
<td>1.32</td>
<td>0.98-1.79</td>
<td>1.88</td>
<td>1.38-2.58</td>
</tr>
<tr>
<td>France</td>
<td>5.19E-13</td>
<td>0.675</td>
<td>1</td>
<td>1.69</td>
<td>0.99-2.92</td>
<td>1.13</td>
<td>0.65-1.98</td>
</tr>
<tr>
<td>Netherlands</td>
<td>5.08E-05</td>
<td>0.601</td>
<td>1</td>
<td>1.06</td>
<td>0.65-1.74</td>
<td>1.35</td>
<td>0.83-2.20</td>
</tr>
<tr>
<td>Combined</td>
<td>&lt;2E-16</td>
<td>0.645</td>
<td>1</td>
<td>1.43</td>
<td>1.26-1.61</td>
<td>1.79</td>
<td>1.58-2.02</td>
</tr>
<tr>
<td>% Cases per Quintile</td>
<td>37.90</td>
<td>46.06</td>
<td>51.15</td>
<td>56.56</td>
<td>63.75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Risk scores for the 16 confirmed loci were computed using the odds ratio estimated from the genome-wide case-control genotype data. Individuals were split into quintile on the basis of their risk scores. The odds ratios quantify the effect of the computed risk quintile on the probability of being a PD case (one-degree-of-freedom logistic trend test with the PD status as a binary outcome variable and the quintiles, coded as 1-5, as covariates). The first quantile group was taken as a reference group. OR: odds ratio, CI: confidence interval.

doi:10.1371/journal.pgen.1002142.003
samples were provided and in which preparation/hybridization
batch the samples were processed. A total of 670 candidate QTL
associations were tested: 87 expression QTLs in the cerebellum
samples, 83 expression QTLs in the frontal cortex samples, 249
methylation QTLs in the cerebellum samples and 249 methylation
QTLs in the frontal cortex samples. Multiple test correction was
undertaken using false discovery rate adjusted p-values<0.05 to
dictate significance, with the p-value adjustment undertaken in each
series separately, stratified by brain region and assay. A complete list
of all QTL associations tested is included in Table S3.

Supporting Information

Table S1  Summary of results for fixed and random effects meta-
analysis, as estimates of effect heterogeneity across cohorts and
SNP used at the Do et al replication stage.
(XLSX)

Table S2  Summary of the quality control parameters applied to
the GWA datasets included in this study.
(XLSX)

Table S3  Complete list of tested QTL associations (expression and methylation).
(XLSX)

Text S1  Membership of the Wellcome Trust Case Control Consortium 2.
(DOC)

Acknowledgments

This study utilized the high-performance computational capabilities of the
Biowulf Linux cluster at the National Institutes of Health, Bethesda, Maryland
(http://biowulf.nih.gov/). DNA panels and samples from the NINDS Human
.nih.gov/) were used in this study, as well as clonal data. The submitters
that contributed samples are acknowledged in detailed descriptions of each panel
(http://ccr.coicell.org/sections/Collections/NINDS/?SId=10).


Consortium 2.

and methylation).

undertaken using false discovery rate adjusted p-values

A total of 670 candidate QTL

association undertaken in each

series separately, stratified by brain region and assay. A complete list

of all QTL associations tested is included in Table S3.

support for generating the genome-wide molecular data.

The UK brain samples for the gene expression studies were obtained
from the MRC Sudden Death Brain Bank in Edinburgh. This study makes
possible to use part of the 3C cohort and Drs. M. Lathrop and D.

Acknowledgments

This study utilized the high-performance computational capabilities of the
Biowulf Linux cluster at the National Institutes of Health, Bethesda, Maryland
(http://biowulf.nih.gov/). DNA panels and samples from the NINDS Human
.nih.gov/) were used in this study, as well as clonal data. The submitters
that contributed samples are acknowledged in detailed descriptions of each panel
(http://ccr.coicell.org/sections/Collections/NINDS/?SId=10).

The authors thank The French Parkinson’s Disease Genetics Study Group: Y. Aigle, M. Anheim, A-M. Bonnet, M. Borg, A. Brice, E. BrunossoL, J.-C. Corvol, Ph. Damier, A. Destee, A. Durr, F. Durif, S. Klebe, E. Lehnmann, M. Martinez, P. Pollak, O. Rascol, F. Tison, C. Tranchant, M. Verin, F. Viallet, and M. VidalHait. The authors thank the members of the French 3C consortium: Drs Annick Alperovitch, Claudine Berr, Christophe Tzourio, and Jean-Charles Lambert for giving us the possibility to use part of the 3C cohort and Drs. M. Lathrop and D. Zelenika for their support in generating the genome-wide molecular data.

The UK brain samples for the gene expression studies were obtained
from the MRC Sudden Death Brain Bank in Edinburgh. This study makes
use of GWA data generated by the Wellcome Trust Case-Control
consortium 2 (WTCCC2) on UK PD cases and on UK controls from
UK brain samples for the gene expression studies were obtained
from the MRC Sudden Death Brain Bank in Edinburgh. This study makes
possible to use part of the 3C cohort and Drs. M. Lathrop and D.

Acknowledgments

This study utilized the high-performance computational capabilities of the
Biowulf Linux cluster at the National Institutes of Health, Bethesda, Maryland
(http://biowulf.nih.gov/). DNA panels and samples from the NINDS Human
.nih.gov/) were used in this study, as well as clonal data. The submitters
that contributed samples are acknowledged in detailed descriptions of each panel
(http://ccr.coicell.org/sections/Collections/NINDS/?SId=10).

The authors thank The French Parkinson’s Disease Genetics Study Group: Y. Aigle, M. Anheim, A-M. Bonnet, M. Borg, A. Brice, E. BrunossoL, J.-C. Corvol, Ph. Damier, A. Destee, A. Durr, F. Durif, S. Klebe, E. Lehnmann, M. Martinez, P. Pollak, O. Rascol, F. Tison, C. Tranchant, M. Verin, F. Viallet, and M. VidalHait. The authors thank the members of the French 3C consortium: Drs Annick Alperovitch, Claudine Berr, Christophe Tzourio, and Jean-Charles Lambert for giving us the possibility to use part of the 3C cohort and Drs. M. Lathrop and D. Zelenika for their support in generating the genome-wide molecular data.

The UK brain samples for the gene expression studies were obtained
from the MRC Sudden Death Brain Bank in Edinburgh. This study makes
use of GWA data generated by the Wellcome Trust Case-Control
consortium 2 (WTCCC2) on UK PD cases and on UK controls from
the 1958 Birth Cohort (58BC) and National Blood Service (NBS). UK
population control data was made available through WTCCC1. We thank
Jeffrey Barrett for assistance with the design of the Immunochip.

The authors of this manuscript are the following:

Yongheng Bi, Christophe Tzourio, and Jean-Charles Lambert for giving us the possibility to use part of the 3C cohort and Drs. M. Lathrop and D. Zelenika for their support in generating the genome-wide molecular data.

The authors thank The French Parkinson’s Disease Genetics Study Group: Y. Aigle, M. Anheim, A-M. Bonnet, M. Borg, A. Brice, E. BrunossoL, J.-C. Corvol, Ph. Damier, A. Destee, A. Durr, F. Durif, S. Klebe, E. Lehnmann, M. Martinez, P. Pollak, O. Rascol, F. Tison, C. Tranchant, M. Verin, F. Viallet, and M. VidalHait. The authors thank the members of the French 3C consortium: Drs Annick Alperovitch, Claudine Berr, Christophe Tzourio, and Jean-Charles Lambert for giving us the possibility to use part of the 3C cohort and Drs. M. Lathrop and D. Zelenika for their support in generating the genome-wide molecular data.

The UK brain samples for the gene expression studies were obtained
from the MRC Sudden Death Brain Bank in Edinburgh. This study makes
use of GWA data generated by the Wellcome Trust Case-Control
consortium 2 (WTCCC2) on UK PD cases and on UK controls from
the 1958 Birth Cohort (58BC) and National Blood Service (NBS). UK
population control data was made available through WTCCC1. We thank
Jeffrey Barrett for assistance with the design of the Immunochip.

The authors of this manuscript are the following:

Yongheng Bi, Christophe Tzourio, and Jean-Charles Lambert for giving us the possibility to use part of the 3C cohort and Drs. M. Lathrop and D. Zelenika for their support in generating the genome-wide molecular data.

PLoS Genetics | www.plosgenetics.org 7 June 2011 | Volume 7 | Issue 6 | e1002142
Birmingham Hospitals NHS Trust, Birmingham, United Kingdom, 34
Department of Clinical Neurosciences, University College London Institute of Neurology, London, United Kingdom, 35
Institut National de la Sante et de la Recherche Medicale, CIC-9503, Hotel Pitié-Salpêtrière, Paris, France, 36
University of Aberdeen, Division of Applied Health Sciences, Population Health Section, Aberdeen, United Kingdom, 37
Centre Hospitalier Universitaire Nantes, CIC0004, Service de Neurologie, Nantes, France, 38
Institut National de la Sante et de la Recherche Médicale, U987, Université Victor Segalen, Bordeaux, France, 39
Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom, 40
Klinik für Neurologie, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Christian-Albrechts-Universität Kiel, Kiel, Germany, 41
Parkinson’s Disease Research Group, Faculty of Medicine, Imperial College London, London, United Kingdom, 42
Service de Neurologie, Hôpital Gabriel Montpied, Clermont-Ferrand, France, 43
AD-SP, Pitie-Salpetriere Hospital, Department of Genetics and Cytogenetics, Paris, France, 44
Cambridge Centre for Brain Repair, University of Cambridge, Cambridge, United Kingdom, 45
Institute of Neurology, University College London, London, United Kingdom, 46
Department of Psychiatry, Department of Neurology, Washington University School of Medicine, St. Louis, Missouri, United States of America, 47
14 deCODE genetics, Reykjavik, Iceland, 48
Department of Neurology, Leiden University Medical Center, Leiden, The Netherlands, 49
Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands, 50
American Association of Retired Persons, Washington DC, United States of America, 51
Queen Square Brain Bank for Neurological Disorders, Institute of Neurology, University College London, London, United Kingdom, 52
Department of Clinical Neurology, John Radcliffe Hospital, Oxford, United Kingdom, 53
Department of Neurology, Radiology, Neurosurgery, and Plastic Kinesiology, Bioengineering, Pennsylvania State University-Milton S. Hershey Medical Center, Hershey, Pennsylvania, United States of America, 54
Institute of Epidemiology, Helmholtz Zentrum München, German Research Centre for Environmental Health, Neuherberg, Germany, 55
Department of Geriatrics, Landspitali University Hospital, Reykjavik, Iceland, 56
Institute of Human Genetics, Helmholtz Zentrum München, German Research Centre for Environmental Health, Neuherberg, Germany, 57
Psychology Department, Unit of Functional Neurosurgery, University College London Institute of Neurology, London, United Kingdom, 58
Section on Molecular Neurogenetics, Medical Genetics Branch, NHGRI, National Institutes of Health, Bethesda, Maryland, United States of America, 59
Medical Research Council Centre for Neuropsychiatric Genetics and Genomics, Cardiff University School of Medicine, Cardiff, United Kingdom, 60
Neurosciences Department, Queen’s University Belfast, Belfast City Hospital, University of Belfast, Northern Ireland, United Kingdom, 61
Neurogenetics Unit, University College London Institute of Neurology/National Hospital for Neurology and Neurosurgery, London, United Kingdom, 62
Service de Neurologie, Centre Hospitalier Universitaire de Grenoble, Grenoble, France, 63
Translational Neurology, Biogen Idec, Cambridge, Massachusetts, United States of America, 64
Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands, 65
University of Cambridge, Department of Clinical Neurosciences, Addenbrooke’s Hospital, Cambridge, United Kingdom, 66
Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, 67
Department of Neurology, University of Rochester, Rochester, New York, United States of America, 68
Department of Pathology, University of Edinburgh, Edinburgh, United Kingdom, 69
University of Oxford, Department of Clinical Neurology, John Radcliffe Hospital, Oxford, United Kingdom, 70
Clinical Research Department, The Parkinson’s Institute and Clinical Center, Sunnyvale, California, United States of America, 71
Service de Neurologie, Hôpital Haut-Lévêque, Pessac, France, 72
Department of Medical and Molecular Genetics, King’s College London, London, United Kingdom, 73
Department of Neurology, Cardiff University, Cardiff, United Kingdom, 74
Department of Psychiatry and Medical Research Centre, South Manchester University Hospitals NHS Trust, Manchester, United Kingdom, 75
Trust Behavioural and Clinical Neurosciences Institute, University of Cambridge, Cambridge, United Kingdom.

Author Contributions


References