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 $p<5\times10^{-10}$, *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 = 399) and methylation (n = 292), 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 (UKPDC), 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 NIHR Biomedical Research Centre. This work was also supported by Parkinson's UK (Grants 8047 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/Z/08/Z). 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 (S 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 Pétursson, 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-09-2-0128. Funding to support collection of a portion of the samples was obtained from the National Institutes of Health (grants NS057105 and RR024992), 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 ForschungszentrumfürUmwelt und Gesundheit (GSF), 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 #01GS08134; 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 (HelMA, 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 framework (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 *LRKK2* [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: *ACMSD*, *STK39*, *MCCC1/LAMP3*, *SYT11*, 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

Author Summary

This paper describes the largest case-control analysis of Parkinson's disease to date, with a combined sample set of over 12,000 cases and 21,000 controls. After combining our findings with an independent replication dataset of more than 3,000 cases and 29,000 controls, we found five additional PD risk loci in addition to the 11 loci previously identified in earlier consortium efforts. This successful study further demonstrates the power of the GWA scan experimental design to find new loci contributing to disease risk, even in the context of complex disorders like Parkinson's disease. These new findings provide insights into the etiology of PD and will promote a better understanding of its pathogenesis.

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 GWA PD 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 GWA 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 $p<10^{-3}$. 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 12,386 cases and 21,026 controls (Table 1) from the IPDGC. We exchanged summary statistics for these most significant hits with an additional large, case-control replication dataset (3,426 PD cases and 29,624 controls) in an attempt to demonstrate independent replication.

On the basis of stage 1+2 results, seven new SNPs passed our defined genome-wide significance threshold ($p<5\times10^{-8}$, 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 1q32/*PARK16* [7], 4q21/*STBD1*, 7p15/*GPNMB*, 8p22/*FGF20* [16] and 16p11/*STX1B*.

rs708723/1q32 has been previously reported as PD associated (*PARK16*, [7,8]) but this SNP lacked the unequivocal evidence of association in European samples ($p=9.47\times10^{-10}$ in stage 2 only). To understand the potential biological consequences of risk variation at this locus we tested whether rs708723 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* ($p=1.8\times10^{-7}$) and *RAB7L1* ($p=7.2\times10^{-4}$). We also found correlations with the methylation state of CpG sites located in the *FLJ3269* gene ($p=3.9\times10^{-22}$).

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/*GPNMD* 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 *NUPL2* (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/*NMD3* and 8q21/*MMP16*) 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 ($p=9.7\times10^{-8}$ 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 *LRKK2* [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 ($p=2.2\times10^{-3}$) 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 phosphorylation sites and to be highly expressed in the cardiac muscle [26]; but an involvement in PD pathogenesis has yet to be suggested.

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	1039	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

At the 16p11/*STX1B* locus, notwithstanding the fact that other genes are in the associated region, *STX1B* is the most plausible candidate. It has been previously shown to be directly implicated in the process of calcium-dependent synaptic transmission in rat brain [17], having been suggested to play a role in the excitatory pathway of synaptic transmission. Since parkin, encoded by *PARK2*, negatively regulates the number and strength of excitatory synapses [27] , it makes *STX1B* a very interesting candidate from a biologic perspective.

FGF20 at 8p22 has been suggested to be involved in PD [16], albeit negative results in smaller cohorts have followed the original finding [28]. *FGF20* is a neurotrophic factor that exerts strong neurotrophic properties within brain tissue, and regulates central nervous development and function [29]. It is preferentially expressed in the substantia nigra [30], and it has been reported to be involved in dopaminergic neurons survival [30].

The ImmunoChip data provide limited resolution for the detection of multiple independent association signals in these regions. A previous study [31] reported some evidence of allelic heterogeneity at the 1q32/*PARK16* locus but the ImmunoChip data do not support this result. A previous study [11] also reported two independent associations at the 4q22/*SNCA* locus and our data are consistent with this scenario. However, the newly reported secondary association (rs2245801) is in low LD ($r^2=0.21$) with rs2301134, the SNP reported in [11] as an independent association. Taken together, these findings suggest that at least three independent associations exist at *SNCA*/4q22. A more exhaustive fine-mapping analysis using either sequencing of large cohorts or targeted genotyping arrays will also be required to fully explore this locus.

As yet, we do not know which of the variants and which genes within each region are exerting the pathogenic effect. We cannot exclude that some of the currently reported variants are in fact tagging high penetrance, but rare, mutations [32]. Nevertheless, the successful identification of these 16 risk loci further demonstrates the power of the GWA study design, even in the context of disorders like

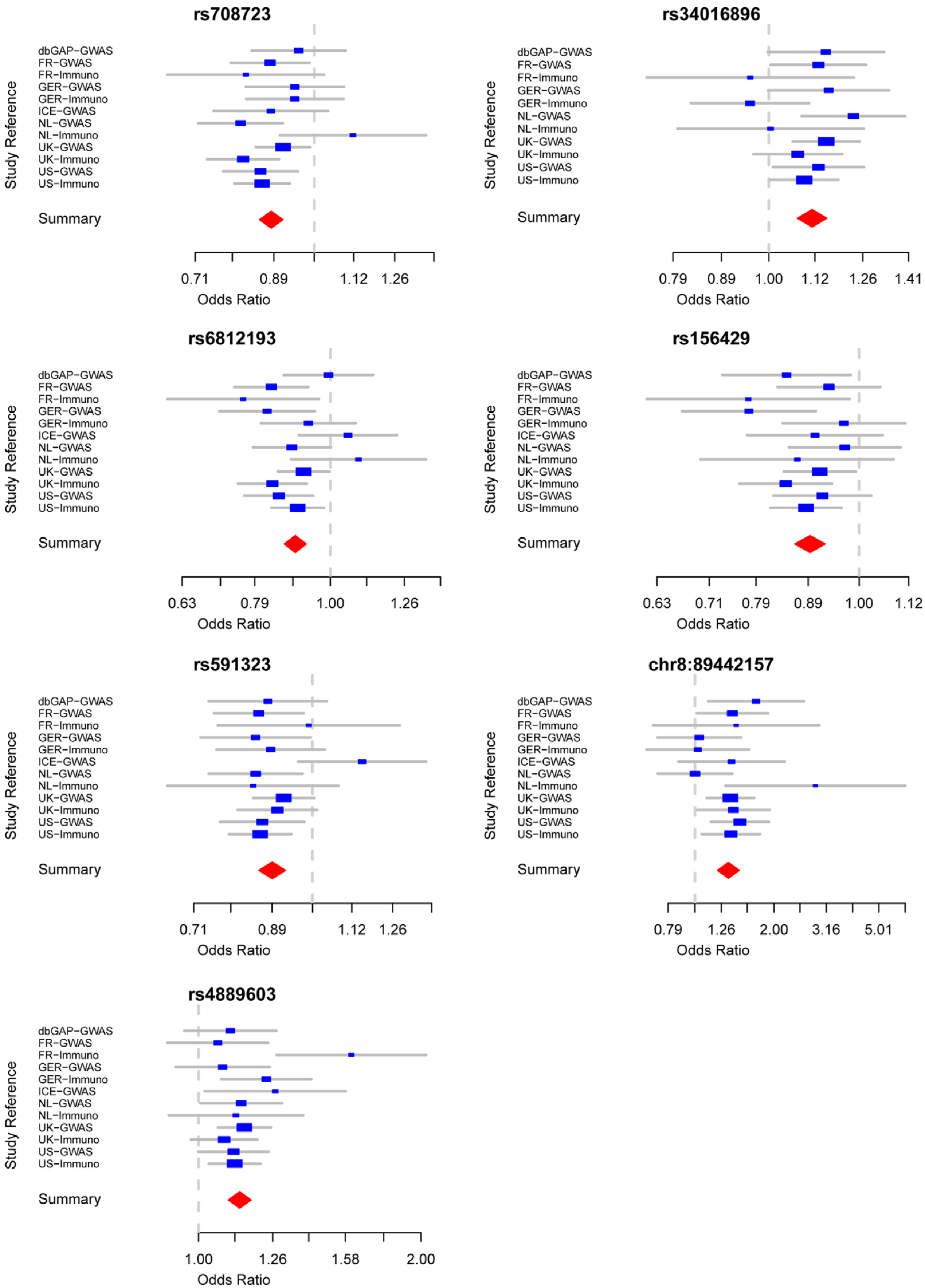
rs708723
Study Reference
dbGAP−GWAS
FR−GWAS
FR−Immuno
GER−GWAS
GER−Immuno
ICE−GWAS
NL−GWAS
NL−Immuno
UK−GWAS
UK−Immuno
US−GWAS
US−Immuno
Summary
0.71
0.89
1.12
1.26
Odds Ratio
rs34016896
0.79
0.89
1.00
1.12
1.26
1.41
rs6812193
0.63
0.79
1.00
1.26
rs156429
0.63
0.71
0.79
0.89
1.00
1.12
rs591323
0.71
0.89
1.12
1.26
chr8:89442157
0.79
1.26
2.00
3.16
5.01
rs4889603
1.00
1.26
1.58
2.00

Figure 1. Forest plots detailing effect estimates from the combined analysis of all data contributed by the International Parkinson Disease Genomics Consortium (joint estimates describing constituent effects of Stage 1+Stage 2).
doi:10.1371/journal.pgen.1002142.g001

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-heterogeneity 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 MACHv1.0.16 [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 2. Summary statistics for the seven SNPs that pass genome-wide significance ($p<5\times10^{-8}$) in the combined stage 1+2 analysis and that have either not been reported in published PD association studies.

					Stage 1		Stage 2		Stage 1+2	Do et al		Combined
SNP	**Chrom**	**Gene(s)**	**Alleles**	**MAF**	**OR (95%CI)**	***P***	**OR (95%CI)**	***P***	***P***	**OR (95%CI)**	***P***	***P***
rs708723	1q32	*RAB7L1/PARK16*	T>C	0.439	0.905 (0.862–0.95)	6.68×10^{-5}	0.863 (0.824–0.905)	9.47×10^{-10}	1.00×10^{-12}	0.758 (0.65–0.88)	2.12×10^{-6}	8.82×10^{-15}
rs34016896	3q26	*NMD3*	C>T	0.305	1.14 (1.09–1.2)	3.00×10^{-7}	1.08 (1.02–1.14)	0.00399	1.81×10^{-8}	1.002 (0.95–1.06)	0.954	1.31×10^{-6}
rs6812193	4q21	*STBD1*	C>T	0.36	0.886 (0.843–0.932)	2.52×10^{-6}	0.906 (0.864–0.95)	5.29×10^{-5}	7.46×10^{-10}	0.839 (0.79–0.89)	7.55×10^{-10}	1.17×10^{-17}
rs156429	7p15	*GPNMB*	A>G	0.403	0.894 (0.849–0.942)	2.15×10^{-5}	0.893 (0.852–0.937)	3.86×10^{-6}	3.27×10^{-10}	0.901 (0.85–0.95)	0.000193	3.05×10^{-13}
rs591323	8p22	*FGF20*	G>A	0.271	0.884 (0.836–0.935)	1.59×10^{-5}	0.875 (0.83–0.923)	$8.49E\times10^{-7}$	7.45×10^{-11}	0.932 (0.88–0.99)	0.023	1.92×10^{-11}
chr8:89442157	8q21	*MMP16*	C>T	0.0247	1.38 (1.21–1.57)	1.10×10^{-6}	1.29 (1.12–1.49)	0.000451	2.26×10^{-9}	0.969 (0.86–1.09)	0.589	2.36×10^{-5}
rs4889603	16p11	*STX1B*	A>G	0.413	1.12 (1.06–1.18)	4.13×10^{-5}	1.15 (1.1–1.21)	8.21×10^{-9}	2.66×10^{-12}	1.070 (1.01–1.13)	0.014	6.98×10^{-13}

1q32/PARK16 has been reported previously but is included because these data provide for the first time unequivocal evidence of association. P-values are computed using a one-degree-of-freedom regression trend test, including two principal components as covariates and combining the results across cohorts using a score test methodology. P-values are two-tailed and odds ratios are reported for the minor alleles. The notation X>Y indicates that X is the major allele and Y the minor allele. Allele frequencies were estimated using the UK control data. OR: odds ratio.
doi:10.1371/journal.pgen.1002142.t002

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.

Assay	Region	SNP	Region	Gene Tagged by Probe	Illumina Probe	Alleles	Effect Estimate	Standard Error	Unadjusted P	False Discovery Rate Adjusted P
Expression	Frontal Cortex	rs156429	7p15/*GPNMB*	*NUPL2*	ILMN_1789616	A>G	0.083	0.018	3.6E-06	1.0E-04
		rs156429	7p15/*GPNMB*	*NUPL2*	ILMN_2115154	A>G	0.078	0.017	3.1E-06	1.0E-04
		rs708723	1q32/*PARK16*	*NUCKS1*	ILMN_1680692	T>C	0.155	0.03	1.8E-07	1.5E-05
		rs708723	1q32/*PARK16*	*RAB7L1*	ILMN_1813685	T>C	−0.062	0.018	7.2E-04	1.2E-02
		rs4889603	16p11/*STX1B*	*ZNF668*	ILMN_1739236	A>G	0.062	0.015	4.1E-05	8.7E-04
		rs4889603	16p11/*STX1B*	*MYST1*	ILMN_1804679	A>G	−0.053	0.018	3.4E-03	4.8E-02
	Cerebellum	rs156429	7p15/*GPNMB*	*NUPL2*	ILMN_1789616	A>G	0.133	0.025	1.0E-07	3.7E-06
		rs156429	7p15/*GPNMB*	*NUPL2*	ILMN_2115154	A>G	0.131	0.023	1.2E-08	1.0E-06
		rs708723	1q32/*PARK16*	*NUCKS1*	ILMN_1680692	T>C	0.13	0.029	5.3E-06	1.1E-04
		rs708723	1q32/*PARK16*	*RAB7L1*	ILMN_1813685	T>C	−0.106	0.02	1.3E-07	3.7E-06
		rs4889603	16p11/*STX1B*	*ZNF668*	ILMN_1739236	A>G	0.075	0.02	1.3E-04	2.3E-03
		rs4889603	16p11/*STX1B*	*BCL7C*	ILMN_2371147	A>G	0.066	0.022	2.6E-03	3.8E-02
Methylation	Frontal Cortex	rs156429	7p15/*GPNMB*	*GPNMB*	cg17274742	A>G	−0.027	0.005	5.1E-07	3.2E-05
		rs156429	7p15/*GPNMB*	*GPNMB*	cg22932819	A>G	−0.009	0.002	1.6E-07	1.3E-05
		rs6812193	4q21/*STBD1*	*GENX-3414*	cg17010112	C>T	0.008	0.002	9.4E-04	3.0E-02
		rs708723	1q32/*PARK16*	*FLJ32569*	cg14159672	T>C	−0.219	0.022	3.1E-24	3.9E-22
		rs708723	1q32/*PARK16*	*FLJ32569*	cg14893161	T>C	−0.176	0.017	3.9E-25	9.6E-23
		rs4889603	16p11/*STX1B*	*BCL7C*	cg07896225	A>G	−0.002	0.001	9.7E-04	3.0E-02
		rs4889603	16p11/*STX1B*	*STX1B*	cg25033993	A>G	0.012	0.003	8.2E-05	3.4E-03
	Cerebellum	rs156429	7p15/*GPNMB*	*GPNMB*	cg17274742	A>G	−0.015	0.003	2.1E-06	1.3E-04
		rs708723	1q32/*PARK16*	*FLJ32569*	cg14159672	T>C	−0.246	0.023	3.0E-27	3.7E-25
		rs708723	1q32/*PARK16*	*FLJ32569*	cg14893161	T>C	−0.202	0.018	2.6E-28	6.4E-26

doi:10.1371/journal.pgen.1002142.t003

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

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

			1st quintile		*2nd quintile*		*3rd quintile*		*4th quintile*		*5th quintile*	
Study	*Trend P-value*	*AUC*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
USA	<2E-16	0.614	1	–	1.54	1.29–1.84	1.92	1.61–2.29	2.21	1.85–2.65	3.03	2.52–3.64
UK	<2e-16	0.636	1	–	1.34	1.05–1.71	1.79	1.41–2.28	2.35	1.86–2.99	3.11	2.46–3.96
Germany	1.29E-11	0.692	1	–	1.32	0.98–1.79	1.88	1.38–2.58	1.88	1.38–2.56	2.57	1.88–3.53
France	5.19E-13	0.675	1	–	1.69	0.99–2.92	1.13	0.65–1.98	3.30	1.95–5.67	5.92	3.42–10.52
Netherlands	5.08E-05	0.601	1	–	1.06	0.65–1.74	1.35	0.83–2.20	1.91	1.18–3.11	2.36	1.45–3.86
Combined	<2E-16	0.645	1	–	1.43	1.26–1.61	1.79	1.58–2.02	2.22	1.96–2.50	3.02	2.67–3.42
% Cases per Quintile				**37.90**		**46.06**		**51.15**		**56.56**		**63.75**

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.t004

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, 85 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 Genetics Resource Center DNA and Cell Line Repository (http://ccr.coriell.org/ninds) were used in this study, as well as clinical data. The submitters that contributed samples are acknowledged in detailed descriptions of each panel (http://ccr.coriell.org/sections/Collections/NINDS/?SsId = 10).

The authors thank The French Parkinson's Disease Genetics Study Group: Y. Agid, M. Anheim, A.-M. Bonnet, M. Borg, A. Brice, E. Broussolle, J.-C. Corvol, Ph. Damier, A. Destée, A. Dürr, F. Durif, S. Klebe, E. Lohmann, M. Martinez, P. Pollak, O. Rascol, F. Tison, C. Tranchant, M. Vérin, F. Viallet, and M. Vidailhet. The authors thank the members of the French 3C consortium: Drs Annick Alpérovitch, 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: Vincent Plagnol[1], Michael A. Nalls[2], Jose M. Bras[3], Dena G. Hernandez[2,3], Manu Sharma[4], Una-Marie Sheerin[3], Mohamad Saad[4,5], Javier Simón-Sánchez[6], Claudia Schulte[7,8], Suzanne Lesage[9,10,11], Sigurlaug Sveinbjörnsdóttir[12,13,14], Philippe Amouyel[15,16], Sampath Arepalli[1], Gavin Band[17], Roger A. Barker[18], Céline Bellinguez[17], Yoav Ben-Shlomo[19], Henk W. Berendse[20], Daniela Berg[7,8], Kailash Bhatia[21], Rob M. A. [de Bie][22], Alessandro Biffi[23,24,25], Bas Bloem[26], Zoltan Bochdanovits[6], Michael Bonin[27], Kathrin Brockmann[7,8], Janet Brooks[1], David J. Burn[28], Gavin Charlesworth[3], Honglei Chen[29], Patrick F. Chinnery[30], Sean Chong[2], Carl E. Clarke[31,32,33], Mark R. Cookson[2], J. Mark Cooper[34], Jean Christophe Corvol[9,10,11,35], Carl Counsell[36], Philippe Damier[37], Jean-François Dartigues[38], Panos Deloukas[39], Günther Deuschl[40], David T. Dexter[41], Karin D. van Dijk[20], Allissa Dillman[2], Frank Durif[42], Alexandra Dürr[8,9,10,43], Sarah Edkins[39], Jonathan R. Evans[44], Thomas Foltynie[45], Colin Freeman[17], Jianjun Gao[29], Michelle Gardner[3], J. Raphael Gibbs[2,3], Alison Goate[46], Emma Gray[39], Rita Guerreiro[3], Ómar Gústafsson[47], Clare Harris[36], Garrett Hellenthal[17], Jacobus J. van Hilten[48], Albert Hofman[49], Albert Hollenbeck[50], Janice Holton[51], Michele Hu[52], Xuemei Huang[53], Heiko Huber[7,8], Gavin Hudson[30], Sarah E. Hunt[39], Johanna Huttenlocher[15], Thomas Illig[54], Pálmi V. Jónsson[55], Cordelia Langford[44], Andrew Lees[51], Peter Lichtner[56], Patricia Limousin[57], Grisel Lopez[58], Delia Lorenz[40], Alisdair McNeill[34], Catriona Moorby[31], Matthew Moore[2], Huw Morris[59], Karen E. Morrison[31,60], Ese Mudanohwo[61], Sean S. O'Sullivan[51], Justin Pearson[59], Richard Pearson[17], Joel S. Perlmutter[46], Hjörvar Pétursson[27,47], Matti Pirinen[17], Pierre Pollak[62], Bart Post[26], Simon Potter[39], Bernard Ravina[63], Tamas Revesz[51], Olaf Riess[27], Fernando Rivadeneira[49,64], Patrizia Rizzu[6], Mina Ryten[3], Stephen Sawcer[65], Anthony Schapira[34], Hans Scheffer[66], Karen Shaw[51], Ira Shoulson[67], Ellen Sidransky[58], Rohan de Silva[3], Colin Smith[68], Chris C. A. Spencer[69], Hreinn Stefánsson[47], Stacy Steinberg[47], Joanna D. Stockton[31], Amy Strange[17], Zhan Su[17], Kevin Talbot[69], Carlie M. Tanner[70], Avazeh Tashakkori-Ghanbaria[39], François Tison[71], Daniah Trabzuni[3], Bryan J. Traynor[2], André G. Uitterlinden[49,64], Jana Vandrovcova[3], Daan Velseboer[22], Marie Vidailhet[9,10,11], Damjan Vukcevic[17], Robert Walker[68], Bart van de Warrenburg[26], Michael E. Weale[72], Mirdhu Wickremaratchi[73], Nigel Williams[59], Caroline H. Williams-Gray[18], Sophie Winder-Rhodes[74], Kári Stefánsson[47], Maria Martinez[4,5], Peter Donnelly[17], Andrew B. Singleton[2], John Hardy[3], Peter Heutink[6], Alexis Brice[9,10,11,43], Thomas Gasser[7,8], Nicholas W. Wood[1,3]*

1 UCL Genetics Institute, University College London, London, United Kingdom, **2** Laboratory of Neurogenetics. National Institute on Aging, National Institutes of Health, Bethesda, Maryland, United States of America, **3** Department of Molecular Neuroscience, Institute of Neurology, University College London, London, United Kingdom, **4** Institut National de la Sante et de la Recherche Medicale, UMR 1043, Centre de Physiopathologie de Toulouse-Purpan, Toulouse, France, **5** Paul Sabatier University, Toulouse, France, **6** Department of Clinical Genetics, Section of Medical Genomics, VU University Medical Centre, Amsterdam, The Netherlands, **7** Department for Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany, **8** Deutsches Zentrum fur Neurodegenerative Erkrangungen (German Center for Neurodegenerative Diseases), Tübingen, Germany, **9** Institut National de la Sante et de la Recherche Medicale, UMR_S975 (Formerly UMR_S679), Paris, France, **10** Université Pierre et Marie Curie-Paris, Centre de Recherche de l'Institut du Cerveau et de la Moelle épinière, UMR-S975, Paris, France, **11** Centre National de la Recherche Scientifique, UMR 7225, Paris, France, **12** Department of Neurology, Landspítali University Hospital, Reykjavík, Iceland, **13** Department of Neurology, Mid Essex Hospital, Broomfield Hospital, Chelmsford, Essex, United Kingdom, **14** Queen Mary College, University of London, London, United Kingdom, **15** Institut National de la Sante et de la Recherche Medicale, U744, Lille, France, **16** Institut Pasteur de Lille, Université de Lille Nord, Lille, France, **17** Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, United Kingdom, **18** Department of Neurology, Addenbrooke's Hospital, University of Cambridge, Cambridge, United Kingdom, **19** Department of Social Medicine, Bristol University, Bristol, United Kingdom, **20** Department of Neurology and Alzheimer Center, VU University Medical Center, Amsterdam, The Netherlands, **21** Department of Motor Neuroscience, University College London Institute of Neurology, London, United Kingdom, **22** Department of Neurology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, **23** Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts, United States of America, **24** Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts, United States of America, **25** Program in Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts, United States of America, **26** Department of Neurology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, **27** Department of Medical Genetics, Institute of Human Genetics, University of Tübingen, Tübingen, Germany, **28** Newcastle University Clinical Ageing Research Unit, Campus for Ageing and Vitality, Newcastle upon Tyne, United Kingdom, **29** Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, North Carolina, United States of America, **30** Neurology Department, The Medical School, Newcastle upon Tyne, Newcastle University, United Kingdom, **31** School of Clinical and Experimental Medicine, University of Birmingham, Edgbaston, Birmingham, United Kingdom, **32** Department of Neurology, City Hospital, Sandwell, United Kingdom, **33** West

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, Hôpital 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 Medicale, U897, 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** AP-HP, Pitié-Salpêtrière 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** Departments of Neurology, Radiology, Neurosurgery, Pharmacology, Kinesiology, and 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, Landspítali University Hospital, Reykjavík, Iceland, **56** Institute of Human Genetics, Helmholtz Zentrum München, German Research Centre for Environmental Health, Neuherberg, Germany, **57** Sobell 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 Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, 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 Council/Wellcome Trust Behavioural and Clinical Neurosciences Institute, University of Cambridge, Cambridge, United Kingdom.

Author Contributions

Conceived and designed the experiments: V Plagnol, MA Nalls, M Martinez, P Donnelly, J Hardy, P Heutink, A Brice, T Gasser, AB Singleton, NW Wood. Analyzed the data: V Plagnol, MA Nalls, JM Bras. Contributed reagents/materials/analysis tools: DG Hernandez, M Sharma, U-M Sheerin, J Simón-Sánchez, C Schulte, S Lesage, S Sveinbjörnsdóttir, P Amouyel, S Arepalli, G Band, RA Barker, C Bellinguez, Y Ben-Shlomo, HW Berendse, D Berg, K Brockmann, RMA de Bie, A Brice, A Biffi, B Bloem, Z Bochdanovits, M Bonin, K Bhatia, J Brooks, DJ Burn, G Charlesworth, H Chen, PF Chinnery, S Chong, CE Clarke, A Dürr, A Dillman, DT Dexter, F Tison, F Durif, KD van Dijk, M Saad, MR Cookson, JM Cooper, JC Corvol, C Counsell, P Damier, J-F Dartigues, P Deloukas, G Deuschl, S Edkins, JR Evans, T Foltynie, C Freeman, J Gao, M Gardner, JR Gibbs, R Guerreiro, A Goate, E Gray, O Gústafsson, C Harris, G Hellenthal, JJ van Hilten, A Hofman, A Hollenbeck, J Holten, J Huttenlocher, M Hu, X Huang, H Huber, G Hudson, SE Hunt, T Illig, PV Jónsson, C Langford, A Lees, P Lichtner, P Limimousin, G Lopez, D Lorenz, A McNeill, C Moorby, H Morris, KE Morrison, E Mudhanohwo, SS O′Sullivan, J Pearson, R Pearson, JS Perlmutter, H Pétursson, M Pirinen, P Pollak, B Post, S Potter, B Ravina, T Revesz, O Riess, F Rivadeneira, P Rizzu, M Ryten, S Sawcer, A Schapira, H Scheffer, K Shaw, I Shoulson, E Sidransky, R de Silva, C Smith, CCA Spencer, H Stefánsson, S Steinberg, JD Stockton, A Strange, Z Su, K Talbot, CM Tanner, A Tashakkori-Ghanbaria, D Tison, BJ Traynor, AG Uitterlinden, J Vandrovcova, D Velseboer, M Vidailhet, D Vukcevic, R Walker, B van de Warrenburg, ME Weale, M Wickremaratchi, N Williams, CH Williams-Gray, S Winder-Rhodes, K Stefánsson, M Moore, P Donnelly, AB Singleton, J Hardy, P Heutink, T Gasser, NW Wood. Wrote the manuscript: V Plagnol, MA Nalls, AB Singleton, NW Wood.

References

1. Zimprich A, Müller-Myhsok B, Farrer M, Leitner P, Sharma M, et al. (2004) The PARK8 locus in autosomal dominant parkinsonism: confirmation of linkage and further delineation of the disease-containing interval. American journal of human genetics 74: 11–19.
2. Paisán-Ruíz C, Jain S, Evans W, Gilks W, Simón J, et al. (2004) Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 44: 595–600.
3. Valente EM, Abou-Sleiman P, Caputo V, Muqit M, Harvey K, et al. (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 304: 1158–1160.
4. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, et al. (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276: 2045–2047.
5. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, et al. (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 392: 605–608.
6. Bonifati V, Rizzu P, van Baren M, Schaap O, Breedveld G, et al. (2003) Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 299: 256–259.
7. Simón-Sánchez J, Schulte C, Bras J, Sharma M, Gibbs R, et al. (2009) Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nature Genetics 41: 1308–1312.
8. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, et al. (2009) Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nature Genetics 41: 1303–1307.
9. Saad M, Lesage S, Saint-Pierre A, Corvol J-C, Zelenika D, et al. (2011) Genome-wide association study confirms BST1 and suggests a locus on 12q24 as risk loci for Parkinson's disease in the European population. Human Molecular Genetics 20: 615–627.
10. Simon-Sanchez J, van Hilten J, van de Warrenburg B, Post B, Berendse H, et al. (2011) Genome-wide association study confirms extant PD risk loci among the Dutch. European Journal of Human Genetics aop.
11. (2011) Dissection of the genetics of Parkinson's disease identifies an additional association 5′ of SNCA and multiple associated haplotypes at 17q21. Human Molecular Genetics 20: 345–353.
12. Pankratz N, Wilk J, Latourelle J, DeStefano A, Halter C, et al. (2009) Genomewide association study for susceptibility genes contributing to familial Parkinson disease. Human genetics 124: 593–605.
13. Hamza T, Zabetian C, Tenesa A, Laederach A, Montimurro J, et al. (2010) Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. Nature Genetics 42: 781–785.
14. Li Y, Willer C, Ding J, Scheet P, Abecasis G (2010) MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genetic epidemiology 34: 816–834.

15. Nalls M, Plagnol V, Hernandez D, Sharma M, Sheerin U-M, et al. (2011) Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet 377: 641–649.
16. van der Walt J, Noureddine M, Kittappa R, Hauser M, Scott W, et al. (2004) Fibroblast growth factor 20 polymorphisms and haplotypes strongly influence risk of Parkinson disease. American journal of human genetics 74: 1121–1127.
17. Smirnova T, Stinnakre J, Mallet J (1993) Characterization of a presynaptic glutamate receptor. Science 262: 430–433.
18. Wang G, van der Walt J, Mayhew G, Li Y-J, Züchner S, et al. (2008) Variation in the miRNA-433 binding site of FGF20 confers risk for Parkinson disease by overexpression of alpha-synuclein. American journal of human genetics 82: 283–289.
19. Wider C, Dachsel J, Soto A, Heckman M, Diehl N, et al. (2009) FGF20 and Parkinson's disease: no evidence of association or pathogenicity via alpha-synuclein expression. Movement disorders 24: 455–459.
20. Gilks W, Abou-Sleiman P, Gandhi S, Jain S, Singleton A, et al. (2005) A common LRRK2 mutation in idiopathic Parkinson's disease. Lancet 365: 415–416.
21. Zollner S, Pritchard J (2007) Overcoming the winner's curse: estimating penetrance parameters from case-control data. American journal of human genetics 80: 605–615.
22. Plagnol V, Smyth D, Todd J, Clayton D (2008) Statistical independence of the colocalized association signals for type 1 diabetes and RPS26 gene expression on chromosome 12q13. Biostatistics (Oxford, England).
23. Shimizu F, Katagiri T, Suzuki M, Watanabe TK, Okuno S, et al. (1997) Cloning and chromosome assignment to 1q32 of a human cDNA (RAB7L1) encoding a small GTP-binding protein, a member of the RAS superfamily. Cytogenetics and cell genetics 77: 261–263.
24. Koshimura K, Ohue T, Akiyama Y, Itoh A, Miwa S (1992) L-dopa administration enhances exocytotic dopamine release in vivo in the rat striatum. Life sciences 51: 747–755.
25. Cabin D, Shimazu K, Murphy D, Cole N, Gottschalk W, et al. (2002) Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking alpha-synuclein. The Journal of neuroscience 22: 8797–8807.
26. Grundt K, Haga IV, Aleporou-Marinou V, Drosos Y, Wanvik B, et al. (2004) Characterisation of the NUCKS gene on human chromosome 1q32.1 and the presence of a homologous gene in different species. Biochemical and biophysical research communications 323: 796–801.
27. Helton T, Otsuka T, Lee M-C, Mu Y, Ehlers M (2008) Pruning and loss of excitatory synapses by the parkin ubiquitin ligase. Proceedings of the National Academy of Sciences of the United States of America 105: 19492–19497.
28. Clarimon J, Xiromerisiou G, Eerola J, Gourbali V, Hellström O, et al. (2005) Lack of evidence for a genetic association between FGF20 and Parkinson's disease in Finnish and Greek patients. BMC neurology 5.
29. Jeffers M, Shimkets R, Prayaga S, Boldog F, Yang M, et al. (2001) Identification of a novel human fibroblast growth factor and characterization of its role in oncogenesis. Cancer research 61: 3131–3138.
30. Ohmachi S, Mikami T, Konishi M, Miyake A, Itoh N (2003) Preferential neurotrophic activity of fibroblast growth factor-20 for dopaminergic neurons through fibroblast growth factor receptor-1c. J Neurosci Res 72: 436–443.
31. Tucci A, Nalls M, Houlden H, Revesz T, Singleton A, et al. (2010) Genetic variability at the PARK16 locus. European journal of human genetics : EJHG 18: 1356–1359.
32. Dickson S, Wang K, Krantz I, Hakonarson H, Goldstein D (2010) Rare Variants Create Synthetic Genome-Wide Associations. PLoS Biol 8: e1000294. doi:10.1371/journal.pbio.1000294.
33. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira M, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. American journal of human genetics 81: 559–575.
34. Price A, Patterson N, Plenge R, Weinblatt M, Shadick N, et al. (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics 38: 904–909.
35. (2010) A map of human genome variation from population-scale sequencing. Nature 467: 1061–1073.