Published in final edited form as:

Neurobiol Aging. 2016 September ; 45: 213.e3–213.e9. doi:10.1016/j.neurobiolaging.2016.06.001.

Genome Wide assessment of Parkinson's disease in a Southern Spanish population

S Bandrés-Ciga^{1,*}, TR Price^{2,*}, FJ Barrero³, F Escamilla-Sevilla⁴, J Pelegrina³, S Arepalli², D Hernández², B Gutiérrez⁵, J Cervilla⁵, M Rivera⁵, AM Rivera², J Ding², F Vives¹, MA Nalls², AB Singleton², and R Durán¹

¹Department of Physiology and Institute of Neurosciences Federico Olóriz, Centro de Investigaciones Biomedicas (CIBM), University of Granada, 18016, Granada, Spain

²Laboratory of Neurogenetics, NIA, NIH, 20892, Bethesda, MD USA

³Movement Disorders Unit, University Hospital San Cecilio, 18012, Granada, Spain

⁴Movement Disorders Unit, Department of Neurology, Instituto de Investigación Biosanitaria (IBS), University Hospital Virgen de las Nieves, 18014, Granada, Spain

⁵Department of Psychiatry and Institute of Neurosciences Federico Olóriz, Centro de Investigaciones Biomedicas (CIBM), University of Granada, 18016, Granada, Spain; CIBER en Salud Mental (CIBERSAM), University of Granada, Granada, Spain

Abstract

Here, we set out to study the genetic architecture of Parkinson's disease (PD) through a Genome-Wide Association Study (GWAS) in a Southern Spanish population. 240 PD cases and 192 controls were genotyped on the NeuroX array. We estimated genetic variation associated with PD risk and age at onset (AAO). Risk profile analyses for PD and AAO were performed using a weighted genetic risk score (GRS). Total heritability was estimated by genome-wide complex trait analysis. Rare variants were screened with single-variant and burden tests. We also screened for variation in known PD genes. Finally, we explored runs of homozygosity and structural genomic variations. We replicate PD association (uncorrected p-value < 0.05) at the following loci:

Corresponding author: Dr. Raquel Durán Ogalla, Department of Physiology and Institute of Neurosciences Federico Olóriz, Centro de Investigaciones Biomedicas (CIBM), University of Granada, 18016, Granada, Spain. Tel: +34 686 63 0291, rduran@ugr.es.

*These authors contributed equally to this work.

DISCLOSURE STATEMENT

The authors have no financial disclosure to report. No pharmaceutical entity has collaborated in this study, and no financial purpose exists.

AUTHOR CONTRIBUTIONS

* Sara Bandrés Ciga and Timothy Ryan Price contributed equally to this work.

Study concept, design, revision and critique: Andrew Singleton, Michael Nalls, Dena Hernández, Raquel Durán, Francisco Vives.

Execution of the laboratory work: Sampath Arepalli, Sara Bandrés Ciga.

Data analysis: Timothy Ryan Price, Sara Bandrés Ciga, Alberto Rivera, Jing-Hui Ding.

Neurological-clinical assessment, patient and control recruitment, sample collection: Francisco Barrero-Hernández, Francisco Escamilla-Sevilla, Javier Pelegrina, Blanca Gutiérrez, Jorge Cervilla, Margarita Rivera.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACMSD/TMEM163, *MAPT*, *STK39*, *MIR4697* and *SREBF/RAI1*. Subjects in the highest GRS quintile showed significantly increased risk of PD versus the lowest quintile (OR=3.6, p-value < $4e^{-7}$), but no significant difference in AAO. We found evidence of runs of homozygosity in two PD-associated regions: one intersecting the *HLA-DQB1* gene in six patients and one control; and another intersecting the *GBA-SYT11* gene in one PD case. The *GBA* N370S and the *LRRK2* G2019S variants were found in 8 and 7 cases respectively, replicating previous work. A structural variant was found in one case in the *PARK2* gene locus. This current work represents a comprehensive assessment at a genome-wide level characterizing a novel population in PD genetics.

Keywords

Parkinson's disease; Genetics; GWAS; Southern Spain

INTRODUCTION

Over the last several years, genetic investigation of Parkinson's disease (PD) has successfully identified many disease-causing mutations (Polymeropoulos, et al., 1997, Kitada, et al., 1998, Paisán-Ruiz, et al., 2004, Zimprich, et al., 2004, Bonifati, et al., 2003, Valente, et al., 2004, Zimprich, et al., 2011, Vilarino-Guell, et al., 2011) providing considerable insight into the molecular mechanisms underlying the etiology and pathogenesis of this multifactorial and complex disease.

Importantly, Genome Wide Association (GWA) studies (Edwards, et al., 2010, Hernandez, et al., 2012, Pihlstrom, et al., 2013, Saad, et al., 2011, Satake, et al., 2009, Simón-Sánchez, et al., 2009, Simón-Sánchez, et al., 2001) and large scale meta-analyses (Nalls, et al., 2014, Nalls, et al., 2011, Do, et al., 2011, International Parkinson's Disease Genomics Consortium and Welcome Trust Case Control Consortium 2, 2011, Lill, et al., 2012, Pankratz, et al., 2012) have been applied to identify and replicate risk loci that fit the common disease, common variant hypothesis in PD (Reich, et al., 2001). Despite the significant number of novel risk loci identified so far, only a small portion of the heritable component for PD has been explained, suggesting there is a substantial unknown genetic component to be discovered (Keller, et al., 2012). The polygenic nature of the illness and the hypothetically large number of loci involved with risk variants of small effect size make analysis of the genetic contribution to disease phenotype particularly difficult.

It has been widely suggested that studies in populations with limited genetic heterogeneity are valuable for studying the genetic basis of disease (Hernandez, et al., 2012). Moreover, replication studies in independent populations are absolutely necessary to test the robustness of such association reports. Andalusia, given its geographical location on the southernmost region of Spain, represents a cross-link between Europe and Africa. The interactions and interbreeding across the Mediterranean Sea and North Africa have contributed to shape a genetic profile which to date has been poorly studied in the context of PD genetics. Motivated by these considerations, we set out to study the genetic architecture of PD in this population by performing a genome-wide association study.

The purpose of this project was to identify novel putative candidate loci associated with PD and to investigate whether SNPs previously identified as risk variants contribute to PD risk in the Southern Spanish population. Furthermore, we use genetic risk profiling to aggregate risk across the previously established risk loci. In the same way, we aim to understand whether this genetic risk is associated with age at disease onset. We also attempt to identify recessive founder variants by exploring runs of homozygosity enriched in cases and explore whether disease-associated rare variants are present in our cohort. Finally, we estimate PD heritability attributable to genetic variation assessed by our genotyping platform and evaluate the role of copy number variants (CNVs) as risk factors for PD in these subjects.

METHODS

We included a group of 240 PD patients treated at the Movement Disorders Unit of the Service of Neurology in both Hospital Clínico San Cecilio and Hospital Virgen de las Nieves of Granada (Spain). PD was diagnosed at least by 2 experienced neurologists in the field of movement disorders following the criteria of the UK PD Society Brain Bank (Gelb, et al., 1999). Our control group was comprised of 192 healthy individuals with no signs or symptoms of parkinsonism who were extensively assessed to rule out any sign of neurological condition. The study was approved by the local ethics committee, and written informed consent was taken from each participant.

Samples were genotyped using the NeuroX Array (Illumina), a powerful and reliable tool for the investigation of genetic factors associated with neurodegenerative disorders (Nalls, et al., 2015a). It comprises standard Illumina exome content of approximately 240,000 variants densely covering previously published PD GWAS-associated loci, ancestry informative markers, markers for determination of identity by descent (IBD), X chromosome single nucleotide polymorphisms (SNPs) for sex determination and over 24,000 custom content variants focusing on rare variants related to neurodegenerative diseases.

After genotyping, genotypes were clustered using Illumina GenomeStudio using a previously developed cluster file for NeuroX (Nalls, et al., 2015a). Stringent quality control (QC) analysis was performed as follows: samples with call rates of less than 95% and whose genetically determined sex from X chromosome heterogeneity did not match that from clinical data were excluded from the analysis. Samples exhibiting heterozygosity greater than 6 standard deviations from the population mean were excluded. Once preliminary sample-level QC was completed, SNPs with minor allele frequency (MAF) < 0.01, Hardy-Weinberg Equilibrium (HWE) p-value < 1E-5 and missingness rates > 5 % were excluded. Palindromic SNPs were excluded as well. SNPs thought to be in linkage disequilibrium (LD) in a sliding window of 50 adjacent SNPs which scrolled through the genome at a rate of 5 overlapping SNPs were also removed from the following analyses. Genetic variants passing QC numbered 33074 SNPs, 28393 of which remained after LD pruning. Next, samples were clustered using principal component analysis (PCA) to evaluate European ancestry as compared to the HapMap3 CEU/TSI populations (International HapMap Consortium, 2003) (Figure S1). Confirmed European ancestry samples were extracted and principal components (PCs) 1 and 2 were used as covariates in all analysis. Those samples cryptically related at the level of first cousins or closer (sharing proportionally more than

12.5 % of alleles) were dropped from the following analysis. Samples passing QC numbered 206 cases and 172 controls.

For all SNPs (non-LD pruned) and samples passing QC as detailed above, a logistic regression model adjusted for sex, age and first and second genotype PCs as covariates was used to estimate risk associated with the disease. A linear regression model adjusted for the same covariates was used to explore the influence of genetic variation on the age at onset of the disease (AAO).

Risk profile analysis for PD and AAO was performed as described in detail elsewhere (Kara, et al., 2014, Nalls, et al., 2015b). Briefly, a cumulative genetic risk score (GRS) was calculated incorporating 30 SNPs previously associated with PD (Nalls, et al., 2014). Risk allele dosages were counted and a GRS was generated across all loci. All SNPs were weighted by their published odds ratios, giving greater weight to alleles with higher risk estimates.

The dataset was divided into quintiles based on the GRS. A regression was performed regressing disease (logistic regression) or AAO (linear regression) against quintile membership, and odds ratios were reported comparing the reference group (lowest risk quintile) to the remaining 4 quintiles. Risk profiling analysis was adjusted for PCs 1 and 2 to account for population substructure.

In an attempt to estimate PD heritability attributable to genome-wide assayed genetic variation, we used the genome-wide complex trait analysis (GCTA) package (Yang, et al., 2001). This analysis has the ability to detect contribution to disease risk of variants which do not reach genome significance because of small effect size, though not to identify the specific variants contributing to disease risk. First, a genetic relationship matrix (GRM) is calculated by estimating pairwise genetic relations between the individuals in the cohort. Then, a linear mixed model is applied to estimate phenotypic variance explained by genome-wide SNPs passing QC, correcting for PCs 1 and 2, age, and gender.

For Identity by state (IBS) comparisons, PLINK 1.07 (Purcell, et al., 2007) was used to calculate case and control specific rates of proportional allele sharing both genome-wide and within defined segments. We used a pairwise approach to estimate overall allelic similarity between each pairing of subjects. Then, case-control labels were permuted 2000 times to assess whether case/control pairings are more different in the actual data than in permuted data, suggesting segregation of cases and controls into distinct populations. In these analyses, cryptically related PD individuals removed in previous steps were included in order to identify shared rare recessive variants.

Subsequently, we tested for association between rare variants ($MAF < 0.05$) and PD using a single-variant test under an additive genetic model. This approach evaluates if there is any association between each independent variant and PD, employing a significance threshold of 5×10^{-8} . The minimum minor allele count (MAC) considered for the analysis was 3, and we adjusted for the top 20 PCs, age, and sex. In addition to testing each variant individually, we evaluated cumulative effects of multiple genetic variants by collapsing rare variants within a

gene level using the SKAT-O algorithm at default settings as part of the EPACTS package (information at <http://genome.sph.umich.edu/wiki/EPACTS>) (Lee, et al., 2012).

Moreover, we screened for variants with MAF <1% in known PD-causing genes (Table S1), checking all variants with two approaches: a putative dominant model (allele present only in cases and not in controls) and a putative recessive model (allele present with two copies only in cases and not in two copies in controls).

Finally, detection of structural genomic variations was performed using PennCNV-1.0.3 (Illumina Inc., San Diego, CA) and visualised with R. Two metrics were assessed with this utility: B allele frequency and log R ratio. These two statistics allow visualization of copy number changes and are described in detail elsewhere (Matarin, et al., 2008). Briefly, log R ratio gives an indirect measure of copy number of each SNP by plotting the ratio of observed to expected hybridization intensity. An R value above 1 is indicative of an increase in copy number (duplication or triplication), and an R value below 1 suggests a decrease (deletion). B allele frequency plots the proportion of times an allele is called A or B at each genotype: thus the expected ratios are 1.0 (B/B), 0.5 (A/B) and 0.0 (A/A). Significant deviations from these figures in contiguous SNPs are indicative of a CNV. While this metric exhibits a high level of variance for individual SNPs, it does provide a measure of CNV when log R ratio values for numerous adjacent SNPs are visualized.

RESULTS

Descriptive statistics of the Spanish cohort are summarized in Table 1. Visualization of the top 2 genetic PCs of our Spanish samples show them to be similar overall to HapMap European populations (Figure S1).

Our GWA failed to detect any genetic variant significant at $p < 5e^{-8}$ (Figure 1). Although we did not show any association signal with PD at this stringent p-value, we managed to marginally replicate association on the basis of a nominal uncorrected p-value < 0.05 with the following loci previously identified in a large-scale PD GWAS meta-analysis: *ACMSD/TMEM163*, *MAPT*, *STK39*, *MIR4697* and *SREBF/RAI1* (Table 2), (Figure S2), (Nalls, et al., 2011). Similarly, genome-wide assessment for AAO of PD did not reveal any genetic variant to be associated at p values < $5e^{-8}$ (Figure 2), (Figure S3).

Our cumulative risk attributable to all known GWAS loci associated with PD showed significantly different genetic risk scores (GRS) in this cohort (p-value < $4e^{-7}$). Individuals in the highest quintile of genetic risk were found to be 3.6 times more likely to develop PD than those in the lowest quintile (Table 3) (Figure S4). No significant association was found between GRS and AAO of disease (p=0.52, data not shown).

Using GCTA, we estimated total heritability explained by the genotyped SNPs on the NeuroX array to be $13.7\% \pm 40\%$ (0–92% at 95% CI). Our NeuroX array is more focused on a lower number of exonic variants compared to earlier analyses, which may contribute to the lower estimate reported here (Keller, et al., 2012). The lack of precision in this estimate likely reflects the low power of the current study, and thus the results of this heritability analysis should be viewed with caution.

In a genome-wide assessment of differential IBS between cases and controls, we found no evidence of excess of homozygosity in all cases vs. all controls. However, we did find evidence of runs of homozygosity in two regions previously associated with PD: one intersecting the *HLA-DQB1* gene in six patients and one control; and another intersecting the *GBA-SYT11* gene in one PD case. A run of at least 98.5% homozygosity from chr6:32,333,827–32,680,928 (~347 kb) was consistently reported across subjects showing homozygosity in the *HLA-DQB1* locus. This region directly overlaps the previously reported GWAS hit (Nalls, et al., 2014). Our single case with a run of homozygosity in *GBA-SYT11* exhibited a 99.6% homozygous run of 11233 kb (chr1:149,906,413–161,139,738).

Single-variant tests could not identify any prospective novel rare variant associated with PD (Figure S5). When focusing on the cumulative effects of multiple genetic rare variants by grouping them by a gene level we also detect no significant association with PD (Figure S6, Figure S7, Figure S8).

However, we explored rare variants in PD known genes present in cases but absent in our controls (Table 4). The *GBA* N370S risk variant was found in 8 cases (7 heterozygous and 1 homozygous) and the *LRRK2* G2019S pathogenic mutation was identified in 7 patients, all in heterozygosity. Variants of unknown pathogenicity were also detected in *PINK1* (Valente, et al., 2004), *PARK2* (Bonifati, et al., 2003), *GIGYF2* (Lautier, et al., 2008), *DNAJC13* (Vilarino-Guell, et al., 2014), *SYNJ1* (Krebs, et al., 2013), and *FBXO7* (Shojaee, et al., 2008).

In addition to providing SNP variation data, the genotyping assay used generates metrics that allow detection of CNV. We evaluated both the log R ratio and the B allele frequency plots across the genome in all samples and identified a *PARK2* heterozygous deletion (Chr6: 162,206,852–162,206,882) in a PD case (Figure S9).

DISCUSSION

Here, we describe what is to our knowledge the first GWAS for PD in a Spanish cohort, and the second in a Southern European population (Kara, et al., 2014). Although we are aware that the sample size itself is a limitation, it comprised most PD cases from the province of Granada. Therefore, one of the strengths of this study is that no potential problems of population stratification are expected as the sample comes from a relatively homogeneous ancestry background.

Our results suggest that there is no detectable high risk variant for PD present in our population, consistent with results of other GWASes with small sample size (Hernández, et al., 2012, Fung, et al., 2006). However, we have replicated association of five previously reported PD-related loci (Nalls, et al., 2014) at nominal uncorrected p-value < 0.05, though these associations do not pass multiple corrections at $n=30$ ($0.05/30 \approx 0.002$; Table 2). Our limited replication is likely due at least in part to insufficient statistical power to detect the relatively low effect of these alleles in our smaller cohort. For example, assuming an alpha of 0.002, disease prevalence of 4.4%, allele frequency of 0.2, and relative risk of 1.3, our

power in this study would be 0.065. The presence of population-specific differences in the Southern Spanish sample and PD subphenotypes with distinct genetic etiologies might also be responsible, among other factors, of this lack of association.

This present study ascertains excesses of homozygosity in specific genomic regions overrepresented in cases, suggesting that variants in *HLA-DQB1* and *GBA-SYT11* contribute to idiopathic PD in our population. These sites have been linked to idiopathic PD in the past (Sidransky, et al., 2009, Hamza, et al., 2010), although further work characterizing these loci in patients remains.

Our genetic risk profiling for PD demonstrates between-quintile odds ratios of a similar magnitude reported in other previous studies (Hernandez, et al., 2012, Nalls, et al., 2011, International Parkinson's Disease Consortium, 2011, Kara, et al., 2014), showing that the risk profiles found in other populations of European descent are applicable to the Andalusian population. Consistent with these earlier studies, the area under the curve (AUC), calculated based on a logistic regression of disease status vs. GRS was 0.638. This suggests that the currently known risk loci are not sufficient to predict disease status and thus it is of limited clinical utility when used alone. When used in conjunction with age, gender, olfaction, and family history of PD, however, the GRS has been shown to contribute distinct information content to disease status prediction (Nalls, et al., 2015c).

To date, limited progress has been made in understanding how genetic variation affects PD phenotype. No GWAS has identified and independently replicated common variants associated with AAO on a genomewide scale. Our study, in agreement with the literature, did not reveal any significant variant to be associated with this PD feature. However, recent studies have found that increasing genetic risk scores are related to earlier age at onset (Nalls, et al., 2015b, Lill, et al., 2015) suggesting that early onset forms of the illness are not exclusively caused by highly penetrant Mendelian mutations, but can also be influenced by an accumulation of common polygenic alleles with relatively low effect sizes (Escott-Price, et al., 2015). We show no association between GRS and AAO in this study.

We know that common genetic variation plays a substantial role in PD, but an important proportion of the genetic risk might result from rare alleles. Given the size of our cohort, it is likely that we were unable to find any novel prospective rare variant simply due to their rarity. If a disease-causing rare variant is present in 0.5% of cases, we would expect to see it in only one case in our population, severely limiting statistical power. Indeed, no rare variant or summation of rare variants within genes showed a significant association with PD status. However, when examining known PD gene loci, we identified the *LRRK2* G2019S pathogenic mutation in 7 cases and the *GBA* N370S risk variant in 8 cases, in concordance with our previous findings by sequencing (Bandrés-Ciga S, et al., 2016).

Structural genomic variation analysis revealed that an early-onset PD case (0.48 % of patients) carried a heterozygous CNV mutation in *PARK2*. CNVs constitute a particular challenge in GWAs and we assume there may be additional CNVs conferring risk for the disease poorly covered or undetectable by the array. That could explain the lower frequency

of heterozygous exonic rearrangements in *PARK2* in our study in comparison with other studies (Huttenlocher, et al., 2015, Kay, et al., 2010).

Although our estimates of phenotypic variance provided imprecise results due to limited sample size, there is compelling evidence that a large proportion of heritability in PD has yet to be discovered (Keller, et al., 2012). We also note that reported heritability is likely biased to be high because of the enrichment of neurodegenerative disease-associated loci on the NeuroX chip (Nalls, et al., 2015a). We propose there will be a considerable number of variants that impart risk for disease outside the limits of what can be reliably detected by our genotyping platform.

In conclusion, this current work represents a comprehensive assessment of a Spanish cohort at a genome-wide level, characterizing a novel population in PD genetics. We replicate the association of 5 reported PD-related loci and our genetic risk profile is consistent with previous studies. Moreover, we reveal an excess of homozygosity in cases in the *HLA-DBQ1* and *GBA-SYT11* loci, strengthening evidence for the role of these loci in the etiology of PD. We suggest that pooling datasets of larger samples will be required for further identification and replication of PD genetic associations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

FUNDING AGENCIES

This work was supported in part by the Intramural Research Program of the National Institute on Aging, National Institutes of Health, part of the Department of Health and Human Services (project ZO1 AG000949), the following grants from the Junta de Andalucía; PI0322/2009 to the group CTS-6682-; CVI-6476, PSI2014-57643 to the CTS-438 group and a research award from the Andalusian Society of Neurology. Sara Bandrés Ciga held a FPU fellowship and a short-term stay grant from the Spanish Ministry of Education and Science.

The authors are grateful to the participants in this study without whom this work would not have been possible.

References

- Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MCJ, Squitieri F, Ibanez P, Joosse M, van Dongen JW, Vanacore N, van Swieten JC, Brice A, Meco G, van Duijn CM, Oostra BA, Heutink P. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science*. 2003; 299:256–259. [PubMed: 12446870]
- Bandrés-Ciga S, Mencacci NE, Durán R, Barrero FJ, Escamilla-Sevilla F, Morgan S, Hehir J, Vives F, Hardy J, Pittman AM. Analysis of the genetic variability in Parkinson's disease from Southern Spain. *Neurobiol Aging*. 2016; 37:210.e1–5. [PubMed: 26518746]
- Do CB, Tung JY, Dorfman E, Kiefer AK, Drabant EM, Francke U, Mountain JL, Goldman SM, Tanner CM, Langston JW, Wojcicki A, Eriksson N. Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. *PLoS Genet*. 2011; 7:e1002141. [PubMed: 21738487]
- Duran R, Mencacci NE, Angeli AV, Shoaib M, Deas E, Houlden H, Mehta A, Hughes D, Cox TM, Deegan P, Schapira AH, Lees AJ, Limousin P, Jarman PR, Bhatia KP, Wood NW, Hardy J, Foltynie T. The glucocerebrosidase E326K variant predisposes to Parkinson's disease, but does not cause Gaucher's disease. *Mov Disord*. 2013; 28:232–236. [PubMed: 23225227]

- Edwards TL, Scott WK, Almonte C, Burt A, Powell EH, Beecham GW, Wang L, Züchner S, Konidari I, Wang G, Singer C, Nahab F, Scott B, Stajich JM, Pericak-Vance M, Haines J, Vance JM, Martin ER. Genome-wide association study confirms SNPs in SNCA and the MAPT region as common risk factors for Parkinson disease. *Ann Hum Genet.* 2010; 74:97–109. [PubMed: 20070850]
- Escott-Price V, Nalls MA, Morris HR, Lubbe S, Brice A, Gasser T, Heutink P, Wood NW, Hardy J, Singleton AB, Williams NM. International Parkinson's Disease Genomics Consortium, IPDGC consortium members. Polygenic risk of Parkinson disease is correlated with disease age at onset. *Ann Neurol.* 2015; 77:582–591. [PubMed: 25773351]
- Fung HC, Scholz S, Matarin M, Simón-Sánchez J, Hernandez D, Britton A, Gibbs JR, Langefeld C, Stiebert ML, Schymick J, Okun MS, Mandel RJ, Fernandez HH, Foote KD, Rodríguez RL, Peckham E, De Vrieze FW, Gwinn-Hardy K, Hardy JA, Singleton A. Genome-wide genotyping in Parkinson's disease and neurologically normal controls: first stage analysis and public release of data. *Lancet Neurol.* 2006; 5:911–916. [PubMed: 17052657]
- Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. *Arch Neurol.* 1999; 56:33–39. [PubMed: 9923759]
- Hamza TH, Zabetian CP, Tenesa A, Laederach A, Montimurro J, Yearout D, Kay DM, Doheny KF, Paschall J, Pugh E, Kusel VI, Collura R, Roberts J, Griffith A, Samii A, Scott WK, Nutt J, Factor SA, Payami H. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nat Genet.* 2010; 42:781–785. [PubMed: 20711177]
- Hernandez DG, Nalls MA, Ylikotila P, Keller M, Hardy JA, Majamaa K, Singleton AB. Genome wide assessment of young onset Parkinson's disease from Finland. *PLoS ONE.* 2012; 7:e41859.doi: 10.1371/journal.pone.0041859 [PubMed: 22911860]
- Huttenlocher J, Stefansson H, Steinberg S, Helgadottir HT, Sveinbjörnsdóttir S, Riess O, Bauer P, Stefansson K. Heterozygote carriers for CNVs in PARK2 are at increased risk of Parkinson's disease. *Hum Mol Genet.* 2015; 24:5637–5643. [PubMed: 26188007]
- International HapMap Consortium. The International HapMap Project. *Nature.* 2003; 426:789–796. [PubMed: 14685227]
- Nalls MA, Plagnol V, Hernandez DG, Sharma M, Sheerin U-M, Saad M, Simón-Sánchez J, Schulte C, Lesage S, Sveinbjörnsdóttir S, Stefánsson K, Martinez M, Hardy J, Heutink P, Brice A, Gasser T, Singleton AB, Wood NW. International Parkinson Disease Genomics Consortium. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet.* 2011; 377:641–649. [PubMed: 21292315]
- International Parkinson's Disease Genomics Consortium (IPDGC), Wellcome Trust Case Control Consortium 2 (WTCCC2). A two-stage meta-analysis identifies several new loci for Parkinson's disease. *PLoS Genet.* 2011; 7:e1002142. [PubMed: 21738488]
- Kara E, Xiomerisiou G, Spanaki C, Bozi M, Koutsis G, Panas M, Dardiotis E, Ralli S, Bras J, Letson C, Edsall C, Pliner H, Arepalli S, Kalinderi K, Fidani L, Bostantjopoulou S, Keller MF, Wood NW, Hardy J, Houlden H, Stefanis L, Plaitakis A, Hernandez D, Hadjigeorgiou GM, Nalls MA, Singleton AB. Assessment of Parkinson's disease risk loci in Greece. *Neurobiol Aging.* 2014; 35:442.e9–442.e16. [PubMed: 24080174]
- Kay DM, Stevens CF, Hamza TH, Montimurro JS, Zabetian CP, Factor SA, Samii A, Griffith A, Roberts JW, Molho ES, Higgins DS, Gancher S, Moses L, Zareparsis S, Poorkaj P, Bird T, Nutt J, Schellenberg GD, Payami H. A comprehensive analysis of deletions, multiplications, and copy number variations in PARK2. *Neurology.* 2010; 75:1189–1194. [PubMed: 20876472]
- Keller MF, Saad M, Bras J, Bettella F, Nicolaou N, Simón-Sánchez J, Mittag F, Büchel F, Sharma M, Gibbs JR, Schulte C, Moskvina V, Durr A, Holmans P, Kilarski LL, Guerreiro R, Hernandez DG, Brice A, Ylikotila P, Stefánsson H, Majamaa K, Morris HR, Williams N, Gasser T, Heutink P, Wood NW, Hardy J, Martinez M, Singleton AB, Nalls MA. International Parkinson's Disease Genomics Consortium (IPDGC), Wellcome Trust Case Control Consortium 2 (WTCCC2). Using genome-wide complex trait analysis to quantify “missing heritability” in Parkinson's disease. *Hum Mol Genet.* 2012; 21:4996–5009. [PubMed: 22892372]
- Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature.* 1998; 392:605–608. [PubMed: 9560156]

- Krebs CE, Karkheiran S, Powell JC, Cao M, Makarov V, Darvish H, Di Paolo G, Walker RH, Shahidi GA, Buxbaum JD, De Camilli P, Yue Z, Paisán-Ruiz C. The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive Parkinsonism with generalized seizures. *Hum Mutat.* 2013; 34:1200–1207. [PubMed: 23804563]
- Lautier C, Goldwurm S, Dürr A, Giovannone B, Tsiaras WG, Pezzoli G, Brice A, Smith RJ. Mutations in the GIGYF2 (TNRC15) gene at the PARK11 locus in familial Parkinson disease. *Am J Hum Genet.* 2008; 82:822–833. [PubMed: 18358451]
- Lee S, Emond MJ, Bamshad MJ, Barnes KC, Rieder MJ, Nickerson DA, Christiani DC, Wurfel MM, Lin X. NHLBI GO Exome Sequencing Project—ESP Lung Project Team. Optimal unified approach for rare-variant association testing with application to small-sample case-control whole-exome sequencing studies. *Am J Hum Genet.* 2012; 91:224–237. [PubMed: 22863193]
- Lill CM, Hansen J, Olsen JH, Binder H, Ritz B, Bertram L. Impact of Parkinson's disease risk loci on age at onset. *Mov Disord.* 2015; 30:847–850. [PubMed: 25914293]
- Lill CM, Roehr JT, McQueen MB, Kavvoura FK, Bagade S, Schjeide BMM, Schjeide LM, Meissner E, Zauft U, Allen NC, Liu T, Schilling M, Anderson KJ, Beecham G, Berg D, Biernacka JM, Brice A, DeStefano AL, Do CB, Eriksson N, Factor SA, Farrer MJ, Foroud T, Gasser T, Hamza T, Hardy JA, Heutink P, Hill-Burns EM, Klein C, Latourelle JC, Maraganore DM, Martin ER, Martinez M, Myers RH, Nalls MA, Pankratz N, Payami H, Satake W, Scott WK, Sharma M, Singleton AB, Stefansson K, Toda T, Tung JY, Vance J, Wood NW, Zabetian CP, Young P, Tanzi RE, Khoury MJ, Zipp F, Lehrach H, Ioannidis JPA, Bertram L. 23andMe Genetic Epidemiology of Parkinson's Disease Consortium, International Parkinson's Disease Genomics Consortium, Parkinson's Disease GWAS Consortium, Wellcome Trust Case Control Consortium 2). Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: The PDGene database. *PLoS Genet.* 2012; 8:e1002548. [PubMed: 22438815]
- Matarin M, Simon-Sanchez J, Fung HC, Scholz S, Gibbs JR, Hernandez DG, Crews C, Britton A, De Vrieze FW, Brott TG, Brown RD, Worrall BB, Silliman S, Case LD, Hardy JA, Rich SS, Meschia JF, Singleton AB. Structural genomic variation in ischemic stroke. *Neurogenetics.* 2008; 9:101–108. [PubMed: 18288507]
- Nalls MA, Bras J, Hernandez DG, Keller MF, Majounie E, Renton AE, Saad M, Jansen I, Guerreiro R, Lubbe S, Plagnol V, Gibbs JR, Schulte C, Pankratz N, Sutherland M, Bertram L, Lill CM, DeStefano AL, Faroud T, Eriksson N, Tung JY, Edsall C, Nichols N, Brooks J, Arepalli S, Pliner H, Letson C, Heutink P, Martinez M, Gasser T, Traynor BJ, Wood N, Hardy J, Singleton AB. International Parkinson's Disease Genomics Consortium (IPDGC), Parkinson's Disease meta-analysis consortium. NeuroX, a fast and efficient genotyping platform for investigation of neurodegenerative diseases. *Neurobiol Aging.* 2015a; 36:1605.e7–12. [PubMed: 25444595]
- Nalls MA, Escott-Price V, Williams NM, Lubbe S, Keller MF, Morris HR, Singleton AB. International Parkinson's Disease Genomics Consortium (IPDGC). Genetic risk and age in Parkinson's disease: Continuum not stratum. *Mov Disord.* 2015b; 30:850–854. [PubMed: 25778492]
- Nalls MA, McLean CY, Rick J, Eberly S, Hutten SJ, Gwinn K, Sutherland M, Martinez M, Heutink P, Williams NM, Hardy J, Gasser T, Brice A, Price TR, Nicolas A, Keller MF, Molony C, Gibbs JR, Chen-Plotkin A, Suh E, Letson C, Fiandaca MS, Mapstone M, Federoff HJ, Noyce AJ, Morris H, Van Deerlin VM, Weintraub D, Zabetian C, Hernandez DG, Lesage S, Mullins M, Conley ED, Northover CAM, Frasier M, Marek K, Day-Williams AG, Stone DJ, Ioannidis JPA, Singleton AB. Parkinson's Disease Biomarkers Program and Parkinson's Progression Marker Initiative investigators. Diagnosis of Parkinson's disease on the basis of clinical and genetic classification: a population-based modelling study. *Lancet Neurol.* 2015c; 14:1002–1009. [PubMed: 26271532]
- Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, DeStefano AL, Kara E, Bras J, Sharma M, Schulte C, Keller MF, Arepalli S, Letson C, Edsall C, Stefansson H, Liu X, Pliner H, Lee JH, Cheng R, Ikram MA, Ioannidis JPA, Hadjigeorgiou GM, Bis JC, Martinez M, Perlmutter JS, Goate A, Marder K, Fiske B, Sutherland M, Xiomerisiou G, Myers RH, Clark LN, Stefansson K, Hardy JA, Heutink P, Chen H, Wood NW, Houlden H, Payami H, Brice A, Scott WK, Gasser T, Bertram L, Eriksson N, Foroud T, Singleton AB. International Parkinson's Disease Genomics Consortium (IPDGC), Parkinson's Study Group (PSG) Parkinson's Research: The Organized GENetics Initiative (PROGENI), 23andMe GenePD, NeuroGenetics Research Consortium (NGRC), Hussman Institute of Human Genomics (HIHG), Ashkenazi Jewish Dataset Investigator, Cohorts for Health Aging Research in Genetic Epidemiology (CHARGE), North American Brain

Expression Consortium (NABEC), United Kingdom Brain Expression Consortium (UKBEC), Greek Parkinson's Disease Consortium, Alzheimer Genetic Analysis Group. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet.* 2014; 46:989–993. [PubMed: 25064009]

- Paisán-Ruiz C, Jain S, Evans EW, Gilks WP, Simón J, van der Brug M, López de Munain A, Aparicio S, Gil AM, Khan N, Johnson J, Martinez JR, Nicholl D, Carrera IM, Pena AS, de Silva R, Lees A, Martí-Massó JF, Pérez-Tur J, Wood NW, Singleton AB. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron.* 2004; 44:595–600. [PubMed: 15541308]
- Pankratz N, Beecham GW, DeStefano AL, Dawson TM, Doheny KF, Factor SA, Hamza TH, Hung AY, Hyman BT, Iverson AJ, Krainc D, Latourelle JC, Clark LN, Marder K, Martin ER, Mayeux R, Ross OA, Scherzer CR, Simon DK, Tanner C, Vance JM, Wszolek ZK, Zabetian CP, Myers RH, Payami H, Scott WK, Foroud T. PD GWAS Consortium. Meta-analysis of Parkinson's disease: identification of a novel locus, RIT2. *Ann Neurol.* 2012; 71:370–384. [PubMed: 22451204]
- Pihlstrøm L, Axelsson G, Bjørnarå KA, Dizdar N, Fardell C, Forsgren L, Holmberg B, Larsen JP, Linder J, Nissbrandt H, Tysnes OB, Ohman E, Dietrichs E, Toft M. Supportive evidence for 11 loci from genome-wide association studies in Parkinson's disease. *Neurobiol Aging.* 2013; 34:1708.e7–13. [PubMed: 23153929]
- Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science.* 1997; 276:2045–2047. [PubMed: 9197268]
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007; 81:559–575. [PubMed: 17701901]
- Reich DE, Lander ES. On the allelic spectrum of human disease. *Trends Genet.* 2001; 17:502–510. [PubMed: 11525833]
- Saad M, Lesage S, Saint-Pierre A, Corvol JC, Zelenika D, Lambert JC, Vidailhet M, Mellick GD, Lohmann E, Durif F, Pollak P, Damier P, Tison F, Silburn PA, Tzourio C, Forlani S, Lorient MA, Giroud M, Helmer C, Portet F, Amouyel P, Lathrop M, Elbaz A, Durr A, Martinez M, Brice A. French Parkinson's Disease Genetics Study Group. Genome-wide association study confirms BST1 and suggests a locus on 12q24 as the risk loci for Parkinson's disease in the European population. *Hum Mol Genet.* 2011; 20:615–627. [PubMed: 21084426]
- Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, Kawaguchi T, Tsunoda T, Watanabe M, Takeda A, Tomiyama H, Nakashima K, Hasegawa K, Obata F, Yoshikawa T, Kawakami H, Sakoda S, Yamamoto M, Hattori N, Murata M, Nakamura Y, Toda T. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genet.* 2009; 41:1303–1307. [PubMed: 19915576]
- Shojaee S, Sina F, Banihosseini SS, Kazemi MH, Kalhor R, Shahidi GA, Fakhrai-Rad H, Ronaghi M, Elahi E. Genome-wide linkage analysis of a Parkinsonian-pyramidal syndrome pedigree by 500 K SNP arrays. *Am J Hum Genet.* 2008; 82:1375–1384. [PubMed: 18513678]
- Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, Bar-Shira A, Berg D, Bras J, Brice A, Chen CM, Clark LN, Condroyer C, De Marco EV, Dürr A, Eblan MJ, Fahn S, Farrer MJ, Fung HC, Gan-Or Z, Gasser T, Gershoni-Baruch R, Giladi N, Griffith A, Gurevich T, Januario C, Kropp P, Lang AE, Lee-Chen GJ, Lesage S, Marder K, Mata IF, Mirelman A, Mitsui J, Mizuta I, Nicoletti G, Oliveira C, Ottman R, Orr-Urtreger A, Pereira LV, Quattrone A, Rogaeva E, Rolfs A, Rosenbaum H, Rozenberg R, Samii A, Samadpour T, Schulte C, Sharma M, Singleton A, Spitz M, Tan EK, Tayebi N, Toda T, Troiano AR, Tsuji S, Wittstock M, Wolfsberg TG, Wu YR, Zabetian CP, Zhao Y, Ziegler SG. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med.* 2009; 361:1651–1661. [PubMed: 19846850]
- Simón-Sánchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisán-Ruiz C, Lichtner P, Scholz SW, Hernandez DG, Krüger R, Federoff M, Klein C, Goate A, Perlmutter J, Bonin M, Nalls MA, Illig T, Gieger C, Houlden H, Steffens M, Okun MS, Racette BA, Cookson MR, Foote KD, Fernandez HH, Traynor BJ, Schreiber S, Arepalli S, Zonozi R, Gwinn K, van der Brug M, Lopez G, Chanock SJ, Schatzkin A, Park Y, Hollenbeck A, Gao J, Huang X, Wood NW, Lorenz D,

Deuschl G, Chen H, Riess O, Hardy JA, Singleton AB, Gasser T. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet.* 2009; 41:1308–1312. [PubMed: 19915575]

Simón-Sánchez J, van Hilten JJ, van de Warrenburg B, Post B, Berendse HW, Arepalli S, Hernandez DG, de Bie RMA, Velseboer D, Scheffer H, Bloem B, van Dijk KD, Rivadeneira F, Hofman A, Uitterlinden AG, Rizzu P, Bochdanovits Z, Singleton AB, Heutink P. Genome-wide association study confirms extant PD risk loci among the Dutch. *Eur J Hum Genet.* 2011; 19:655–661. [PubMed: 21248740]

Singleton AB, Hardy J, Traynor BJ, Houlden H. Towards a complete resolution of the genetic architecture of disease. *Trends Genet.* 2010; 26:438–442. [PubMed: 20813421]

Valente EM, Abou-Sleiman PM, Caputo V, Muqit MMK, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, González-Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G, Wood NW. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science.* 2004; 304:1158–1160. [PubMed: 15087508]

Vilariño-Güell C, Wider C, Ross OA, Dachselt JC, Kachergus JM, Lincoln SJ, Soto-Ortolaza AI, Cobb SA, Wilhoite GJ, Bacon JA, Behrouz B, Melrose HL, Hentati E, Puschmann A, Evans DM, Conibear E, Wasserman WW, Aasly JO, Burkhard PR, Djaldetti R, Ghika J, Hentati F, Krygowska-Wajs A, Lynch T, Melamed E, Rajput A, Rajput AH, Solida A, Wu RM, Uitti RJ, Wszolek ZK, Vingerhoets F, Farrer MJ. VPS35 mutations in Parkinson disease. *Am J Hum Genet.* 2011; 89:162–167. [PubMed: 21763482]

Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet.* 2011; 88:76–82. [PubMed: 21167468]

Zimprich A, Benet-Pagès A, Struhal W, Graf E, Eck SH, Offman MN, Haubenberger D, Spielberger S, Schulte EC, Lichtner P, Rossle SC, Klopp N, Wolf E, Seppi K, Pirker W, Presslauer S, Mollenhauer B, Katzenschlager R, Foki T, Hotzy C, Reinthaler E, Harutyunyan A, Kralovics R, Peters A, Zimprich F, Brücke T, Poewe W, Auff E, Trenkwalder C, Rost B, Ransmayr G, Winkelmann J, Meitinger T, Strom TM. A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. *Am J Hum Genet.* 2011; 89:168–175. [PubMed: 21763483]

Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, Kachergus J, Hulihan M, Uitti RJ, Calne DB, Stoessl AJ, Pfeiffer RF, Patenge N, Carbajal IC, Vieregge P, Asmus F, Müller-Myhsok B, Dickson DW, Meitinger T, Strom TM, Wszolek ZK, Gasser T. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron.* 2004; 44:601–607. [PubMed: 15541309]

HIGHLIGHTS

- Genetic profiling for PD risk was consistent with previous studies.
- An excess of homozygosity in cases in the *HLA-DBQ1* and *GBA-SYT11* loci was found.
- The *GBA* N370S risk variant was found in 8 cases.
- The *LRRK2* G2019S pathogenic mutation was found in 7 cases.
- CNV analysis revealed a heterozygous deletion in *PARK2* in an early-onset PD case.

Figure 1.
Manhattan Plot showing results of PD GWA testing between 206 PD patients and 172 controls from Southern Spain.

Figure 2. Manhattan Plot showing results of PD GWA with age at onset testing between 206 PD patients and 172 controls from Southern Spain.

Table 1

Demographic characteristics of the Spanish cohort

	Samples passing QC	Male/Female	* Age of study enrollment	* Age at onset
Whole sample	378	210/168	63.14 ± 13	
PD cases	206	133/73	67.38 ± 10.51	61.88 ± 13.77
Controls	172	77/95	58.07 ± 13.99	

* Expressed as mean age ± standard deviation

Table 2

Results of the replication association analyses

Nearest gene (s)	SNP	CHR	BP	A1	A2	MAF	OR	SE	L95	U95	STAT	P	OR*	L95*	U95*
<i>ACMSD/TMEM163</i>	rs6430538	2	135539967	C	T	0.4112	1.533	0.1865	1.064	2.21	2.291	0.02195	1.14	1.10	1.18
<i>MAPT</i>	rs17649553	17	43994648	T	C	0.3073	0.6318	0.2055	0.4224	0.9452	-2.234	0.02547	0.77	0.74	0.80
<i>STK39</i>	rs1955337	2	169129145	T	G	0.1789	2.346	0.4031	1.065	5.169	2.115	0.03442	1.20	1.15	1.26
<i>MIR4697</i>	rs329648	11	133765367	T	C	0.4399	1.443	0.1736	1.027	2.028	2.113	0.03458	1.09	1.06	1.13
<i>SREBF/RAII</i>	rs11868035	17	17715101	A	G	0.2917	0.6581	0.2094	0.4365	0.992	-1.998	0.0457	0.93	0.90	0.96
<i>SNCA</i>	rs356181	4	90626139	C	T	0.4505	1.303	0.1633	0.9463	1.795	1.622	0.1049	0.90	0.87	0.93
<i>RIT2</i>	rs12456492	18	40673380	G	A	0.3216	1.349	0.1894	0.9306	1.955	1.58	0.1141	1.10	1.06	1.14
<i>SIPA1L2</i>	rs10797576	1	232664611	T	C	0.1589	2.111	0.5017	0.7898	5.644	1.49	0.1363	1.13	1.08	1.19
<i>RAB7L/NUCKS1</i>	rs823118	1	205723572	C	T	0.3838	1.285	0.1848	0.8947	1.846	1.358	0.1745	0.88	0.86	0.91
<i>CCDC62</i>	rs11060180	12	123303586	G	A	0.3945	0.8088	0.1683	0.5815	1.125	-1.26	0.2075	0.90	0.87	0.93
<i>TMEM175/GAK/DGKQ</i>	rs34311866	4	951947	C	T	0.2055	1.356	0.2974	0.7569	2.429	1.024	0.306	1.27	1.22	1.32
<i>SNCA</i>	rs3910105	4	90682571	C	T	0.4413	0.8579	0.1632	0.623	1.181	-0.9392	0.3476	0.89	0.86	0.91
<i>GPNMB</i>	rs199347	7	23293746	C	T	0.4661	0.8747	0.1551	0.6454	1.185	-0.8631	0.3881	0.89	0.86	0.92
<i>FAM47E/SCARB2</i>	rs6812193	4	77198986	T	C	0.3789	1.153	0.1794	0.8113	1.639	0.7943	0.427	0.89	0.86	0.93
<i>MCCC1</i>	rs12637471	3	182762437	A	G	0.1758	0.7834	0.3182	0.4199	1.462	-0.7672	0.443	0.84	0.81	0.87
<i>DDRGI1</i>	rs55785911	20	3153503	A	G	0.373	0.8852	0.1766	0.6262	1.251	-0.6905	0.4899	0.91	0.88	0.94
<i>VPSI3C</i>	rs2414739	15	61994134	G	A	0.2656	0.8594	0.2269	0.5509	1.341	-0.6681	0.5041	0.89	0.86	0.93
<i>LRRK2</i>	rs7294619	12	40614434	T	C	0.1462	0.8173	0.3631	0.4012	1.665	-0.5558	0.5783	0.86	0.82	0.90
<i>BCKDK/STX1B</i>	rs14235	16	31121793	A	G	0.4232	0.9306	0.1643	0.6745	1.284	-0.4377	0.6616	1.09	1.05	1.13
<i>FGF20</i>	rs591323	8	16697091	A	G	0.2546	0.9082	0.2761	0.5287	1.56	-0.3488	0.7273	0.92	0.88	0.95
<i>BST1</i>	rs11724635	4	15737101	C	A	0.4792	0.9513	0.1622	0.6923	1.307	-0.3076	0.7584	0.89	0.86	0.91
<i>GCHI</i>	rs11158026	14	55348869	T	C	0.2865	1.04	0.1997	0.7028	1.538	0.1946	0.8457	0.88	0.85	0.92
<i>HLA-DBQ1</i>	rs115462410	6	32666660	T	C	0.09245	1.116	0.7542	0.2546	4.895	0.1459	0.884	1.25	1.17	1.34
<i>TMEM175</i>	rs34884217	4	944210	G	T	0.09635	0.9088	0.6654	0.2467	3.348	-0.1437	0.8857	0.74	0.68	0.80
<i>GBA</i>	rs35749011	1	155205634	C	T	0.005222	NA	NA	NA	NA	NA	NA	0.56	0.50	0.63
<i>GBA/STT1</i>	rs71628662	1	155359992	C	T	0.005208	NA	NA	NA	NA	NA	NA	0.56	0.50	0.63

Nearest gene (s)	SNP	CHR	BP	A1	A2	MAF	OR	SE	L95	U95	STAT	P	OR*	L95*	U95*
<i>NOTCH</i>	rs8192591	6	32185796	T	C	0.009115	NA	NA	NA	NA	NA	NA	1.11	1.02	1.21
<i>INPP5F</i>	rs10886515	10	121710488	T	C	0.0248	NA	NA	NA	NA	NA	NA	1.10	1.06	1.14

Risk estimates are based on the dosage of A1 (minor allele). MAF refers to the minor allele.

* Values referring to the PD metanalis (Nalls MA et al., 2014)

Table 3

Genetic risk profile in the Southern Spanish cohort based on SNPs in Table 2.

	Risk quintiles				
	First	Second	Third	Fourth	Fifth
Odds ratio	1	1.61	1.83	3.44	3.65
CI (95%)		(0.85–3.08)	(0.96–3.52)	(1.79–6.76)	(1.89–7.21)
p-value	4.0E-07				
AUC	0.63				

AUC: area under the curve

CI: confidence interval

Table 4

Genetic rare variants (MAF<0.01) identified in PD known genes in cases but not in controls

CHR	GENE	BP	NeuroX SNP	rs ID	Variant	Zygoty	R/A	Cases	Frequency (ExAc)	Polyphen	SIFT
1	<i>PINK1</i>	20971158	exm27115	rs139226733	p.Met318Leu	Het	A/T	1	0.001618	B	D
1	<i>PINK1</i>	20975021	exm27131	rs45515602	p.Ala383Thr	Het	G/A	2	0.0001961	B	T
1	<i>GBA</i>	155205634	exm106217	rs76763715	p.Asn370Ser	Het	T/C	7	0.00363	B	D
						Hom		1			
2	<i>GIGYF2</i> *	233612427	exm275808	rs147623346	Met48Ile	Het	G/T	2	0.0001499	D	U
2	<i>GIGYF2</i>	233655834	exm275883	rs148277228	Asp371Glu	Het	T/A	1	0.0007366	T	U
2	<i>GIGYF2</i>	233656142	exm275892	rs34845648	Pro445His	Het	C/T	1	0.0001132	D	U
3	<i>DNAJC13</i> *	132213932	exm351306	rs35658317	IVS5-1G>A/3942-1 G>A	Het	G/A	1	0.0003347	-	-
3	<i>DNAJC13</i>	132242012	exm351379	rs61731474	p.Asn1952Thr	Het	A/C	1	0.0001349	B	T
3	<i>DNAJC13</i>	132244562	exm351399	rs138693725	p.Ala2057Ser	Het	G/T	1	0.001334	B	T
6	<i>PARK2</i>	184045771	exm593521	rs9456735	Met192Leu	Het	T/G	1	0.0003299	T	PD
12	<i>LRRK2</i>	40629436	exm994436	rs33995463	p.Leu119Pro	Het	T/C	2	0.00202	D	PD
12	<i>LRRK2</i>	40677813	exm994520	rs35173587	Arg793Met	Het	G/T	1	0.005747	D	PD
12	<i>LRRK2</i>	40697802	exm994561	rs143710836	Ala1215Thr	Het	G/A	1	0.0002101	T	B
12	<i>LRRK2</i>	40734202	exm994671	rs34637584	p.Gly2019Ser	Het	G/A	7	0.0006294	PD	D
21	<i>SYNJ1</i>	34004111	exm1566662	rs115648918	Val1345Ile	Het	C/T	3	0.0001713	T	B
21	<i>SYNJ1</i>	34012088	exm1566685	rs145937537	Thr1236Met	Het	G/A	1	0.00216	T	B
21	<i>SYNJ1</i>	34029195	exm1566728	rs114053718	Ile905Thr	Het	A/G	1	0	D	PD
21	<i>SYNJ1</i>	34058146	exm1566794	rs114942253	Ala383Thr	Het	C/T	2	0.01499	B	B
22	<i>FBX07</i>	32875122	exm1602819	rs143041875	Ser93Ala	Het	T/G	3	0.0001498	D	B
22	<i>PLA2G6</i>	38528888	exm1608133	rs11570680	Ala343Thr	Hom	C/T	1	0.02574	T	B

B:Benign, D:Damaging/Deleterious, PD: possibly damaging, T: tolerated, U:Unknown;

Frequency ExAc: Frequency of the rare variants reported for European (Non Finnish) population. R: Reference allele; A: Alternate allele

** Putative PD linked genes