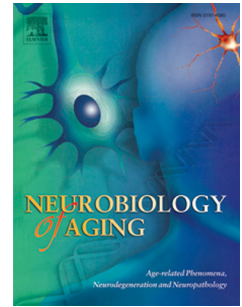


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Title: Establishing the role of rare coding variants in known Parkinson's disease risk loci

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Abstract

Many common genetic factors have been identified to contribute to PD susceptibility, improving our understanding of the related underlying biological mechanisms. The involvement of rarer variants in these loci have been poorly studied. Using International Parkinson's Disease Genomics Consortium datasets, we performed a comprehensive study to determine the impact of rare variants in 26 previously published GWAS loci in PD. We applied Pifix to select the putative causal genes underneath the GWAS peaks, which was based on underlying functional similarities. The Sequence Kernel Association Test was used to analyze the joint effect of rare, common or both types of variants on PD susceptibility. All genes were tested simultaneously as a gene-set and each gene individually. We observed a moderate association of common variants, confirming the involvement of the known PD risk loci within our genetic datasets. Focusing on rare variants we identified additional association signals for *LRRK2*, *STBD1*, and *SPATA19*. Our study suggests an involvement of rare variants within several putatively causal genes underneath previously identified PD GWAS peaks.

Highlights

- Two genetic datasets comprising a total of 7,968 PD cases and 7,655 controls were used to study the exome
- Rare variants in *LRRK2*, *STBD1* and *SPATA19* are suggested to play a role in PD
- Larger sequencing studies are required in future for follow up.

Keywords

Parkinson's disease, common risk loci, rare variants, whole exome sequencing, variant aggregation test

1. Introduction

Genetic factors play an important role in Parkinson's disease (PD) pathogenesis. In addition to the discovery of rare variants using family-based linkage studies, resulting in the identification of, for example *SNCA*, *LRRK2*, *parkin*, *DJ-1*, *PINK1* and *VPS35*, numerous genome-wide association studies (GWAS) have shown that common genetic variants increase PD risk (Bras, et al., 2015). The most recent and largest PD association study (Nalls, et al., 2014b) identified over 20 common risk variants, confirming many previously associated risk factors.

Nevertheless, heritability estimates indicate that additional genetic risk factors remain to be discovered since a relatively large fraction of PD heritability cannot be explained by known PD risk loci or Mendelian genes (Do, et al., 2011, Keller, et al., 2012, Pihlstrom and Toft, 2011). GWAS approaches are primarily designed to identify common risk variants by the usage of genotyping arrays. However, emerging evidence suggests that rare variants may explain part of the missing heritability (Manolio, et al., 2009, Zuk, et al., 2014). Rare variants in protein coding regions are more likely to affect the function of a gene than common variants which tag the causal variants via linkage disequilibrium (LD) and are often located in non-coding regions of the genome (Nelson, et al., 2012, Tennessen, et al., 2012). Therefore, rare variants might be of more importance to complex diseases than predicted by the Common Disease-Common Variant hypothesis (Botstein and Risch, 2003, Lander, 1996, Pritchard and Cox, 2002, Sharma, et al., 2014). In contrast to GWAS, exome sequencing studies aim at systematically analyzing coding regions of the genome to identify causal variants in complex diseases (Kiezun, et al., 2012). Exome studies have been proven to be effective for studying familial diseases (Bamshad, et al., 2011) but an increasing number of applications for populations-based studies have been developed (Cirulli, et al., 2015, Purcell, et al., 2014).

In PD, multiple genes have been shown to harbor both common and rare variants which contribute to disease pathogenesis. *SNCA* and *LRRK2* contain both PD-risk associated rare variants with Mendelian effects as common variants that increase PD risk in sporadic patients (Edwards, et al., 2010, Nalls, et al., 2014b, Nalls, et al., 2011, Paisan-Ruiz, et al., 2004, Polymeropoulos, et al., 1997, Simon-Sanchez, et al., 2009, Zimprich, et al., 2004). *GBA*, for which an association was first seen in families with Gaucher's disease and parkinsonism (Goker-Alpan, et al., 2004), is furthermore shown to play a role in PD by both rare coding variants and common risk variants (Do, et al., 2011, Nalls, et al., 2014b, Pankratz, et al., 2012). Thus, we hypothesize that rare coding variants in the known risk loci for sporadic PD are involved in the genetic etiology of PD. The combined effect of rare variants within recently identified PD risk loci will likely explain an additional portion of PD heritability. We aim to assess this hypothesis by determining the genetic burden of rare variants in the PD risk loci using two exome cohorts of the International Parkinson's Disease Genomics Consortium (IPDGC).

2. Methods

2.1 Subjects

All PD cases included in this study have given written informed consent. Relevant local ethical committees for medical research approved involvement in genetic studies. The PD patients were diagnosed using the UK Brain Bank criteria (Hughes, et al., 1992).

2.2 Whole exome sequencing dataset

The whole exome sequencing (WES) dataset includes 1,167 PD cases and 1,685 controls (post QC) of European ancestry. The PD patients have a tendency towards a young age of onset with an average of 41.2 years (SD = 10.9). 1,201 controls originate from the Rotterdam Study version 1 (RSX1), as we merged the IPGDC WES data with the RSX1 WES data (Hofman, et al., 2015). The samples were sequenced in different batches with two exome capture kits: EZ Exome Library v2.0 (Roche/Nimblegen) and Truseq Exome Enrichment Kit targeting 44.1 Mb and 62 Mb, respectively (Supplementary Table 1). To account for putative technical differences between the different capture kits, we only considered variants that were targeted by both capture protocols and included preQC individual sample missingness (as a reference to sequencing coverage) as covariates during all genetic analyses.

On average, 94.4% of the exome was covered for at least 10x. The 100-bp paired-end reads were sequenced on a HiSeq2000 and aligned to the human reference genome (build hg19) using Barrow Wheeler Aligner (BWA)-MEM (Li and Durbin, 2009). Genome Analysis Toolkit (McKenna, et al., 2010) (GATK) called single nucleotide variants (SNVs) and small insertions/deletions (indels) for each sample, resulting in individual gVCF files. Genotypes of all IPDGC and RSX1 exome samples were then jointly called and recalibrated, allowing to merge the distinct WES datasets in a correct manner. Standard GATK filter steps were applied, together with a minimum genotype quality Phred-score of 20 and depth of 8, to only select high-quality variants. Only bi-allelic calls were considered that were located in regions targeted by both capture kits. Supplementary Table 2 reports the exons that have been excluded due to insufficient coverage within one of the exome capture protocols.

2.3 NeuroX dataset

The NeuroX dataset encompasses 6,801 PD cases and 5,970 controls (post QC) of European ancestry. Overlapping samples with the WES dataset were excluded. The average age of onset of the PD patients is 63.0 years (SD = 12.4). The Exome NeuroX array (Nalls, et al., 2014a) was used consisting of ~240,000 exonic variants standard to the Illumina HumanExome array v1.1 and ~25,000 variants focused on neurologic and neurodegenerative diseases.

2.4 Quality procedures

For individual QC in both the WES and the NeuroX datasets, samples were removed when showing gender ambiguity, dubious heterozygosity/genotype calls, evidence of relatedness, or being a population outlier. The latter two were calculated with LD-pruned common variants. Variant QC procedures were slightly different for the two different datasets. For the WES dataset, variants passed QC when having a minimum call rate $> 85\%$ and being in Hardy-Weinberg equilibrium (HWE p -values $> 1e-8$ based on controls). For the NeuroX dataset, variants were excluded for subsequent analyses with a minimum call rate $< 95\%$, a HWE p -value $< 1e-6$, or with significant differences in missingness rate between cases and controls.

2.5 Causal gene selection within PD risk loci

Based on the most recent and largest GWAS (Lill, et al., 2012, Nalls, et al., 2014b) we selected 26 loci containing at least one top SNP nominated in meta-analysis with $p < 5.00e-08$ (as reported by pdgene.org). The published SNPs associated with PD are not the causal variants but rather tag the unknown causal variants with which they are in LD. As the causal variant (and therefore also the related gene) has not been determined for most of the PD risk loci, we explored the involvement of rare variants in PD susceptibility by using the PrixFixe strategy, which selects one gene per locus based on functional similarities of genes within the LD-blocks from the different loci.

The functional similarity is defined as the degree of shared biological function and is determined by overlapping biological features such as protein domains, transcription factor binding sites, gene-expression, phylogenetic profiles and protein-protein interactions. Based on these features, cofunction networks are generated which connect genes that are likely to share the same underlying molecular pathway. Genes that are strongly connected to other candidate genes obtain a higher PrixFixe score and therefore prioritized as causal gene. As this approach is based on genome-wide datasets and is not performed with disease-related biological assumptions, the PrixFixe strategy aims to prioritize genes without the usual text mining bias caused by literature-based knowledge (Edwards, et al., 2011).

The most significantly associated SNPs from the recent meta-analysis by Nalls et al. (Nalls, et al., 2014b) were used as seeding SNPs to define the LD region per PD locus. If a SNP was not applicable to be used as seeding SNP (not present in either the current dbSNP 137 or HapMap public resources), the next strongest associated SNP or a SNP in high LD ($r^2 > 0.8$) within the same locus was used as a seed. We were unable to define a legitimate seeding SNP for 3 loci (rs71628662, rs591323, rs2414739). LD-regions were based on the CEU phase III population with a minimal R^2 of 0.5. The final Prixfixe gene-set consists of 23 genes for downstream analyses (Table 1).

2.6 Variant selection

To enrich both genetic datasets for deleterious variants we selected multiple subsets of variants, differing in the method and stringency to select pathogenic variants. Based on variant annotation with ANNOVAR (Wang, et al., 2010), 3 distinct subsets of variants were created, including: 1) all exonic variants (disruptive, splicing, (non)synonymous and (non)frameshift indels), 2) amino-acid changing variants (same as previous except for synonymous) 3) amino-acid changing (AAchanging) variants that are predicted to be deleterious. The latter subset includes variants that are predicted to be pathogenic (CADD-score ≥ 12.37 (Amendola, et al., 2015)) by Combined Annotation Dependent Depletion (CADD) v1 (Kircher, et al., 2014). Figure 1 displays a workflow of the classification of the different variant subsets. The exonic subset was exclusively tested for the gene-set analysis to determine the involvement of the known PD risk loci in the WES and NeuroX dataset.

2.7 Variant aggregation analysis

The Sequence Kernel Association Test (SKAT) (Ionita-Laza, et al., 2013, Wu, et al., 2011) was used to perform burden analyses. The MAF threshold, separating the rare and common variants, was based on the total sample size using the formula ($T = 1/(V(2n))$) suggested by SKAT (Ionita-Laza, et al., 2013), therefore resulting in the MAF thresholds of 0.013 and 0.006 for the WES dataset and NeuroX dataset, respectively. We performed polygenetic burden analyses for exclusively rare variants, exclusively common variants and both types of variants together. The common variants were pruned (PLINK (Purcell, et al., 2007) indep settings 50 5 1.5) aiming to only consider independent variants in our genetic analyses. For the gene-sets we performed a two-sided SKAT test allowing variants within a gene-set to have different directions and magnitudes of effects, which is in concordance with both damaging and protective effect estimates observed for the 26 published PD loci. To test individual genes we performed a one-sided burden test, as we hypothesized that variants in individual genes are likely to have the same direction of effect. We also performed a two-sided SKAT analysis per gene in case we were interested which genes are driven an observed rare variant association in the total gene-set.

To correct for confounding factors (e.g. population stratification and technical artifacts), we included 20 multi-dimensional scaling components, gender and individual missingness rate pre QC (as a reference to the individual WES coverage) for the WES dataset. As the NeuroX dataset is more homogeneous, we corrected for the first 4 MDS components and gender. Empirical p -values were calculated for significant sample results ($p < 0.05$). For the gene-set analysis, the original sample p -value of the gene-set of interest was compared to p -values of 1,000 randomly drawn gene-sets of the same size. For the individual gene associations, empirical p -values were calculated using the

resampling method implemented by SKAT, by 10,000 permutations of the affection status. Empirical p -values are calculated by $(n_1+1)/(n+1)$, where n_1 = the number of resampling p -values smaller than the original sample p -value and n = the number of resampling.

2.8 Power calculations

We estimated the power of our study design to detect rare variant associations. Supplementary Table 3 displays the parameters that were chosen for the calculations. For both datasets, the PD prevalence was set to 0.0057 (Pringsheim, et al., 2014). As approximately half of the loci in PDgene.org have an odds ratio below 1, the percentage protective effect was set to 50%. A thousand simulations ($\alpha = 0.025$) were performed on a haplotype matrix of SKAT, mimicking linkage disequilibrium structure of European ancestry, comprising 10,000 haplotypes over 200 kb regions.

3. Results

3.1 WES and rare variants

First, we analyzed the WES dataset as it represents all exonic variants, of which the study design has 65% power to detect a rare variant association signal considering individual genes. Testing the aggregated effect of grouped variants within a gene-set has the potential to increase power. Supplementary Table 4 shows the results of the gene-set analyses in the WES dataset. Common exonic variants are moderately associated to PD. The nominal p -value is significant, but the empirical p -value exceeds 0.05. Although we anticipated a significant association of common variants, we attribute the moderate association to a relatively low sample size (compared to the original GWAS), and the selection of genes (by Prefixe) with variants in moderate LD with the original highest SNP. The gene-set association is absent when focusing on the common amino-acid changing and CADD variants, which is probably due to a decrease in power as the number of variants drops.

No rare variant, or common & rare variant associations were observed for the gene-set in either of the functional variant categories (nominal $p \geq 0.223$; Supplementary Table 4). An alternative approach to study the putative rare variant associations is to test each gene individually within the gene-set. Table 2 displays the 3 strongest associated genes per variant subset and approach. Using the AAchanging variants category, we observed a significant association for *STBD1* ($p = 0.046$).

3.2 NeuroX and rare variants

The NeuroX dataset contains previously identified exonic variants, of which a large proportion is rare (Nalls, et al., 2014a). In contrast to the WES data sets, our NeuroX cohort has enough power (estimated at 96%), due to the larger sample size (6,804 cases 5,970 controls), to detect a rare variant association signal. Similarly, to the WES dataset, a moderate common variant association is

detected (nominal $p = 0.031$). In contrast to the WES dataset, we do observe significant associations of the gene-set with PD, even when only considering rare variants (AAchanging = 0.007; CADD = 0.002; Supplementary Table 5a).

To discover whether specific genes drive this observed rare variant association as observed in our cohort, the variants were grouped per gene and two-sided tested for their association to PD. *LRRK2* is the gene driving the association observed in the total gene-sets (Supplementary Table 6). Focusing on the CADD subset, this association (nominal $p = 5.17 \times 10^{-13}$) is considerably stronger than the second most significant *SPATA19* (nominal $p = 0.050$). The NeuroX array custom content is primarily driven by neurodegenerative diseases; therefore, NeuroX chip biases towards capture of in-depth genetic variability within genes, which are known to cause disease pathogenesis. (Tennessen, et al., 2012) Likewise, NeuroX harbors many variants of the known PD genes. For example, NeuroX contains 32 harmful (predicted by CADD) *LRRK2* variants, while only 2 harmful variants are present for *SPATA19*. The variants in *LRRK2* are overrepresented and biasing the results of the total gene-sets. We, therefore, performed the same gene-set analyses on the NeuroX dataset excluding the variants of *LRRK2* (Supplementary Table 5b), resulting in the absence of a rare variant association in the NeuroX dataset (nominal $p \geq 0.28$). This suggests that the previously observed association of rare variants within the total gene-set to PD was solely driven by *LRRK2*.

The two-sided SKAT analysis per gene aimed at the discovery of genes driving the rare variant association in the total gene-set. Next, we were interested to explore the genetic burden of rare variants for each gene individually when assuming all rare variants to have the same direction of effect (one-sided BURDEN test). Table 3 shows again that *LRRK2* is the strongest associated gene. Furthermore, *SPATA19* ($p = 0.017$) is significantly associated when specifically considering rare CADD variants.

3.3 Directionality of effect

We further explored the significant individual association signals (empirical $p < 0.05$) for *LRRK2* and *STBD1*, and *SPATA19*. By focusing on the variant level we aimed to comprehend the direction of effect estimates. *LRRK2* showed a significant burden of 32 rare damaging variants in the NeuroX dataset. Single-marker association analysis of *LRRK2* variants revealed that the observed association ($p = 3.17 \times 10^{-13}$) is attributed to the p.G2019S (rs34637584), the most common cause of monogenetic forms of PD. Interestingly, this particular variant was present in 78 cases (MAF = 0.006). Performing the rare variant aggregation test on 31 pathogenic *LRRK2* variants, excluding p.G2019S, resulted in no association ($p = 0.98$) to PD, and thus suggesting that the observed rare variant association in *LRRK2* was solely driven by the p.G2019S variant. As this variant is only present in 7 cases in the WES dataset (MAF = 0.003) with a single-marker p -value of 0.002 (*LRRK2* mutations

generally observed in late-onset PD), it explains the discrepancy of results for *LRRK2* locus as observed in the WES dataset, while it showed a strong association in the NeuroX dataset.

In addition to the rare variant association test in *LRRK2*, we explored the presence of the previously published common *LRRK2* haplotype with a protective effect of 3 exonic variants (N551K-R1398H-K1423K) (Ross, et al., 2011). K1423K is not included in the NeuroX genotyping array, but is in high linkage-disequilibrium ($r^2 = 1.00$) with R1398H. We therefore tested the N551K-R1398H (G-A) haplotype and confirmed the protective effect (OR = 0.89, $p = 0.027$) of this haplotype for the PD cases, showing a minor haplotype frequency of 6.2% in cases and 6.9% in controls. All 3 variants were detected in the WES dataset, allowing to test the full haplotype (G-A-A). Although the haplotype association was not significant in the WES dataset (OR = 0.81, $p = 0.223$), the trend of effect is similar with a minor haplotype frequency of 7.0% in cases and 7.5% in controls. The smaller sample size of the WES dataset is a plausible reason for not obtaining a significant association.

Next, the WES-based *STBD1* and NeuroX-based *SPATA19* were investigated for their variant frequencies. Single-marker association analysis showed no significant results for the 8 variants within *STBD1*. It therefore appears that the observed rare variant association is not caused by one exclusive variant but is rather the effect of multiple rare variants. Seven of the 8 variants are control-specific as they are only present in 10 control individuals. In contrast, only 1 variant is present in a single case. The direction of effect of the variants that are generating the *STBD1* gene association is therefore implied to be protective. The significant gene-based association for *SPATA19* is relatively strong considering that it is driven by only 2 CADD variants that are present in 7 cases and 0 controls. The absence of *SPATA19* CADD variants in controls suggests that the association signal is damaging.

4. Discussion

To establish the influence of rare variants in sporadic PD risk loci, we explored two independent PD datasets (WES and NeuroX) enriched for coding rare variants. We used the PrixFixe strategy to select the most likely causal genes underlying the PD loci peaks, which is based on overlapping biological functional similarities. We tested both the effect of rare variants in the gene-sets at once, as each gene individually. Aggregating variants simultaneously across a set of genes has the potential to increase power to detect an association signal, given that the selected genes are enriched for a group of genes that are genuinely involved in the disease pathogenesis.

The average age of onset within the cases of the WES dataset (~41 years) is 20 years younger than in the meta-analysis of the most recent PD GWAS (~61 years) where the PD risk loci were based on. As some rare genetic risk factors (*DJ-1*, *parkin* *PINK1*) (Bras, et al., 2015) are specific for young onset PD (YOPD), we acknowledge the putative existence of YOPD-specific common genetic risk factors within the WES dataset. However, risk factors related to late onset sporadic PD might also

play a role in YOPD. PD risk loci, such as *SNCA* and *GBA* (Klein and Westenberg, 2012; Nalls, et al., 2014b) overlap between late and young onset. We therefore expect that our WES dataset is an adequate dataset to study the rare exonic variants in PD risk loci. Furthermore, YOPD is often genetically explained through rare variants (Bras, et al., 2015). The YOPD patient group in the WES dataset could therefore be enriched for cases which are genetically influenced by rare variants, possibly increasing the likelihood of detecting rare variant association.

Using gene-set approach in the WES dataset, we did not detect a burden of rare variants when comparing PD subjects to controls. However, it is undetermined whether the absence of a rare variant association is genuine or due to insufficient power. A genuine rare variant association might furthermore be impeded by the gene-set composition. By using PrioFix, we increase the likelihood of selecting the truly involved PD genes underneath the known PD risk loci, yet unrelated genes might still be included, possibly diluting an association signal. In contrast, with the gene based association test for the genes selected with the PrioFix strategy we observed a rare variant association for *STBD1*, implying that rare variants in this gene could decrease the risk to develop PD. *STBD1* has its function in lysosomal-mediated autophagy to specifically guide glycogen to lysosomes for sequestration and degeneration (Jiang, et al., 2011). It, therefore, seems that variants in *STBD1* could have beneficial effects for the removal of glycogen. The lysosomal-mediated autophagy has been implied to be involved in PD through the association of multiple genes, such as *LRRK2*, *ATP13A2* and *GBA* (Trinh and Farrer, 2013). However, the involvement of *STBD1* in PD pathogenesis has to be carefully considered, as we currently did not have an adequate independent dataset to replicate the association that was generally based on singletons. The NeuroX genotyping array typically includes variants that have been observed in previous datasets, minimizing the probability to detect similar singletons with an extremely low minor allele frequency. Only 3 of the 8 *STBD1* variants of the WES dataset, were present within the NeuroX dataset reducing the power to detect the single gene association. Hence, further genetic validation studies are warranted to establish the role of *STBD1* in PD. Once a legitimate replication is realized, functional assays on lysosomal-mediated autophagy should further decipher the contribution of *STBD1*, preferably in relation to well-established PD genes.

We detected a strong association of rare variants within the gene-sets for the NeuroX dataset. However, subsequent analyses showed that these associations were dominated by *LRRK2* variants. Association analysis on variant level revealed that the *LRRK2* gene signal was driven by the known p.G2019S variant. This observation highlights the importance of cataloguing the individual rare variants to fully resolve the impact of rare variants in disease susceptibility for PD. As shown for the *LRRK2* association and even the total gene-set association, it is driven by only 1 variant, which also could have been detected with the performance of a simple single-marker association test.

Besides the pathogenic association signal of rare variant G2019S, we observed a significant protective effect of a previously published common haplotype (Ross, et al., 2011). This observation supports the theory that other variants with opposite effects could interact and potentially influence the penetrance of pathogenic *LRRK2* variants, such as G2019S. Besides *LRRK2*, we furthermore detected a NeuroX-based burden of rare CADD variants for *SPATA19* that increases PD risk ($p = 0.017$). This association signal is relatively strong considering that it is driven by only 2 CADD variants that are present in 7 cases and 0 controls. As *SPATA19* is involved in spermatogenesis (Nourashrafeddin, et al., 2014), and the GTEx portal displays specific high expression for the testis, it diminishes the likelihood that defects of this gene would contribute to neurodegeneration. Further genetic and functional studies are warranted to decipher a role of this gene in PD.

In contrast to selecting the physically closest gene to the strongest SNP within each PD locus, we followed a comprehensive strategy to define true causal gene, which is based on biological similarities. As we expect that only one gene per locus is the true causal gene, we did not define a gene-set including all the genes underneath the GWAS loci assuming the overrepresentation of non-causal genes would dilute a putative association signal. We acknowledge that the ultimate strategy to test the effect of rare variants in the PD loci would be to sequence all genes in a large cohort, and test the effect of rare variants in each gene individually. Furthermore, sequencing rather than genotyping will define novel rare variants and contribute to cataloguing the influence of rare variants underneath the PD risk loci. Acknowledging these caveats, our study suggests for the first time that, apart from *LRRK2*, *SNCA* and *GBA*, other common PD risk loci might harbor rare variants that contribute to PD risk.

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Conflicts of interest

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Figures

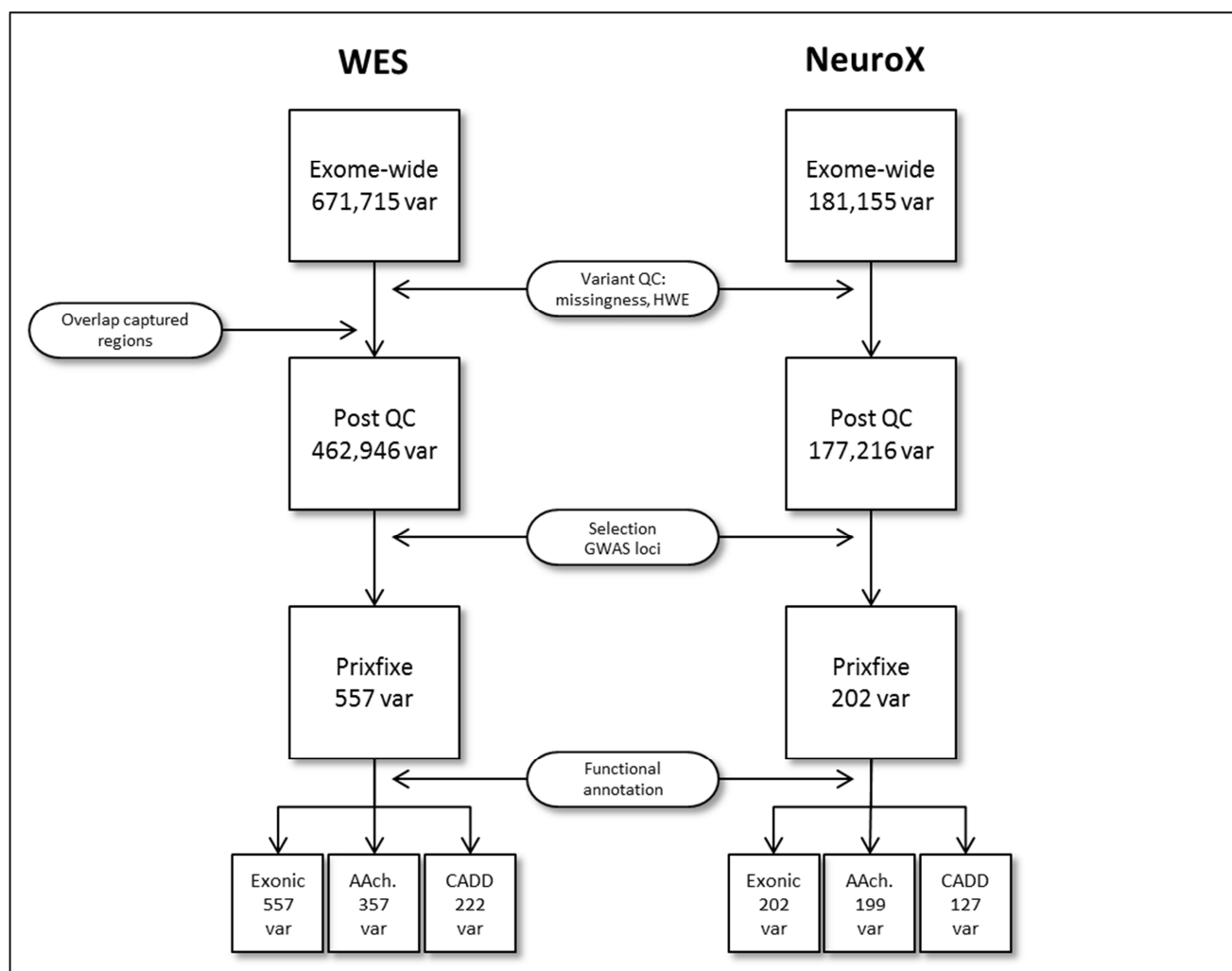


Figure 1. Flowchart of variant subset classification. The variants undergo multiple analyses procedures, including quality control, selection of variants within PD loci and functional annotation. Each genetic dataset (WES and NeuroX) is tested for 6 different variant categories, differing in causal gene selection approach and functionality of variant.

Tables

Table 1. Selected set of genes.

Polymorphism	Location (hg19)	P-value	Seeding SNP	Prixfixe gene
rs71628662	chr1:155359992	6.86×10^{-28}	NA	
rs823118	chr1:205723572	1.96×10^{-16}	rs823114	<i>RAB7L1</i>
rs10797576	chr1:232664611	1.76×10^{-10}	rs2182431	<i>SIPA1L2</i>
rs6430538	chr2:135539967	3.35×10^{-19}	rs6430538	<i>ACMSD</i>
rs1955337	chr2:169129145	1.67×10^{-20}	rs2390669	<i>STK39</i>
rs12637471	chr3:182762437	5.38×10^{-22}	rs12637471	<i>LAMP3</i>
rs11724635	chr4:15737101	4.26×10^{-17}	rs11724635	<i>FBXL5</i>
rs6812193	chr4:77198986	1.85×10^{-11}	rs6812193	<i>STBD1</i>
rs356182	chr4:90626111	1.85×10^{-82}	rs356219	<i>SNCA</i>
rs34311866	chr4:951947	6.0×10^{-41}	rs748483	<i>MFSD7</i>
rs9275326	chr6:32666660	5.81×10^{-13}	rs9275311	<i>HLA-DRB5</i>
rs199347	chr7:23293746	5.62×10^{-14}	rs199347	<i>GPNMB</i>
rs591323	chr8:16697091	3.17×10^{-8}	NA	
rs117896735	chr10:121536327	1.21×10^{-11}	rs10886515	<i>RGS10</i>
rs329648	chr11:133765367	8.05×10^{-12}	rs329648	<i>SPATA19</i>
rs3793947	chr11:83544472	2.59×10^{-08}	rs1400313	<i>DLG2</i>
rs11060180	chr12:123303586	3.08×10^{-11}	rs11060180	<i>HIP1R</i>
rs76904798	chr12:40614434	4.86×10^{-14}	rs2708435	<i>LRRK2</i>
rs7155501	chr14:55347827	1.25×10^{-10}	rs2878174	<i>LGALS3</i>
rs1555399	chr14:67984370	5.70×10^{-16}	rs7155830	<i>ARG2</i>
rs2414739	chr15:61994134	3.59×10^{-12}	NA	
rs14235	chr16:31121793	3.63×10^{-12}	rs14235	<i>PRSS8</i>
rs17649553	chr17:43994648	6.11×10^{-49}	rs17649553	<i>MAPT</i>
rs12456492	chr18:40673380	2.15×10^{-11}	rs12456492	<i>RIT2</i>
rs62120679	chr19:2363319	2.52×10^{-09}	rs2074546	<i>PLEKHJ1</i>
rs55785911	chr20:3153503	3.30×10^{-10}	rs2295545	<i>AVP</i>

P-value = Meta p-value as reported on pdegene.org. Seeding SNP = input SNP for PrixFixe software. Prixfixe gene = genes selected based on underlying functional similarities, which is determined by overlapping biological features such as protein domains, transcription factor binding sites, gene expression, phylogenetic profiles and literature-based protein-protein interactions.

Table 2. Gene-based rare variant association results for WES dataset.

Variant type	Gene	<i>p</i> -value (emp)	<i>n</i> variants	maf cases	maf controls
AAchanging	<i>STBD1</i>	0.018 (0.046)	8	0.05%	0.32%
	<i>HIP1R</i>	0.082	20	0.61%	0.53%
	<i>STK39</i>	0.126	4	0.20%	0.00%
CADD	<i>STBD1</i>	0.105	5	0.05%	0.16%
	<i>SPATA19</i>	0.122	4	0.19%	0.06%
	<i>GPNUMB</i>	0.141	18	0.85%	0.92%

p-value = theoretical *p*-value; (emp.) = empirical *p*-value calculated by comparison to 10000 permutations of affection status. AAchanging = amino acid changing variants; CADD = variants predicted pathogenic

Table 3. Gene-based rare variant association results for neuroX dataset.

Variant type	Gene	<i>p</i> -value (emp)	<i>n</i> variants	maf cases	maf controls
AAchanging	<i>LRRK2</i>	0.0004 (0.0005)	48	1.70%	1.13%
	<i>RIT2</i>	0.051	2	0.00%	0.03%
	<i>PRSS8</i>	0.098	1	0.04%	0.01%
CADD	<i>LRRK2</i>	0.0003 (0.0005)	32	1.38%	0.86%
	<i>SPATA19</i>	0.014 (0.017)	2	0.05%	0.00%
	<i>RIT2</i>	0.051	2	0.00%	0.03%

p-value = theoretical *p*-value; (emp.) = empirical *p*-value calculated by comparison to 10000 permutations of affection status. AAchanging = amino acid changing variants; CADD = variants predicted pathogenic

Supplemental data**Table 1.** WES capture protocols

	cases	controls	
	IPDGC	IPDGC	RSX1
Nimblegenenv2	252	37	1201
Truseq	912	446	0
Mixed	3	1	0
Total	1167	484	1201

Mixed = samples that have been captured using the 2 distinct capture kits.

Table 2. Exclusion of exons based on capture inc

	gene	exon	Source
PD meta	ASH1L	21	Truseq
	DLG2	1+2	Nimblegenenv2
	TMEM229	1+2	Nimblegenenv2
	TMEM175	1	Nimblegenenv2

Table 3. Parameters for power calculations.

Arguments	WES	NeuroX
Subreg. Length	3205	3205
Prevalence PD	0.0057	0.0057
% protective effect	50	50
n samples	2852	12771
Case proportion	0.41	0.53
Causal MAF cutoff	0.013	0.006
% causal variants	40	52

Subregion length = the average length of transcripts corresponding to the genes included in the gene-sets. % protective effect = % of causal variants with a negative coefficient. Causal MAF cutoff is similar to common/rare variant cut-off. % causal variants = % of CADD variants

Table 4. Gene-set association results of WES dataset.

Gene-set	Variant type	Rare		Common		Common & rare	
		<i>p</i> -value (emp.)	<i>n</i> variants	<i>p</i> -value (emp.)	<i>n</i> variants	<i>p</i> -value (emp.)	<i>n</i> variants
Prixfixe	exonic			0.014 (0.074)	29		
	AAchanging	0.227	343	0.319	14	0.223	357
	CADD	0.189	212	0.414	10	0.247	222

p-value = nominal *p*-value; (emp.) = empirical *p*-value calculated by comparison to 1000 randomly drawn gene-sets of same size. *P*-values in bold are significant. MAF cut-off to separate rare and common variants is 0.013 on sample size).

Table 5. Gene-set association results of neuroX dataset.

Gene-set	Variant type	Rare		Common		Common & rare	
		<i>p</i> -value (emp.)	<i>n</i> variants	<i>p</i> -value (emp.)	<i>n</i> variants	<i>p</i> -value (emp.)	<i>n</i> variants
a. <i>LRRK2</i> included	exonic			0.031 (0.101)	18		
	AAchanging	1.06 x 10⁻⁵ (0.007)	176	0.0084 (0.053)	23	8.45 x 10⁻⁷ (0.026)	199
	CADD	5.99 x 10⁻⁷ (0.002)	114	0.0032 (0.034)	13	8.58 x 10⁻⁸ (0.020)	127
b. <i>LRRK2</i> excluded	exonic			0.243	13		
	AAchanging	0.28	128	0.154	16	0.367	144
	CADD	0.70	82	0.197	8	0.411	90

p-value = theoretical *p*-value; (emp.) = empirical *p*-value calculated by comparison to 1000 randomly drawn gene-sets of same size. Boldfaced *p*-values are significant. MAF cut-off to separate rare and common variants is 0.006 (based on sample size).

Table 6. Gene-based rare variant association results for neuroX dataset.

Variant type	Gene	<i>p</i> -value	<i>n</i> variants
AAchanging	<i>LRRK2</i>	4.32×10^{-13}	48
	<i>PRSS8</i>	0.098	1
	<i>RIT2</i>	0.129	2
CADD	<i>LRRK2</i>	5.17×10^{-13}	32
	<i>SPATA19</i>	0.050	2
	<i>HIP1R</i>	0.091	9

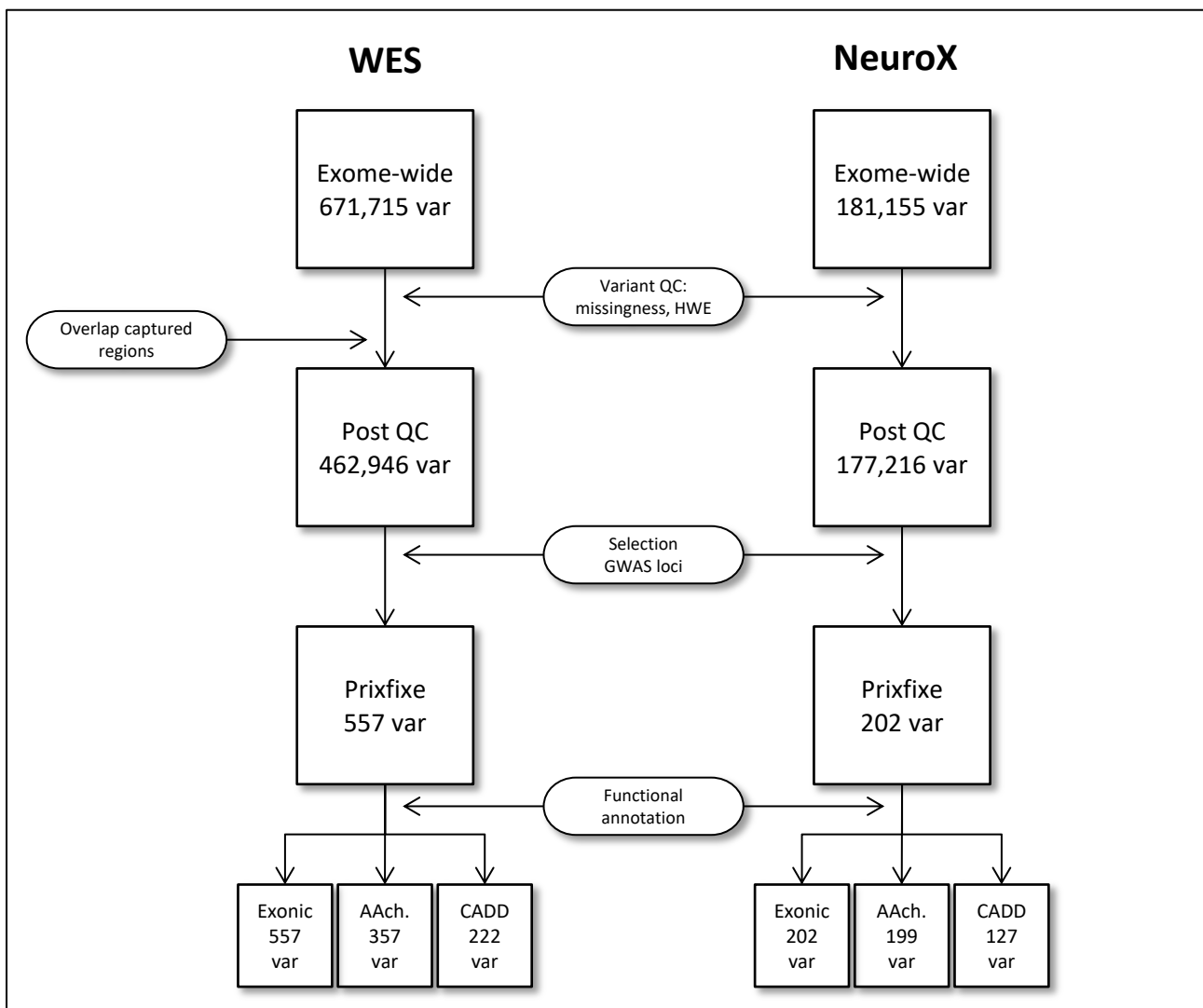
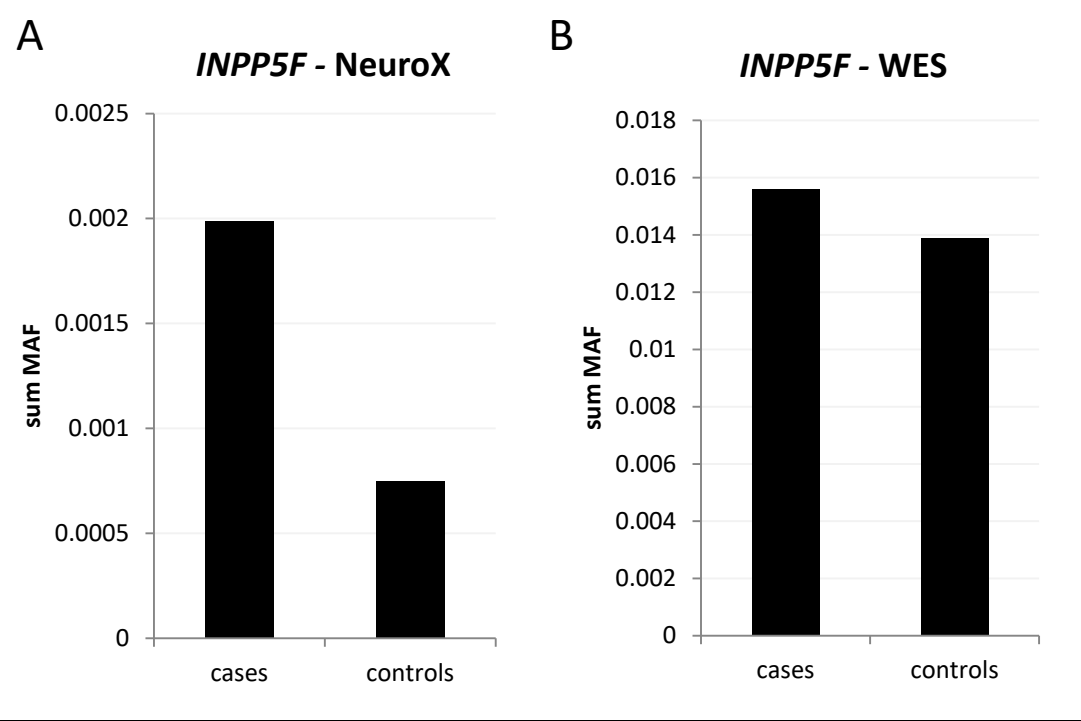


Figure 1. Flowchart of variant subset classification. The variants undergo multiple analyses procedures, including quality control, selection of variants within PD loci and functional annotation. Each genetic dataset (WES and NeuroX) is tested for 6 different variant categories, differing in causal gene selection approach and functionality of variant.



Highlights

- Two genetic datasets comprising a total of 7,968 PD cases and 7,655 controls were used to study the exome
- Rare variants in *LRRK2*, *STBD1* and *SPATA19* are suggested to play a role in PD
- Larger sequencing studies are required in future for follow up.