

Excessive burden of lysosomal storage disorder gene variants in Parkinson's disease

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Abstract

Mutations in the *glucocerebrosidase* gene (*GBA*), which cause Gaucher disease, are also potent risk factors for Parkinson's disease. We examined whether a genetic burden of variants in other lysosomal storage disorder genes is more broadly associated with Parkinson's disease susceptibility. The sequence kernel association test was used to interrogate variant burden among 54 lysosomal storage disorder genes, leveraging whole exome sequencing data from 1,156 Parkinson's disease cases and 1,679 control subjects. We discovered a significant burden of rare, likely damaging lysosomal storage disorder gene variants in association with Parkinson's disease risk. The association signal was robust to the exclusion of *GBA*, and consistent results were obtained in 2 independent replication cohorts, including 436 cases and 169 controls with whole exome sequencing and an additional 6,713 cases and 5,964 controls with exome-wide genotyping. In secondary analyses designed to highlight the specific genes driving the aggregate signal, we confirmed associations at the *GBA* and *SMPD1* loci and newly implicate *CTSD*, *SLC17A5*, and *ASAHI* as candidate Parkinson's disease susceptibility genes. In our discovery cohort, the majority of Parkinson's disease cases (56%) have at least one putative damaging variant in a lysosomal storage disorder gene, and 21% carry multiple alleles. Our results highlight several promising new susceptibility loci and reinforce the importance of lysosomal mechanisms in Parkinson's disease pathogenesis. We suggest that multiple genetic hits may act in combination to degrade lysosomal function, enhancing Parkinson's disease susceptibility.

Key Words

Parkinson's disease

Lysosomal storage disorders

1 Genetics

2 Whole exome sequencing

3

4 **Abbreviations**

5 CADD Combined Annotation Dependent Depletion

6 GBA Glucocerebrosidase

7 IPDGC International Parkinson’s Disease Genomics Consortium

8 LSD Lysosomal Storage Disorder

9 MAF Minor allele frequency

10 PPMI Parkinson’s Progression Markers Initiative

11 QC Quality control

12 RSX-1 Rotterdam Study exome dataset version 1

13 SKAT-O Sequence kernel association test – optimized

14 WES Whole exome sequencing

15

16 **Introduction**

17 Parkinson’s disease is a common neurodegenerative disorder with evidence for a substantial
18 genetic etiology (Kalia and Lang, 2015). Studies in families as well as large population-based
19 cohorts have implicated more than 30 genes (Bras *et al.*, 2015; Singleton *et al.*, 2013;
20 Verstraeten *et al.*, 2015); however, the risk alleles identified to date explain only a fraction of
21 Parkinson’s disease heritability estimates (Do *et al.*, 2011; Hamza and Paymi, 2010; Keller *et al.*,
22 2012), suggesting the involvement of additional loci. Beyond discovering the responsible genes,
23 a major challenge remains to understand the mechanisms by which these factors alter disease

onset and/or progression, including whether they act independently or interact within coherent biologic pathways.

Substantial evidence highlights the importance of lysosomal mechanisms in Parkinson's disease susceptibility and pathogenesis (Kalia and Lang, 2015; Moors *et al.*, 2016; Vekrellis *et al.*, 2011; Wong and Krainc, 2016). Prior to its discovery as a Parkinson's disease risk locus, the *glucocerebrosidase* gene, *GBA*, was known to cause Gaucher disease, an autosomal recessive lysosomal storage disorder (LSD). Increased risk for Parkinson's disease in heterozygous carriers of *GBA* loss-of-function alleles was first recognized in families of individuals with Gaucher disease (Goker-Alpan *et al.*, 2004; Tayebi *et al.*, 2003). Follow-up studies in large, case-control samples confirmed that heterozygous *GBA* variants confer at least a five-fold increased risk of Parkinson's disease (Aharon-Peretz *et al.*, 2004; Sidransky *et al.*, 2009). *GBA* variants may also modify Parkinson's disease clinical manifestations (Brockmann *et al.*, 2015; Clark *et al.*, 2007; Davis *et al.*, 2016; Winder-Rhodes *et al.*, 2012), causing earlier age-of-onset, higher risk of cognitive impairment, and accelerated progression. LSDs—of which there are more than 50—are strictly Mendelian-inherited, metabolic disorders collectively caused by dysfunction in lysosomal biogenesis or function, and similarly characterized by the abnormal accumulation of non-degraded metabolites in the lysosome (Boustany, 2013; Filocamo and Morrone, 2011). The strong genetic evidence linking Gaucher disease and Parkinson's disease risk leads to the intriguing, generalized hypothesis that LSDs and Parkinson's disease may share a common genetic mechanism. Other LSD genes have therefore become attractive candidate risk factors for Parkinson's disease (Deng *et al.*, 2015; Shachar *et al.*, 2011). Several studies have consistently supported a role for *SMPD1* (Clark *et al.*, 2015; Foo *et al.*, 2013; Gan-Or *et al.*, 2013; 2015; Wu *et al.*, 2014), which causes Niemann-Pick disease, Type A/B. Initial reports evaluating other

LSD genes, including *NPC1*, *NPC2*, *MCOLN1*, *NAGLU* and *ARSB*, have either shown conflicting results or await further replication (Clark *et al.*, 2015; Jansen *et al.*, 2017; Klunenmann *et al.*, 2013; Winder-Rhodes *et al.*, 2012; Zech *et al.*, 2013). LSDs are individually quite rare in populations of European ancestry, as are the known genetic variants established to cause these disorders (Boustany, 2013; Filocamo and Morrone, 2011). However, with the exception of *GBA*, most studies of LSD gene candidates have been small and therefore likely underpowered to detect the effects of rare alleles or those with more modest effect sizes. Genome-wide association studies in large Parkinson's disease case-control cohorts have independently implicated more common risk alleles at another LSD gene, *SCARB2* (Do *et al.*, 2011; Nalls *et al.*, 2014), which encodes a membrane protein required for correct targeting of glucocerebrosidase to the lysosome. Besides this growing genetic evidence, studies in cellular and animal models also implicate the lysosome in the clearance of alpha-synuclein (Cuervo *et al.*, 2004; H. J. Lee, 2004; Vogiatzi *et al.*, 2008), which aggregates to form Lewy body pathology in Parkinson's disease. Reciprocally, alpha-synuclein disrupts neuronal vesicle trafficking and lysosomal function (Cooper *et al.*, 2006; Mazzulli *et al.*, 2011; Moors *et al.*, 2016; Wong and Krainc, 2016).

In this study, we leverage the largest Parkinson's disease whole exome sequencing (WES) dataset currently available to systematically examine the overlap between genes responsible for LSDs and Parkinson's disease. Our results reveal an aggregate burden for genetic variants among 54 genes established to cause LSDs and suggest that many genes besides *GBA* likely contribute to susceptibility for Parkinson's disease.

Materials and Methods

Subjects

Clinical and demographic features for our study cohorts, which have also been described in other recent reports (Giri *et al.*, 2017; Jansen *et al.*, 2017), are shown in Supplemental Table 1. The International Parkinson's Disease Genomics Consortium (IPDGC) WES discovery dataset used for this study consists of 2,835 samples of Northern and Western European ancestry, including 1,156 Parkinson's disease cases and 1,679 controls not known to have Parkinson's disease. Subjects were recruited from academic medical centers across the United States and Europe. Cases were recruited at a mean age of 51.5 years (SD=11.5) and diagnosed with Parkinson's disease at a mean age of 41.2 years (SD=10.8); 40.4% reported a positive family history. Control subjects were on average 63.7 years of age (SD=17.1). 1,201 control exomes originated from the Rotterdam Study exome dataset version 1 (RSX-1) (van Rooj *et al.*, 2017; Giri *et al.*, 2017). The Rotterdam Study is a prospective population-based cohort study based in Rotterdam, the Netherlands. WES was performed on DNA from participants from the RSX-I subcohort, enrolled in 1990, with an average age at baseline of 68.6 (SD=8.6, 54.4% female) (Hofman *et al.*, 2015). All IPDGC and RSX-1 subjects gave written informed consent for participation in genetic research, which was approved by relevant oversight committees and institutional review boards. Subjects with pathogenic variants in established Mendelian Parkinson's disease genes (*SNCA*, *LRRK2*, *VPS35*, *PARK2/parkin*, *PARK7/DJ-1*, or *PINK1*) were excluded from analysis (Jansen *et al.*, 2017). Following quality control filters, the Parkinson's Progression Markers Initiative (PPMI) replication dataset (Parkinson Progression Marker Initiative, 2011) includes 436 cases and 169 controls of Northwest European descent. Cases were recruited at a mean age of 61.7 years (SD 9.7) and diagnosed with Parkinson's disease at an average age of 59.8 years (SD=10.0); 27.1% reported a positive family history. PPMI controls were an average of 61.8

years of age (SD=10.1) at the time of evaluation. Data used in the preparation of this article were obtained from the PPMI database (www.ppmi-info.org/data); for up-to-date information on the study, visit www.ppmi-info.org. Samples analyzed for both the IPDGC and PPMI cohorts were derived from whole blood. The NeuroX cohort has also been previously described in detail (Jansen *et al.*, 2017; Nalls *et al.* 2015). A minority of subjects overlapping with the IPDGC WES discovery sample were removed, such that the NeuroX replication cohort was a completely independent sample, including 6,713 individuals with Parkinson's disease and 5,964 controls. NeuroX cases were diagnosed at an average age of 61.6 (SD=12.4) and controls were evaluated at an average age of 64.1 (SD=14.3).

Sequencing/Genotyping and Quality Control

Data generation and detailed quality control procedures for the IPDGC and RSX-1 samples has recently been reported (Giri *et al.*, 2017; Jansen *et al.*, 2017; van Rooj *et al.*, 2017). WES was performed using the Roche Nimblegen SeqCap v2 or Illumina exome capture kits to prepare sample libraries, followed by paired-end sequencing with Illumina HiSeq2000. The generation of the PPMI WES dataset are described elsewhere (www.ppmi-info.org). Although the datasets originate from different consortia, the same algorithms were used for read processing. The Burrows-Wheeler Aligner-MEM algorithm (Li and Durbin, 2010) was used for alignment of sequencing reads to the human reference genome (hg19). Using Picard tools (<http://broadinstitute.github.io/picard>), Binary Alignment/Map files were generated in a sorted and indexed manner. Alignments were Base-Quality score recalibrated and indels realigned using the Genome Analysis Toolkit (McKenna *et al.*, 2010) v3.3-0, after which single nucleotide variants and small insertions/deletions were called with the HaplotypeCaller to one genomic

Variant Call Format file per individual. The IPDGC and RSX-1 WES datasets (hereafter referred to as simply the IPDGC discovery dataset) were merged by joint variant calling from the individual genomic Variant Call Format files. Variants that were not assigned with the standard Genome Analysis Toolkit quality annotation 'PASS' were excluded for subsequent analyses. 94.4% and 98.0% of the IPDGC and PPMI exomes, respectively, achieved a minimum of 10x coverage.

As previously described (Giri *et al.*, 2017; Jansen *et al.*, 2017), for individual quality control, samples were excluded for ambiguous gender, deviating heterozygosity/genotype calls, low genotype call rates, or cryptic relatedness following identity-by-descent analyses. Population structure was further evaluated using multi-dimensional scaling component analysis based on linkage disequilibrium-pruned, genome-wide common variant markers. Prior to these calculations, our datasets were merged with available genotypes from 1000 Genomes Project (1000GP) ancestry-based population samples, including African (AFR), East Asian (EAS), European (EUR) and the Americas (AMR) (1000 Genomes Project Consortium 2012). Using the European samples as a reference, population outliers were excluded, resulting in the removal of 39 or 9 individuals from the IPDGC and PPMI datasets, respectively. All remaining samples cluster tightly with European ancestry subjects on multi-dimensional scaling plots (Supplemental Figure 1). Genotype and variant quality control was accomplished by removal of low-quality genotypes (Phred-scaled genotype quality score < 20, depth < 8) and variants with low call rates or departure from Hardy-Weinberg equilibrium. Furthermore, for the IPDGC discovery dataset, variants were only considered when located within the overlapping targeted regions of the applied library preparation capture kits. Post-quality control procedures, a total of 462,946 and 192,421 variants were called for the IPDGC and PPMI datasets, respectively.

1 Data generation and quality control for the NeuroX cohort has also previously described
2 in detail (Jansen *et al.* 2017; Nalls *et al.*, 2015). NeuroX consists of 242,901 exonic variants from
3 the Illumina Infinium HumanExome BeadChip and 24,706 custom variants related to neurologic
4 disease. For individual quality control, as above, samples were excluded for gender ambiguity,
5 dubious heterozygosity/genotype calls, evidence of relatedness, or poor clustering on multi-
6 dimensional scaling plots (Supplemental Figure 1). We similarly excluded variants for low call
7 rates, departure from Hardy-Weinberg equilibrium, or for significant differences in missingness
8 rate between cases and controls. Post-quality control, we called 177,028 exonic variants from the
9 NeuroX dataset.

10 Where allowable based on individual consents and institutional review board approval,
11 the datasets used in this study, including WES and NeuroX data from the IPDGC, are publicly
12 available. Data availability is detailed in Jansen *et al.* 2017 and at <http://pdgenetics.org/resources>.
13 Data from PPMI is also available for download at [http://www.ppmi-info.org/access-data-](http://www.ppmi-info.org/access-data-specimens/download-data/)
14 [specimens/download-data/](http://www.ppmi-info.org/access-data-specimens/download-data/).

16 **Variant Selection**

17 Our analyses initially considered 54 LSDs (Table 1), defined based on widely accepted clinical,
18 pathologic, and metabolic criteria (Amberger *et al.*, 2015; Boustany, 2013; Filocamo and
19 Morrone, 2011). All variants within the LSD gene set were extracted from the three datasets. For
20 the IPDGC WES dataset, no variants in the genes *CLN5* and *NEU1* passed the pre-specified
21 maximum missingness criteria of 15%, yielding 1,136 total exonic variants for consideration in
22 these analyses. In addition, there were no non-synonymous variants identified in *SUMF1*.
23 Variants were categorized in nested groups (Figure 1) including (1) nonsynonymous (n=760

1 variants in 51 genes), (2) likely damaging (n=596 variants in 51 genes), or (3) loss-of-function
2 (n=69 variants in 27 genes) (see Table 1 and Supplemental Table 2). Loss-of-function variants
3 included stop gain/loss, frameshift, and splicing mutations falling within two base pairs of exon-
4 intron junctions. Predictions of variant pathogenicity were obtained from ANNOVAR (Wang *et*
5 *al.*, 2010), based on the Combined Annotation Dependent Depletion (CADD) algorithm (v1.3,
6 <http://cadd.gs.washington.edu>) (Kircher *et al.*, 2014). CADD integrates predictions from
7 numerous bioinformatic algorithms into a single “C-score” and ranks all possible nucleotide
8 changes in the genome based on potential to disrupt gene/protein function. In accordance with
9 prior work (Amendola *et al.*, 2015), we selected a stringent CADD C-score \geq 12.37, representing
10 the top ~2% most damaging of all possible nucleotide changes in the genome—this subset is
11 enriched for known pathogenic alleles. For descriptive purposes, all putative damaging variants
12 within the IPDGC discovery cohort were further cross-referenced with ClinVar (Landrum *et al.*,
13 2016) to identify those previously established with pathogenicity for LSDs (Supplemental Table
14 3). For the PPMI cohort, no variants were called in *DNAJC5*, resulting in a dataset of 515 total
15 exonic variants, of which 256 variants from 49 genes were nonsynonymous and 187 variants in
16 47 genes met the CADD criteria for putative damaging changes (Supplemental Table 2). For the
17 NeuroX cohort, all genes in the 54-gene set were represented, resulting in 467 nonsynonymous
18 variants, of which 348 were classified as likely damaging (Supplemental Table 2). Within these
19 categories, variants were filtered based on two minor allele frequency (MAF) thresholds: (a)
20 <1% and (b) <3% (Figure 1). The latter, more relaxed frequency threshold is based on the
21 population prevalence (de Lau and Breteler, 2006; Pringsheim *et al.*, 2014) and known
22 incomplete penetrance of Parkinson’s disease risk alleles (Anheim *et al.* 2012; Marder *et al.*,
23 2015; Rana *et al.* 2013; Trinh *et al.*, 2014). For a subset of individuals in the IPDGC (n=572) and

PPMI (n=566) WES cohorts, array-based genotyping data was also available, allowing us to compute concordance rates for genotyping calls present in both datasets using 2 independent assays (Supplemental Table 4). We observe complete concordance for *GBA* variants as well as nearly perfect concordance (>>99%) for variant genotype calls in the full LSD gene set.

Statistical Analysis

The sequence kernel association test – optimal (SKAT-O) (S. Lee *et al.*, 2012; 2016) was implemented in R using SKAT v1.0.9 to determine the difference in the aggregate burden of rare LSD gene variants between Parkinson’s disease cases and controls. SKAT-O aggregates genetic information across defined genomic regions to test for associations. Covariates were included to adjust analyses for gender and WES coverage (pre-quality control missingness). Twenty multi-dimensional scaling components were also included to account for other possible confounding factors (4 components for analyses of the NeuroX genotyping cohort). An empirical p -value (p) was derived from the distribution of null results based on 10,000 permutation trials in which case/control assignment was randomized. As diagrammed in Figure 1, SKAT-O analysis was initially performed for the complete LSD gene set, considering each class of variants defined based on frequency and functional characteristics. In order to adjust for multiple comparisons, we applied the Bonferroni-Holm stepwise procedure to control for the familywise error rate and establish a corrected statistical significance threshold and adjusted p -value (p_{adj}) based on a significance level, α , of 0.05 (Holm 1979). For those categories with a significant SKAT-O association in the full gene set, a secondary analysis was performed excluding all *GBA* variants in order to confirm the involvement of additional genes. For example, in the IPDGC discovery cohort, we adjusted for $k=5$ or 2 comparisons for the number

of variant categories evaluated in the primary and secondary analyses, respectively. Due to the nested variant categories (Figure 1) and the highly interdependent nature of the respective burden tests, we separately considered those results with an empirical SKAT-O p -value < 0.05 , but not surviving the Bonferonni-Holm correction, as “suggestive”. Unadjusted, empiric SKAT-O p -values for all gene set analyses are included in Supplemental Table 5. Lastly, in order to highlight those loci driving associations detected in the gene set, secondary analyses were also performed using SKAT-O to evaluate variants in each LSD gene independently. For these per gene analyses, which we considered exploratory due to limited statistical power (below), we report all findings with an empirical unadjusted p -value < 0.05 .

To estimate statistical power, we performed 1,000 SKAT simulations of causal subregions within the discovery or replication datasets. We assumed a Parkinson’s disease prevalence of 0.0041 and 0.0017 for the IPDGC and PPMI datasets, respectively, based on their distinct ages of onset (Supplemental Table 1) (Pringsheim *et al.*, 2014). For gene set simulations, subregion length was defined as the sum of individual LSD gene coding region lengths (169.5 kb or 170.4 in IPDGC and PPMI, respectively). For single gene simulations, the average gene length was used (3.5kb or 3.2 kb, respectively). The MAF cutoff for causal variants was set to 0.00035 (based on the frequency of rare *GBA* loss-of-function alleles in the IPDGC data set) or 0.03 for the rare or more common variant models, respectively, and penetrance was assumed to be either 100% or 10%. Because we predict that LSD gene variants associated with Parkinson’s disease will have a damaging effect, all causal variants were assumed to have a positive coefficient (risk rather than protective alleles).

Results

1 Variants were extracted from 54 genes responsible for LSDs, defined based on widely accepted
2 criteria (Table 1), and filtered into nested categories based on 2 frequency thresholds and 3 tiers
3 of functional criteria (Figure 1A). Our overall analytic approach is diagrammed in Figure 1B. To
4 test our hypothesis that an aggregate burden of variants in the LSD gene set contributes to
5 Parkinson's disease, we first implemented SKAT-O within the IPDGC WES discovery cohort
6 (Table 2). Following adjustment for multiple comparisons (see Methods), significant associations
7 were detected for the LSD gene set considering either all non-synonymous variants (category 1b,
8 $p_{adj}=0.014$) or likely damaging variants (category 2b, $p_{adj}=0.0055$), when using the more relaxed
9 frequency threshold of $MAF < 3\%$. When considering only the subset of rare ($MAF < 1\%$)
10 nonsynonymous or likely damaging variants, the SKAT-O result was attenuated and no longer
11 significant (category 1a, $p_{adj}=0.056$ and category 2a, 0.066, respectively). No association was
12 observed when considering only loss-of-function alleles (category 3, $p_{adj}=0.464$), possibly due to
13 the relative paucity of such variants limiting statistical power (Supplemental Table 1). We next
14 repeated analyses with significant results, but excluding all *GBA* variants. As expected, the
15 strength of the associations was attenuated; however, both SKAT-O results including either all
16 nonsynonymous variants ($MAF<3\%$) or the subset of likely damaging variants was robust to the
17 exclusion of *GBA* and remained significant (category 1b, $p_{adj}=0.026$ and category 2b,
18 $p_{adj}=0.0198$). Our results indicate that the association between variant burden and Parkinson's
19 disease risk in the IPDGC discovery cohort is mediated, at least in part, by the effects of LSD
20 genes other than *GBA*, an established Parkinson's disease susceptibility locus.

21 To replicate our findings, we leveraged two independent cohorts, including an additional
22 WES dataset from PPMI (436 Parkinson's disease cases and 169 controls) (Parkinson
23 Progression Marker Initiative, 2011) and the NeuroX exome-wide genotyping dataset from

1 IPDGC (6,713 Parkinson’s disease cases and 5,964 controls) (Nalls *et al.*, 2015). We again
2 implemented SKAT-O to detect a potential variant burden in Parkinson’s disease cases versus
3 controls. In the smaller PPMI replication cohort, we discovered suggestive evidence for an
4 excessive LSD variant burden in Parkinson’s disease (Table 2); however, this finding was not
5 significant following adjustment for multiple comparisons (category 1a, $p_{adj}=0.096$). The
6 association signal—which appeared independent of *GBA* (Supplemental Table 5)—was detected
7 exclusively among rare alleles ($MAF < 1\%$) and only when considering all non-synonymous
8 variants. It is possible that SKAT-O is sensitive to cohort differences between PPMI and the
9 IPDGC, including both sample size and pertinent demographic features (e.g. age of onset and
10 family history; Supplemental Table 1). However, in the substantially larger NeuroX dataset,
11 significant burden associations were detected for the same 2 variant categories implicated by
12 SKAT-O in the IPDGC discovery cohort (Table 2), despite the less comprehensive genotyping
13 coverage compared to WES. A major driver for the robust LSD gene set association in NeuroX
14 (category 1b, $p_{adj}=0.0004$ and category 2b, $p_{adj}=0.0003$) appears to be the more common
15 *GBA*^{E326K} variant ($Freq_{Cases}=0.021$, $Freq_{Controls}=0.011$), which has been reported to be associated
16 with Parkinson’s disease risk in several large studies (Duran *et al.*, 2012; Pankratz *et al.*, 2012).
17 Importantly, consistent with our findings in the IPDGC discovery cohort, the LSD gene set
18 burden association for both of these variant categories remained significant in NeuroX following
19 exclusion of *GBA* (category 1b, $p_{adj}=0.002$ and category 2b, $p_{adj}=0.020$). When considering only
20 the subset of rare ($MAF < 1\%$) variants in the NeuroX dataset, the SKAT-O result for the LSD
21 gene set was attenuated and no longer significant; although, the association in the
22 nonsynonymous variant group remained suggestive, and this association was independent of
23 *GBA* (Supplemental Table 5). In sum, based on analyses in three independent Parkinson’s

disease case-control datasets, we demonstrate a burden of variants in LSD genes associated with Parkinson's disease risk, and this signal is at least partially independent of *GBA*.

To determine which additional LSD genes/variants may be responsible for the observed association with Parkinson's disease risk, we performed exploratory analyses using SKAT-O to assess for potential contribution of variants within each gene considered independently. For these analyses, we returned to the IPDGC discovery dataset, and again focused on likely damaging variants, which showed the strongest association signal in our primary analysis (category 2b). In these gene-based analyses, besides the expected result for *GBA* ($p = 0.0001$) and confirmation of *SMPD1* ($p = 0.029$), we discover evidence of novel aggregate associations for variants in *CTSD* ($p = 0.002$), *SLC17A5* ($p = 0.005$), and *ASAH1* ($p = 0.031$). The specific variants implicated for each of these genes are included in Supplemental Table 3, along with all other putative damaging variants considered in our full LSD gene set analysis. While our datasets are underpowered to definitively assess the contributions of a particular rare variant in any single gene (see Discussion), these results identify the most likely specific loci driving the aggregate LSD gene set association signal detected in the IPDGC discovery sample.

Lastly, we examined the distribution of putative damaging LSD gene variants (MAF < 3%, category 2b) within the IPDGC WES cohort (Figure 2). Consistent with our finding of an excessive variant burden in Parkinson's disease, the distribution of variants appeared modestly right-skewed in cases. The average variant burden among IPDGC cases was 0.9 alleles per individual, which was slightly higher than that seen in controls (0.8 alleles per individual). Given their commonality, the majority of IPDGC cases (56%) have at least one putative damaging variant in an LSD gene, and 21% carry multiple alleles. Notably, only 22 out of 1156 total Parkinson's disease cases are homo- or hemizygous for putative damaging LSD variants

(Supplemental Table 6), suggesting that Mendelian recessive or X-linked inheritance may contribute minimally to the overall burden association. As discussed further below, our findings are consistent with a hypothetical model in which multiple LSD gene variants may interact to influence Parkinson's disease risk.

Discussion

This study reveals an important connection between the genetic factors broadly responsible for LSDs, which are predominantly pediatric Mendelian disorders, and Parkinson's disease, an adult-onset neurodegenerative disorder with complex genetic etiology. Specifically, among 54 genes that cause LSDs, we find evidence for a burden of damaging alleles in association with Parkinson's disease risk. This association persisted after excluding *GBA*, consistent with a contribution from additional LSD genes. More than half of Parkinson's disease cases in our cohort harbor one or more putative damaging variants among the LSD genes. Thus, our results implicate several promising new Parkinson's disease susceptibility loci and reinforce the importance of lysosomal mechanisms in Parkinson's disease pathogenesis.

The strengths of this study include a large Parkinson's disease case/control discovery cohort as well as two independent datasets for replication of our findings. The IPDGC WES discovery sample is characterized by younger-onset Parkinson's disease cases (mean age~41 years) and those with a positive family history, thereby enriching for individuals with a potential genetic contribution. Recruitment of a substantially older IPDGC control group (mean age~64), reduces the possibility of latent, unrecognized Parkinson's disease (i.e. with minimal or absent symptoms), likely further increasing power for genetic discovery. By contrast, our PPMI and NeuroX replication cohorts include older cases (mean age~62 years) and age-matched controls,

1 making them more broadly representative of the older adult population commonly affected by
2 Parkinson's disease. Consistent findings of an excessive LSD variant burden across these 3
3 datasets, especially the large NeuroX sample (n~12,677), strongly enhances the generalizability
4 of our conclusions. To minimize the possibility of population stratification, stringent quality
5 control filters were implemented to ensure a homogeneous European ancestry sample in all study
6 cohorts (Supplemental Figure 1). Nevertheless, it will also be important to examine other ethnic
7 populations in the future, especially those potentially enriched for LSD-causing variants due to
8 genetic bottlenecks.

9 Since our understanding of the characteristics of causal alleles—including in both
10 Parkinson's disease and LSDs—is incomplete, our initial analyses systematically considered
11 multiple variant classes binned into categories based on frequency and putative functional
12 impact. In the IPDGC and PPMI cohorts, WES offers comprehensive characterization of LSD
13 gene variants. By contrast, since the NeuroX data is restricted to those variants included on the
14 genotyping array, it is possible that many potential pathogenic variants would be missed.
15 Nevertheless, a total of 348 putative damaging variants were detected, including alleles for all
16 LSD genes (Supplemental Table 2). Importantly, the selected analytic tool, SKAT-O, is robust to
17 a wide frequency spectrum, including rare and more common alleles, and to variants with
18 different magnitudes and directions of effect (S. Lee *et al.*, 2012; 2016). Our results suggest that
19 consideration of likely damaging alleles based on bioinformatic predictions, including more
20 common LSD variants ($MAF < 3\%$), appeared to offer optimal sensitivity for detection of a
21 significant aggregate variant association. Many of these variants are known to be pathogenic for
22 LSDs (Supplemental Table 3). For example, of the *GBA* variants considered in our analyses,
23 27% of those with annotations available in ClinVar (Landrum *et al.*, 2016) are rated as likely or

definitively pathogenic. Critically, the implementation of burden association tests for joint consideration of LSD genes significantly improves statistical power over single gene and variant tests (Zuk *et al.*, 2014). In populations of European ancestry similar to our study cohorts, loss-of-function alleles, including those established to cause LSDs, are individually rare (Supplemental Table 2), and based on post-hoc simulations (see Methods), we estimate poor power for discovery of rare Parkinson's disease risk alleles at single loci. For example, assuming a rare variant model (MAF = 0.035%, as for *GBA* loss-of-function alleles in our sample) and even assuming full penetrance, the IPDGC discovery cohort has only 30% power to discover an association for a single gene. However, a similar simulation considering the full set of 54 LSD genes was fully powered (100%). Our consideration of higher frequency variants further enhances power for both discovery and replication, especially when coupled with filtering based on potential pathogenicity. For example, allowing for more common variants (MAF < 3%) and assuming 10% of such alleles are causal, we estimate that the smaller PPMI cohort achieves 95% power for replication of a gene set association, whereas negligible power (1%) is available for interrogation of a single gene candidate. We anticipate that larger WES datasets will significantly improve power, including for per gene analyses.

We also performed analyses in the IPDGC cohort to pinpoint the specific drivers from the LSD gene set responsible for increasing Parkinson's disease risk. Our results (i) recapitulate the established association with *GBA*, (ii) strengthen the emerging evidence in support of *SMPD1*, and (iii) newly implicate *SLC17A5*, *ASAHI*, and *CTSD* as candidate Parkinson's disease susceptibility genes. Recessive mutations in *SMPD1* cause Niemann-Pick type A/B disease and this locus has been independently implicated in Parkinson's disease risk based on several published studies (Clark *et al.*, 2015; Foo *et al.*, 2013; Gan-Or *et al.*, 2013; 2015; S. Lee *et al.*,

2012). While our analysis identified 21 candidate, putative damaging *SMPD1* risk alleles (Supplemental Table 3), most appear distinct from those reported in other studies of Parkinson's disease. One notable exception, *SMPD1*p.L304P (also referred to as p.L302P), was previously implicated in a study of Ashkenazi Jewish subjects (Gan-Or *et al.*, 2013). Another non-synonymous variant, p.P332L implicated in the IPDGC sample is at the same amino acid position as a different substitution, p.P332R, that was previously implicated in a Chinese Parkinson's disease cohort (Foo *et al.*, 2013). Among the novel candidate genes, *SLC17A5*, *ASAHI*, and *CTSD*, most of the implicated variants are rare (MAF<1%). Only 2 of these variants (*rs16883930* and *rs141068211* in *SLC17A5* and *ASAHI*, respectively) are present in the 1000 Genomes reference (The 1000 Genomes Project Consortium, 2012), having been previously examined in genome-wide scans, and both were non-associated with Parkinson's disease risk (p>0.05) based on available data (Lill *et al.*, 2012). Mutations in *SLC17A5*, *ASAHI*, and *CTSD* cause the rare LSDs, Salla disease, Farber Lipogranulomatosis, and Neuronal Ceroid Lipofuscinosis (CLN10), respectively. Whereas Sialin (the protein product of *SLC17A5*) is a lysosomal membrane transporter for sialic acid, Acid Ceramidase (*ASAHI*) participates in ceramide metabolism, similar to Glucocerebrosidase and Sphingomyelinase (*SMPD1*). In addition to promoting lysosomal stress, glucosylceramide, which accumulates in Gaucher disease, has been suggested to directly promote the aggregation of alpha-synuclein (Mazzulli *et al.*, 2011; Moors *et al.*, 2016). Interestingly, *CTSD* encodes a lysosomal aspartyl proteinase which has been independently implicated in alpha-synuclein degradation (Cullen *et al.*, 2009; McGlinchey and J. C. Lee, 2015). In sum, the LSD genes and variants implicated by our studies are excellent candidates for further replication, including resequencing and/or genotyping in the largest available Parkinson's disease case/control samples. Although we employed standard

1 quality control procedures for calling variants from WES and genotyping data, definitive
2 confirmation of specific variants will require additional studies.

3 There is a growing recognition of the importance of lysosomal biology in Parkinson's
4 disease pathogenesis (Moors *et al.*, 2016; Wong and Krainc, 2016). First, the lysosome is an
5 important route for alpha-synuclein degradation (Cuervo *et al.*, 2004; H. J. Lee, 2004; Vogiatzi
6 *et al.*, 2008). Genomic variants that elevate alpha-synuclein protein levels—such as rare locus
7 multiplication (Singleton *et al.*, 2003) or a common polymorphism that enhances promoter
8 activity (Soldner *et al.*, 2016)—also increase Parkinson's disease risk. Knockdown of selected
9 LSD genes, including *GBA* or *SCARB2*, in neuronal cells or in mouse models impairs alpha-
10 synuclein clearance (Cooper *et al.*, 2006; Rothaug *et al.*, 2014; Sardi *et al.*, 2011), whereas
11 increasing glucocerebrosidase activity has the opposite effect (Mazzulli, Zunke, Tsunemi, *et al.*,
12 2016; Migdalska-Richards *et al.*, 2016; Sardi *et al.*, 2011). Second, lysosomal autophagy plays a
13 critical role in mitochondrial quality control, and substantial evidence, including from genetics,
14 highlight mitochondrial dysfunction in Parkinson's disease (Haelterman *et al.*, 2014). Third,
15 there is accumulating evidence from numerous experimental models that alpha-synuclein
16 interferes with endoplasmic reticulum-to-Golgi vesicle trafficking, inducing reciprocal
17 disruptions in lysosomal biogenesis (Cooper *et al.*, 2006). Expression of alpha-synuclein
18 impeded trafficking of multiple hydrolases linked to LSDs, including *GBA*, within human
19 dopaminergic neurons (Mazzulli, Zunke, Isacson, *et al.*, 2016). In one recent study, subjects with
20 idiopathic Parkinson's disease, in which *GBA* carriers were excluded, were found to have modest
21 but significantly reduced glucocerebrosidase enzymatic activity based on peripheral blood testing
22 (Alcalay *et al.*, 2015). Fourth, besides *GBA* and the other genes implicated in our study,
23 mutations in *ATP13A2*, a rare cause of recessive juvenile-onset parkinsonism and dementia has

1 been independently implicated to cause the LSD Neuronal Ceroid Lipofuscinosis (Bras *et al.*,
2 2012). Lastly, many other common and rare Parkinson's disease risk alleles, including at
3 *RAB7L1*, *GAK*, *LRRK2*, and *VPS35* have strong functional links to vesicle trafficking, including
4 for lysosomal biogenesis and function. Together, these findings support a model in which partial
5 loss-of-function in genes regulating lysosomal activity, such as those that cause LSDs, may
6 increase vulnerability to alpha-synuclein-mediated mechanisms in Parkinson's disease.

7 While our analyses reveal a robust and replicable LSD variant burden in Parkinson's
8 disease cases, the overall magnitude of the difference between variant frequencies in cases and
9 controls appears modest (Figure 2). We speculate that this is probably an underestimate of the
10 true difference due to several assumptions. Specifically, only a subset of the 54 LSD genes and
11 760 nonsynonymous variants considered in our burden analyses are likely to be truly involved in
12 Parkinson's disease risk. Further, as noted above, while the CADD framework allowed us to
13 prioritize 596 variants as putative damaging alleles, larger Parkinson's disease exome datasets
14 with improved statistical power will be required to resolve the specific LSD genes and variants
15 that contribute to Parkinson's disease risk. Lastly, similar to *GBA* (Anheim *et al.* 2012; Rana *et*
16 *al.* 2013), we expect that many of the other LSD gene variants contributing to Parkinson's
17 disease risk may have individually modest and therefore incompletely penetrant effects, perhaps
18 modified by alleles at other loci (Cooper *et al.* 2013). In sum, the likely (i) incomplete
19 penetrance of many pathogenic variants along with (ii) contamination of our analyses by many
20 benign variants would be expected to inflate estimates for the LSD variant burden among
21 controls and attenuate the overall SKAT-O association.

22 Parkinson's disease heritability remains incompletely explained by the genes and variants
23 identified to date (Do *et al.*, 2011; Hamza and Paymi, 2010; Keller *et al.*, 2012; Verstraeten *et*

1 *al.*, 2015). Besides the likelihood of yet undiscovered loci, alternative explanations for familial
2 aggregation of disease include epigenetic changes due to shared environmental exposures or
3 even false positive diagnoses due to phenocopies (Mullin and Schapira 2015; Pihlstrom 2011). In
4 complex genetic disorders such as Parkinson's disease, the cumulative impact of common and
5 rare variants at multiple genomic loci, as well as non-additive interactions among alleles, likely
6 also play an important role (Cooper *et al.* 2013; Lupski *et al.* 2011). Polygenic modeling
7 approaches have previously demonstrated how common risk alleles can cumulatively impact
8 Parkinson's disease risk and age-of-onset (Escott-Price *et al.*, 2015; Nalls *et al.*, 2014). In
9 addition, a recently published analysis in the IPDGC WES and NeuroX cohorts identified
10 evidence for oligogenic interactions underlying Parkinson's disease risk, including alleles for
11 *GBA* and those for established Mendelian Parkinson's disease genes (Lubbe *et al.*, 2016). In the
12 IPDGC, WES reveals a substantial proportion of Parkinson's disease cases (21%) carrying two
13 or more likely damaging variants in *LSD* genes. Consistent with other reports (Clark *et al.*,
14 2015), our observation suggests the possibility that multiple *LSD* gene variants may interact in a
15 multi-hit, combinatorial manner to degrade lysosomal function, causing the accumulation of
16 alpha-synuclein and potentially other toxic substrates, and increasing susceptibility for
17 Parkinson's disease. Oligogenic interactions such as those proposed here may be an important
18 source for "missing heritability" in Parkinson's disease (Mullin and Schapira 2015; Pihlstrom
19 2011). Recent work has also implicated oligogenic inheritance in other neurologic disorders,
20 including amyotrophic lateral sclerosis (Cady *et al.*, 2015; Kenna *et al.*, 2013; van Blitterswijk *et*
21 *al.*, 2012) and idiopathic peripheral neuropathy (Gonzaga-Jauregui *et al.*, 2015), and further
22 reveals how pleiotropic genes causing early-onset, monogenic disorders may act in combination
23 to additionally trigger late-onset, complex genetic disorders (Cooper *et al.* 2013; Lupski *et al.*

2011). Future studies, including even-larger, case-control cohorts with WES and complementary experiments in Parkinson's disease cellular or animal models, are needed to further investigate whether a variant burden in LSD genes, perhaps in combination with other susceptibility loci, underlies oligogenic risk and contributes substantially to Parkinson's disease heritability.

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Supplementary Material

Supplementary material, including 2 Figures and 6 Tables, is available at Brain online.

Appendix

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Figure Legend

Figure 1 Overall analytic strategy. (Left) Variant categories. Because the number, frequency, and effect sizes of Parkinson's disease risk variants remains incompletely defined, our analyses considered three nested categories based on increasing variant pathogenicity: (1) all non-synonymous variants (Nonsyn), (2) likely damaging variants based on combined annotation dependent depletion (CADD) score, and (3) loss-of-function (LoF) variants. Based on the known prevalence of Parkinson's disease and incomplete penetrance documented for many risk alleles, we also considered 2 frequency thresholds, including rare ($MAF < 1\%$) and somewhat more common ($MAF < 3\%$) variants. (Right) Analysis Flowchart. The Sequence Kernel Association Test-Optimal (SKAT-O) was initially performed for the complete LSD gene set in the IPDGC discovery cohort, considering each variant category separately. For those categories with a significant SKAT-O association in the full gene set, a secondary analysis was performed excluding all *GBA* variants in order to confirm the involvement of additional genes. This was repeated in each of the replication cohorts (PPMI and NeuroX). Lastly, in order to highlight those loci driving associations detected in the gene set, secondary analyses were performed using SKAT-O to evaluate variants in each LSD gene independently.

Figure 2 Distribution of LSD variants in the IPDGC cohort. The number of likely damaging LSD variants ($MAF < 3\%$, $CADD\ C\text{-score} \geq 12.37$) per individual is shown versus the proportional representation in the IPDGC discovery cohort. Cases (Red) and Controls (Blue) are plotted separately. Many individuals harbor multiple LSD alleles, and the distribution is right-skewed among Parkinson's disease cases. The analysis considers variants in all 54 LSD genes. Supplemental Figure 2 shows a similar plot restricted to the 5 top driver genes.

1 Tables

2 **Table 1 LSD Genes and Variants in the IPDGC cohort**

Disease	Gene	Variants ^a
Aspartylglucosaminuria	<i>AGA</i>	13 (10)
Metachromatic Leukodystrophy	<i>ARSA</i>	5 (5)
Maroteaux-Lamy disease	<i>ARSB</i>	11 (10)
Farber Lipogranulomatosis	<i>ASAHI</i>	20 (17)
Kufor-Rakeb syndrome	<i>ATP13A2</i>	24 (18)
Neuronal Ceroid Lipofuscinosis (CLN3)	<i>CLN3</i>	18 (17)
Neuronal Ceroid Lipofuscinosis (CLN5)	<i>CLN5</i>	-
Neuronal Ceroid Lipofuscinosis (CLN6)	<i>CLN6</i>	10 (7)
Neuronal Ceroid Lipofuscinosis (CLN8)	<i>CLN8</i>	9 (4)
Cystinosis	<i>CTNS</i>	13 (12)
Galactosialidosis	<i>CTSA</i>	14 (11)
Neuronal Ceroid Lipofuscinosis (CLN10)	<i>CTSD</i>	7 (4)
Neuronal Ceroid Lipofuscinosis (CLN13)	<i>CTSF</i>	11 (9)
Pycnodysostosis	<i>CTSK</i>	6 (5)
Neuronal Ceroid Lipofuscinosis (CLN4B)	<i>DNAJC5</i>	5 (5)
Fucosidosis	<i>FUCA1</i>	15 (12)
Pompe disease	<i>GAA</i>	15 (10)
Krabbe disease	<i>GALC</i>	36 (30)
Morquio A disease	<i>GALNS</i>	22 (14)
Gaucher disease	<i>GBA</i>	39 (32)
Fabry disease	<i>GLA</i>	9 (7)
GM1-Gangliosidosis/Morquio B	<i>GLB1</i>	8 (4)
GM2-Gangliosidosis	<i>GM2A</i>	1 (1)
I-Cell disease	<i>GNPTAB</i>	39 (31)
Sanfilippo D syndrome	<i>GNS</i>	20 (11)
Neuronal Ceroid Lipofuscinosis (CLN11)	<i>GRN</i>	19 (12)
Sly disease	<i>GUSB</i>	17 (10)
Tay-Sachs disease	<i>HEXA</i>	20 (18)
Sandhoff disease	<i>HEXB</i>	8 (6)
Sanfilippo C syndrome	<i>HGSNAT</i>	18 (15)
Mucopolysaccharidosis Type IX	<i>HYAL1</i>	13 (9)
Hunter syndrome	<i>IDS</i>	9 (8)
Hurler syndrome	<i>IDUA</i>	8 (4)
Neuronal Ceroid Lipofuscinosis (CLN14)	<i>KCTD7</i>	4 (3)
Danon disease	<i>LAMP2</i>	9 (7)
Wolman disease	<i>LIPA</i>	14 (10)
Alpha-Mannosidosis	<i>MAN2B1</i>	12 (11)
Beta-Mannosidosis	<i>MANBA</i>	18 (15)
Mucopolidosis Type IV	<i>MCOLN1</i>	19 (14)
Neuronal Ceroid Lipofuscinosis (CLN7)	<i>MFSN8</i>	18 (14)
Schindler Disease/Kanzaki disease	<i>NAGA</i>	9 (8)
Sanfilippo B syndrome	<i>NAGLU</i>	10 (9)
Sialidosis	<i>NEU1</i>	-
Niemann-Pick Disease Type C1	<i>NPC1</i>	43 (35)
Niemann-Pick Disease Type C2	<i>NPC2</i>	2 (2)
Neuronal Ceroid Lipofuscinosis (CLN1)	<i>PPT1</i>	9 (7)
Sphingolipid-activator deficiency	<i>PSAP</i>	22 (16)
Action mycolonus-renal failure syndrome	<i>SCARB2</i>	10 (7)
Sanfilippo A syndrome	<i>SGSH</i>	10 (8)
Salla disease	<i>SLC17A5</i>	18 (17)
Niemann-Pick Disease Type A/B	<i>SMPD1</i>	25 (21)
GM3-Gangliosidosis	<i>ST3GAL5</i>	11 (11)
Multiple Sulfatase Deficiency	<i>SUMF1</i>	-
Neuronal Ceroid Lipofuscinosis (CLN2)	<i>TPP1</i>	15 (13)

3 ^aThe number of variants (MAF < 3%) in each LSD gene is shown for the IPDGC
4 discovery cohort, including total number of nonsynonymous variants and likely
5 damaging variants based on CADD (in parentheses). Of the 54 LSD genes
6 considered, no exonic variants in *CLN5* or *NEU1* passed quality control filters (see
7 Methods), and no nonsynonymous variants were identified in *SUMF1*.
8 LSD=Lysosomal storage disorder; CADD=Combined Annotation Dependent
9 Depletion.

Table 2 Analyses of LSD Variant Burden in Parkinson's disease

Cohort	Cases (n)	Controls (n)	Variants ^a	(a) MAF < 1%		(b) MAF < 3%	
				n ^b	<i>p</i> _{LSD} ^c	n	<i>p</i> _{LSD} (<i>p</i> -GBA) ^c
<i>Discovery</i>							
IPDGC	1,167	1,685	(1) nonsyn	746 (709)	0.056	760 (721)	0.014 (0.026)
			(2) CADD	585 (555)	0.066	596 (564)	0.0055 (0.0198)
			(3) LoF	69 (65)	0.464	- ^d	-
<i>Replication</i>							
PPMI	436	169	(1) nonsyn	243 (237)	0.096	256 (248)	0.320
			(2) CADD	179 (174)	0.294	187 (180)	0.281
NeuroX	6,713	5,964	(1) nonsyn	452 (443)	0.068	467 (456)	0.0004 (0.002)
			(2) CADD	338 (331)	0.057	348 (339)	0.0003 (0.020)

^aVariants were classified into nested categories (Figure 1A) based on two frequency thresholds, MAF < 1% (a) or 3% (b), and three functional filters, all nonsynonymous (1), CADD likely damaging (2), and LoF (3).

^bn=total number of LSD variant (number of variants excluding *GBA*). In parentheses, the number of variants excluding those in *GBA* are shown.

^cEmpirical SKAT-O *p*-values are based on 10,000 permutations following randomization of case/control status, and adjusted for multiple comparisons using the Bonferroni-Holm method (see Methods). As shown in Figure 1, primary analyses consider the variant burden among 54 LSD genes (*p*_{LSD}). For significant SKAT-O results, secondary analyses were performed excluding all variants in *GBA* (*p*-*GBA*). Unadjusted *p*-values are reported in Supplemental Table 5.

^dNo additional LoF variants met the relaxed frequency threshold (MAF < 3%).

LSD=lysosomal storage disorder; MAF=minor allele frequency; IPDGC=International Parkinson's Disease Genomics Consortium Discovery Cohort; PPMI= Parkinson's Progression Markers Initiative Replication Cohort; NeuroX = NeuroX exome array cohort; nonsyn=nonsynonymous variants; CADD=Combined Annotation Dependent Depletion; LoF= loss of function variants

Figures

Figure 1 Overall Analytic Strategy.

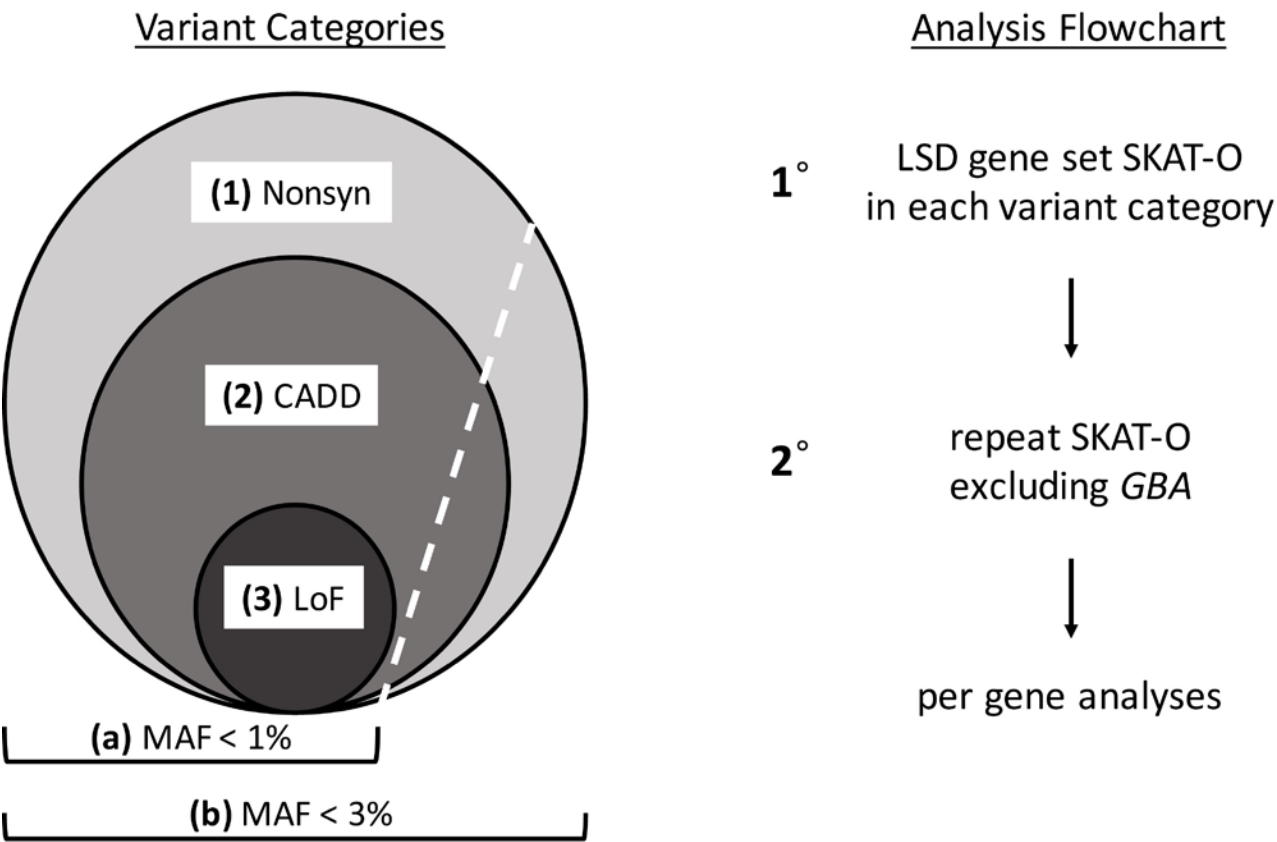


Figure 2 Distribution of LSD variants in the IPDGC cohort.

