Accurate long-read sequencing identified GBA variants as a major genetic risk factor in the Luxembourg Parkinson's study

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Abstract

Heterozygous variants in the glucocerebrosidase *GBA* gene are an increasingly recognized risk factor for Parkinson's disease (PD). Due to the pseudogene *GBAP1* that shares 96% sequence homology with the *GBA* coding region, accurate variant calling by array-based or short-read sequencing methods remains a major challenge in understanding the genetic landscape of *GBA*-related PD. We established a novel long-read sequencing technology for assessing the full length of the *GBA* gene. We used subsequent regression models for genotype-phenotype analyses. We sequenced 752 patients with parkinsonism and 806 healthy controls of the Luxembourg Parkinson's study. All *GBA* variants identified showed a 100% true positive rate by Sanger validation. We found 12% of unrelated PD patients carrying *GBA* variants. Three novel variants of unknown significance (VUS) were identified. Using a structure-based approach, we defined a potential risk prediction method for VUS. This study describes the full landscape of *GBA*-related parkinsonism in Luxembourg, showing a high prevalence of *GBA* variants as the major genetic risk for PD. Our approach provides an important advancement for highly accurate *GBA* variant calling, which is essential for providing access to emerging causative therapies for *GBA* carriers.

1. Introduction

Heterozygous variants in the glucocerebrosidase (*GBA*) gene, which encodes the enzyme β-glucocerebrosidase (GCase), are increasingly recognized as the most common genetic risk factor for the development of Parkinson's disease (PD). Homozygous mutations in *GBA* are causative for the most frequent autosomal-recessive lysosomal storage disorder, Gaucher disease (GD).[1] GD is characterized by a deficiency of the enzyme GCase which is necessary to hydrolyse the β-glucosyl linkage of glucosylceramide lipide (GlcCer) in lysosomes, to yield glucose and ceramide.[2]

The accurate variant calling in the *GBA* gene is challenging due to the presence of the highly homogeneous untranslated pseudogene called *GBAP1*, which is located 16 kilobases (kbp) downstream,[3] and shares 96% sequence homology within the coding region.[4] Furthermore, recombination and structural chromosomal variations within and around the *GBA* locus make the analysis more challenging.[5] Complex alleles, which include several point mutations, are derived from a recombination between functional *GBA* and pseudogene *GBAP1*.[6] RecNciI is the most prevalent recombinant allele, including the amino acid changes p.L483P and p.A495P, and the synonymous variant p.V499V.[6]

Our study aimed at accurately assessing all coding variants in the *GBA* gene among all participants of the Luxembourg Parkinson's study,[7] a case and control cohort including people with PD and atypical parkinsonism. To assess the accuracy of the novel targeted *GBA* sequencing method using Pacific Biosciences (PacBio)[8] technology, we compared this method to genotyping with the NeuroChip array[9] and short-read whole genome sequencing (WGS) data using Sanger sequencing as the gold standard for validation. We identified several types of pathogenic *GBA* variants (severe, mild, and risk) and further characterized genotype-phenotype associations to better understand the influence of each variant type and their effect on disease severity.

2. Results

2.1. Demographic and clinical characteristics

A total of 752 patients (652 PD patients and 100 patients with other forms of parkinsonism) and 806 HC were included (Figure 1). All participants were genotyped using NeuroChip and screened for *GBA* variants using targeted PacBio method, while a subset of 72 patients was screened with WGS. Among the patients, 66.4% (n = 499) were male with a mean age at disease onset of 63.1 ± 16 years. The control group consisted of 52.9% (n = 426) males with a mean age at assessment of 59.3 ± 12.2 years (Supplementary Table 1).

2.2. Targeted PacBio sequencing showed the highest specificity to detect *GBA* variants

To measure the reliability of *GBA* variant detection, we proceeded with two types of comparison. We compared PacBio, WGS and NeuroChip methods for a subset of samples (n=72). Then, we compared the PacBio and NeuroChip methods since they both covered most of the samples (n=1558). We considered as true positives only variants validated via Sanger sequencing (Supplementary Table 2).

First, we evaluated 72 samples screened by all three methods (Figure 2). Using the GBA-targeted PacBio method, we detected six individuals carrying *GBA* variants (p.E365K (n = 3), p.T408M (n = 1), p.N409S (n = 1), RecNciI , n = 1)). All the detected variants were confirmed by Sanger sequencing (true positive rate (TPR) of 100%). With the WGS method, we did not identify any false positive variant call, however, the WGS method failed to detect the RecNciI recombinant allele in one individual (TPR of 83.3% (5/6). Using Neurochip, we detected three potential *GBA* variants carriers (p.T408M (n = 1), p.N431S (n = 1), p.A215D (n=1), however, only one variant (p.T408M) was subsequently confirmed by Sanger sequencing (TPR of 16.6% (1/6) translating into a false detection rate (FDR) of 66.6% (2/3)). Next, we compared the results from 1558 samples screened with both, the *GBA* targeted PacBio method and the NeuroChip array (Figure 3). Using the GBA-targeted PacBio method, we detected 133 *GBA* variants carriers, of which 100% were validated by Sanger sequencing. Through the NeuroChip array, we detected 47 potential *GBA* variant carriers, among which only 36 were validated by Sanger sequencing (TPR of 27% (36/133), resulting in an FDR of 23.4% (11/47)).

2.3. Classification of *GBA* variants

From the 1558 individuals sequenced with the GBA-targeted PacBio method, we identified 124 carriers with at least one *GBA* variant (Supplementary Table 3). *GBA* variants were mostly heterozygous missense, one patient carried a heterozygous stop-gain variant

p.R398*(rs121908309), two PD patients carried a homozygous missense variant p.E365K/p.E365K(rs2230288). We also detected nine different synonymous variants in exonic regions (Supplementary Table 4). The variant p.T408T(rs138498426) is a splice site variant (located within 2bp of the exon boundary) and classified as VUS.[10] The remaining synonymous variants were not further analysed. Additionally, we identified 69 variants in intronic and UTRs regions (Supplementary Table 5) with unclear pathogenic relevance, of which 35 were rare. Based on Neurochip and WGS data, none of the *GBA* carriers carried pathogenic mutations in other PD associated genes as defined by MDSGene.[11]

We classified four combinations of multiple variants per individual as severe (p.N409S-p.L483P; the recombinant allele RecNciI; p.K13R-p.L483P; p.F252I-p.T408M) and one combination of variants as risk (Y61H-T408M) based on the classification of the respective associated pathogenic variants (Table 1).

Overall, we detected 12% (77/644) *GBA* variant carriers among 644 unrelated PD patients and 5% (34/678) in healthy control individuals. We found a frequency of 10.4% (67/644) known pathogenic mutations in PD patients and 4.3% (29/678) in the control group (Table 2). Carriers of severe *GBA* mutations (n=21; OR=11.4; 95% CI=[2.6, 48.8]; p=0.0010) and risk *GBA* variants (n=39; OR=1.6; 95% CI=[1, 2.8]; p=0.0470) had a different risk of developing PD as defined by the indicated OR.

The most common *GBA* variants in PD patients were the risk variants p.E365K (n=23;3.5%) and p.T408M (n=17;2.6%).

2.4. Genotype-phenotype associations in GBA-PD patients

We characterized the clinical phenotype of severe, mild and risk *GBA* carriers and non-carriers only in unrelated PD patients excluding carriers with only one synonymous or VUS variants. The AAO was similar between *GBA* carriers (61.6±11.5) and non-carriers (62.5±11). Severe PD_{GBA} mutations carriers showed a trend towards younger AAO compared to mild and risk (severe: 58.6±13.1 vs mild: 65.4±17 vs risk: 62.5±0.3 years; p=0.29) (Table 3), with a significant risk to develop early onset PD (OR=3.76;p=0.0135).

We compared clinical features between PD patients carrying pathogenic *GBA* variants and PD patients without *GBA* variants. We found that the sense of smell was strongly impaired in carriers (uncorrected p=0.0198) (Supplementary Table 6). Next, we compared patients carrying variants from each category (severe, mild or risk) separately with PD patients without *GBA* variants (Table 4). Carriers of severe *GBA* mutation showed more severe non-motor symptoms when compared to non-GBA carriers, such as MDS-UPDRS Part I (uncorrected p=0.0088) and

hallucinations (uncorrected p=0.015), and also an impaired sense of smell as assessed by Sniffin' Stick test (uncorrected p=0.0403). To show the deleterious impact of the severe variants, we compared carriers of severe variants with patients carrying either mild or risk *GBA* variants (Table 5). We observed here that severe variants carriers have more severe gait disorder and depression and worse MDS-UPDRS Part I and PDQ-39. For all clinical features, there were no significant associations after the correction for multiple comparisons using FDR adjustment.

2.5. VUS and the Glucosylceramidase structure

We detected nine already reported VUS (p.K13R, p.Y61H, p.R78C, p.L213P, p.E427K, p.A495P, p.H529R, p.R534C, p.T408T) and three new VUS (p.A97G, p.A215 and p.R434C). According to our strategy developed for VUS *GBA* variants classification, where we assign the pathogenicity based on the REVEL, the CADD, the dbscSNV scores, as well as whether the patients carrying the variants. We suggest to sub-classify the variants p.Y61H, p.L213P, p.A215D, and p.R434C as severe variants. The variant p.L213P changes the Leucine amino acid into proline, which is known to be the 'helix breaker' amino acid that can induce a bend into the protein structure[12](Supplementary Figure 1). The p.L213P and p.A215D variants are in the catalytic site of the enzyme in the triose-phosphate isomerase (TIM) barrel structure. The p.Y61H variant (Figure 4.A) is next in sequence and in structure to the known severe PD variant p.C62W and the patient carrying this variant had an AAO of 38 years, indicating an early-onset likely severe form of PD. The p. R434C variant is close to a known severe (p.V433L) and mild (p.W432R, p.N435T) PD variants in the 3D structure. We propose to sub-classify the variants p.H529R and p.R534C as mild, as they are both found only in PD patients. The variants p.K13R, p.R78C, p.E427K, and p.A495P are sub-classified as risk variants. The variant p.K13R is located in the signal peptide region. The variant p.R78C was annotated as "PD susceptibility" in HGMD with deleterious impact in CADD. The variant p.E427K was annotated as linked to "parkinsonism" in ClinVar and "reduced activity" in HGMD. We suggest to classify the variant p.A97G as probably benign because it is localized in a coil-bend structure and is not close to any known pathogenic variants.

The synonymous variant p.T408T was found in two cases and one healthy control individual. Two established splice-site prediction scores (dbscSNV: ada_score 0.9797 and rf_score 0.85) agreed in their prediction that the variant is likely to affect splicing. HGMD classified the variant as disease mutation (DM) (Supplementary Table 4). Therefore, we propose to classify the variant as a risk variant.

In total, we propose to classify four VUS variants as severe (p.Y61H, p.L213P, p.A215D, and p.R434C), two as mild (p.H529R and p.R534C), five as risk (p.K13R, p.R78C, p.E427K, p.A495P and p.T408T) and one as benign (p.A97G) (Figure 4.B).

3. Discussion

Our study showed, for the first time, the utility of targeted PacBio sequencing as a highly sensitive and specific method to identify known and novel *GBA* variants. The PacBio method demonstrated a very high efficiency by targeting the entire length of the *GBA* gene with 100% reliability and solves the problems arising from the presence of the *GBAP1* pseudogene. The effectiveness of the target PacBio method to investigate relevant genes with homologous pseudogenes has also been proven in several other studies.[12–15] The comparative study that we conducted with the different screening methods for *GBA* mutations will help researchers to be more accurate and comprehensive implying a more critical appraisal of the results obtained by NeuroChip and WGS with more false positive and false negative results.

GBA mutations were identified as the most common genetic risk factor for the development of PD. A heterozygous *GBA* variant was typically observed in 4%–12% of PD patients in different populations worldwide, with the highest prevalence of 20% described in Ashkenazi Jewish PD patients. [16,17] Important variation is due to ethnicity, the investigated mutations and the sequencing method used. Our study describes the landscape of *GBA* carriers in the studied Luxembourgish population showing the high prevalence of *GBA* mutations that could be the major genetic risk factor of PD in Luxembourg. The frequency of GBA mutation in PD in our study was 12% and we observed a significantly higher proportion of pathogenic (severe, mild and risk) *GBA* variants in PD patients compared to HC (10.4% *vs* 4.3%;OR=2.6;CI=[1.6,4.1],p=0.0001). Compared to previous studies, our study highlights that using the new PacBio sequencing method, the Luxembourg Parkinson's study cohort showed a comparable frequency of PD_{GBA} carriers reported so far in similarly sized Italian[18] and Spanish[19] cohorts (Supplementary Table 7) . When comparing previous reports of *GBA* variants in different populations, we want to highlight the fact that only cohorts that used full Sanger sequencing were able to detect the RecNciI recombinant allele so far. This once more emphasizes the accuracy of the PacBio sequencing methods for detecting rare and complex *GBA* variants. Additionally, we confirmed that severe variants showed a higher OR than risk variants, which supports the concept of graded risk for different *GBA* variants in PD_{GBA} carriers. [20]

The most prevalent *GBA* variant in the Luxembourg Parkinson's study cohort was p. E365K, and the frequency of this variant was similar to what was described in the Irish[20], Spanish[19] and New Zealand[5] populations. It is interesting to note that homozygous carriers of the p.E326K variant do not develop GD.[21] The variant is associated with PD, and multiple studies have found enrichments varying from 1.60 to 3.34.[22–24]Furthermore, carriers of the risk variants p.E365K and p.T408M were associated with atypical parkinsonism, as these variants were the only ones also present in patients with DLB and PSP in our cohort. Whether this is simply related to the higher frequency of these risk variants in the general population or does have a specific impact on the phenotype needs to be determined in larger studies focusing on *GBA* variants in atypical parkinsonism[25].

We present a concept for classifying VUS in the *GBA* gene according to the localisation in relation to known variants in sequence and 3D structure, which may help to provide access to future targeted therapies for these patients. Here additional *in vitro* and *ex vivo* studies are needed to functionally validate the impact of these VUS on GCase function in neurons derived from stem cells or in enzyme-activity assays in CSF of affected carriers of these VUS.

Additionally, we observed that the average AAO in PD was about four years younger in severe *GBA* carriers compared to non-GBA carriers. This was also observed in previous studies, which showed that PD_{GBA} patients generally have an earlier AAO compared to non-carriers with a median onset in the early fifties.[26,27]

Recent studies have shown that PD_{GBA} carriers have a higher prevalence of cognitive impairment[18,28,29] and non-motor symptoms including neuropsychiatric disturbances[18,19], autonomic dysfunction[28], and sleep disturbances such as RBD[30]. Although not significant after *p*-value adjustment, we found a similar trend and noticed that motor symptoms such as gait disorder, non-motor such as depression and hallucinations symptoms were associated with a more aggressive clinical phenotype in severe *GBA* carriers, supporting the effect of differential *GBA* variant severity.[19,31]

In conclusion, this study showed the utility of targeted PacBio sequencing to identify known and novel *GBA* variants with high accuracy. These findings offer important access to variant-specific counselling. Furthermore, our study describes the full landscape of *GBA* related PD in the current Luxembourgish population showing the high prevalence of *GBA* variants as the major genetic risk in PD.

4. Methods

4.1. Clinical Cohort

At the time of analysis, the Luxembourg Parkinson's study comprised 1558 participants (752 patients of parkinsonism and 806 healthy controls (HC) in the frame of the National Centre for Excellence in Research on Parkinson's disease program (NCER-PD).

All patients complied with the diagnostic criteria of typical PD or atypical parkinsonism as assessed by neurological examination following the United Kingdom Parkinson's Disease Society Brain Bank (UKPDSBB) diagnostic criteria[32]: 652 fulfilled the criteria for PD, 60 for progressive supranuclear palsy (PSP) including corticobasal syndrome as a subtype of PSP (PSP-CBS), 25 for Dementia with Lewy Body (DLB), 14 for Multiple System Atrophy (MSA), and one for Fronto-temporal dementia with parkinsonism (FTDP). All patients and HC underwent a comprehensive clinical assessment of motor and non-motor symptoms, neuropsychological profile and medical history along with comorbidities. The clinical symptoms assessed, and scales applied are defined in the Supplemental Information[33]. All individuals provided written informed consent. The patients were reassessed at regular follow-up visits. We considered early-onset PD patients those with age at onset (AAO) equal to or younger than 45 years[34]. The genotype-phenotype analysis was based on the assessment of the first visit. The study has been approved by the National Research Ethics Committee (CNER Ref: 201407/13).

4.2. Genetic analysis

4.2.1. NeuroChip array

Genotyping was carried out on the InfiniumR NeuroChip Consortium Array[9] (v.1.0 and v1.1; Illumina, San Diego, CA USA). For rare variants analysis, standard quality control (QC) procedures were conducted, using PLINK v1.9[35], to remove variants if they had a low genotyping rate (<95%) and Hardy-Weinberg equilibrium p-value < $1x10^{-6}$. As an additional quality filter, we applied a study-wide allele frequency threshold of <1% in our cohort for rare variants.

4.2.2. GBA-targeted PacBio long-read amplicon sequencing

The targeted *GBA* gene screening was performed by single-molecule real-time (SMRT) long read sequencing[8] using Sequel II instrument (PacBio). The targeted *GBA* gene coordinates were chr1:155,232,501-155,241,415 (USCS GRCh38/hg38). Long-distance PCR was performed using GBA-specific primer sequences (Forward: 5'-GCTCCTAAAGTTGTCACCCATACATG-3' and Reverse: 5'-CCAACCTTTCTTCCTTCTTCTCAA-3')[36] and the 2x KAPA HiFi Hot Start ReadyMix

(Roche). For sample multiplexing, dual asymmetric barcoding was used based on a different 16-bp long index sequence upstream of each of the reverse and forward primers to allow the generation of uniquely barcoded amplicons in one-step PCR amplification. QC was performed prior to pooling. Pools of amplicons were purified with AMPure PacBio beads. A total of 1700 ng of purified amplicon pool was used as input for the SMRTbell library using the SMRTbell Express Template Prep Kit 2.0 (PacBio). Binding of the polymerase and diffusion loading on SMRTCell 8M was prepared according to SMRTLink instructions with CCS reads as sequencing mode (version SMRT Link: 9.0.0.92188). We generated high-quality consensus reads using the PacBio Sequel II sequencer on Circular Consensus Sequencing mode using the pbccs (v6.0.0) tool. The methods replicates both strands of the target DNA.[37] We demultiplexed and mapped reads from each sample to the human reference genome GRCh38 using minimap2[38] from the pbmm2 package (v1.4.0) (https://github.com/PacificBiosciences/pbmm2). For variant calling, we used the DeepVariant[39] (1.0) with models optimized for CCS reads. Finally, we selected variants with quality above 30 (QUAL>30).

4.2.3. Whole genome sequencing

The TruSeq Nano DNA Library Prep Kit (Illumina, San Diego, CA, USA) and MGIEasy FS DNA Prep kit (BGI, China) were used according to the manufacturer's instructions to construct the WGS library. Paired-end sequencing was performed with the Illumina NovaSeq 6000[40] and on the MGI G400 sequencers. A QC of the raw data was performed using FastQC (version 0.11.9).[41] To call the variants, we used the Bio-IT Illumina Dynamic Read Analysis for GENomics (DRAGEN) DNA pipeline[42] v3.8[43] with standard parameters. To select the high-quality variants, we annotated and selected variants using VariantAnnotator and SelectVariants modules of the Genome Analysis Toolkit (GATK 4)[44] pipeline and applied the following additional filtering steps: VariantFiltration module for SNVs (QD<2, FS>60, MQ<40, MQRankSum<−12, ReadPosRankSum<−8, DP<10.0, QUAL<30, VQSLOD<0, ABHet>0.75 or <0.25, SOR>3 and LOD<0), and insertions-deletions (QD<2, FS>200, QUAL<30, ReadPosRankSum<−20, DP<10 and GQ_MEAN<20).

4.3. Variant annotation and validation

Variant annotation was done with ANNOVAR,[45] using the Genome Aggregation Database (gnomAD r2.1)[46], the Human Gene Mutation Database (HGMD)[47] and ClinVar[48], and the Combined Annotation Dependent Depletion (CADD)[49] and REVEL[50] to score the

pathogenicity of missense variants.[51] For variants in splice sites, we used the ada_score and rf_score from dbscSNV (version 1.1)[52]. Ada_score ≥ 0.6 or rf_score ≥ 0.6 indicate that the variant is likely to affect splicing.

Rare variants were selected according to minor allele frequency < 1% in gnomAD for the Non-Finnish European (NFE) population in the 'non-neuro' gnomAD subset. Then, exonic and splicing variants (+/- 2bp from the exon boundary) were selected for autosomal dominant (*LRRK2*, *SNCA*, *VPS35*, *GBA*) and autosomal recessive (*PRKN*, *PINK1*, *PARK7*, *ATP13A2*) PD genes. Rare variants within these genes were then confirmed by Sanger sequencing.[53]

4.4. *GBA* variant nomenclature

All variants in *GBA* were annotated based on GRCh37 and were numbered according to the current variant nomenclature guidelines (http://varnomen.hgvs.org), based on the primary translation product (NM_001005742), which includes the 39-residue signal peptide.

4.5. *GBA* variant classification

GBA variants classification was done according to the PD literature based on the work of Höglinger and colleagues in 2022.[10] Exonic or splice-site variants that are not mentioned in the paper were subclassified as 'severe' *GBA* variants if there were annotated as pathogenic in ClinVar, otherwise they were subclassified as variant with unknown significance (VUS).[55]

4.6 Statistical analysis

To assess the frequency of different *GBA* variant types and analyse the genotype-phenotype associations in the Luxembourg Parkinson's Study, we considered only unrelated individuals and retained only one proband per family. For cases, we kept the patient with the earliest AAO. We excluded HC with first-degree PD relatives (parents, sibs, and offspring) and age at assessment (AAA) less than 60 years, to account for age-dependent penetrance. Therewith, reduce the gap of age between cases and HC. Thus, 1420 unrelated individuals (742 patients and 678 HC) were selected for the statistical analysis.

We used regression models to assess the effect of PD_{GBA} carrier status on the clinical variables. We excluded individuals carrying only VUS or synonymous variants. To this aim, we performed three types of association tests: (1) all PD_{GBA} pathogenic variant carriers (severe, mild and risk) *vs* $PD_{GBA\text{-}non\text{-}carriers}$, (2) for each sub-group of PD_{GBA} pathogenic variant carriers *vs* $PD_{GBA\text{-}non\text{-}carriers}$, (3) severe PD_{GBA} pathogenic variant carriers *vs* combined mild and risk PD_{GBA} pathogenic variant carriers. The effect of each factor was expressed as the Beta (β)

regression coefficient. The odds ratio (OR) along with a 95% confidence interval (CI) was used to assess whether a particular exposure is a risk factor for a particular outcome. Regression models were adjusted for AAA, sex, and disease duration. FDR adjusted p-value < 0.05 was considered as statistically significant.

4.7 Structure-based evaluation of VUS

To evaluate VUS variants, we implemented a method to assign the pathogenicity based on the REVEL and CADD scores for missense variants and the dbscSNV scores (ada_score and rf_score) for splice variants according to dbNFSP definition[54], as well as whether the patients carrying the variants.We reclassified a VUS (1) as 'severe' if the variant was present only in patients and with deleterious effect in all scores or present only in patients with early onset PD, (2) as 'mild' if the variant was present only in patients and with tolerated effect in all scores, (3) as ' risk' if present in patients and HCs or with tolerated and deleterious effect in either score or annotated as 'PD susceptibility' in HGMD, and (4) as 'benign' if present only in HC. We mapped the known pathogenic missense variants and newly identified VUS identified in our cohort together with all reported population variants from gnomAD onto the *GBA* protein sequence and the 3D structure. We used an X-ray structure of GCase at 2.0 Å resolution (PDB structure accession code 1ogs; https://www.rcsb.org/) (Supplementary Figure 2). The analysis of the 3D structure was carried out by PyMOL (http://www.pymol.org). VUS were evaluated as risk variant if they were 2bp positions away in sequence or had a C-alpha distance of less than 5 ångström in 3D from another known pathogenic variant similar to the approach used in Johannesen et al.[55]

5. Data availability

The dataset for this manuscript is not publicly available as it is linked to the Luxembourg Parkinson's Study and its internal regulations. Any requests for accessing the dataset can be directed to request.ncer-pd@uni.lu.

6. References

1. Hruska KS, LaMarca ME, Scott CR, Sidransky E. Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). *Hum Mutat*. 2008;29(5):567-583. doi:10.1002/HUMU.20676
2. Arturo-Terranova D, Giraldo LJM, Satizábal JM. Frequency of gba gene variants in complex disease patients in Southwestern Colombia. *Genetics and Molecular Research*. 2021;20(2). doi:10.4238/gmr18818
3. Horowitz M, Wilder S, Horowitz Z, Reiner O, Gelbart T, Beutler E. The human glucocerebrosidase gene and pseudogene: structure and evolution. *Genomics*. 1989;4(1):87-96. doi:10.1016/0888-7543(89)90319-4
4. Do J, McKinney C, Sharma P, Sidransky E. Glucocerebrosidase and its relevance to Parkinson disease. *Mol Neurodegener*. 2019;14(1). doi:10.1186/s13024-019-0336-2
5. Graham OEE, Pitcher TL, Liau Y, et al. Nanopore sequencing of the glucocerebrosidase (GBA) gene in a New Zealand Parkinson's disease cohort. *Parkinsonism Relat Disord*. 2020;70:36-41. doi:10.1016/j.parkreldis.2019.11.022
6. Zimran A, Horowitz M. *RecTL: A Complex Allele of the Glucocerebrosidase Gene Associated With a Mild Clinical Course of Gaucher Disease*.; 1994.
7. Hipp G, Vaillant M, Diederich NJ, et al. The Luxembourg Parkinson's Study: A Comprehensive Approach for Stratification and Early Diagnosis. *Front Aging Neurosci*. 2018;10. doi:10.3389/FNAGI.2018.00326
8. Korlach J, Bjornson KP, Chaudhuri BP, et al. Real-time DNA sequencing from single polymerase molecules. *Methods Enzymol*. 2010;472:431-455. doi:10.1016/S0076-6879(10)72001-2
9. Blauwendraat C, Faghri F, Pihlstrom L, et al. NeuroChip, an updated version of the NeuroX genotyping platform to rapidly screen for variants associated with neurological diseases. *Neurobiol Aging*. 2017;57:247.e9-247.e13. doi:10.1016/J.NEUROBIOLAGING.2017.05.009
10. Höglinger G, Schulte C, Jost WH, et al. GBA-associated PD: chances and obstacles for targeted treatment strategies. *J Neural Transm*. Published online May 31, 2022. doi:10.1007/s00702-022-02511-7
11. Lill CM, Mashychev A, Hartmann C, et al. Launching the movement disorders society genetic mutation database (MDSGene). *Mov Disord*. 2016;31(5):607-609. doi:10.1002/MDS.26651
12. Qiao W, Yang Y, Sebra R, et al. Long-Read Single Molecule Real-Time Full Gene Sequencing of Cytochrome P450-2D6. *Hum Mutat*. 2016;37(3):315-323. doi:10.1002/HUMU.22936
13. Buermans HPJ, Vossen RHAM, Anvar SY, et al. Flexible and Scalable Full-Length CYP2D6 Long Amplicon PacBio Sequencing. *Hum Mutat*. 2017;38(3):310-316. doi:10.1002/HUMU.23166
14. Borràs DM, Vossen RHAM, Liem M, et al. Detecting PKD1 variants in polycystic kidney disease patients by single-molecule long-read sequencing. *Hum Mutat*. 2017;38(7):870-879. doi:10.1002/HUMU.23223
15. Frans G, Meert W, van der Werff Ten Bosch J, et al. Conventional and Single-Molecule Targeted Sequencing Method for Specific Variant Detection in IKBKG while Bypassing the IKBKGP1 Pseudogene. *J Mol Diagn*. 2018;20(2):195-202. doi:10.1016/J.JMOLDX.2017.10.005

16. Ruskey JA, Greenbaum L, Roncière L, et al. Increased yield of full GBA sequencing in Ashkenazi Jews with Parkinson's disease. *Eur J Med Genet*. 2019;62(1). doi:10.1016/j.ejmg.2018.05.005
17. Gan-Or Z, Amshalom I, Kilarski LL, et al. *Differential Effects of Severe vs Mild GBA Mutations on Parkinson Disease*.; 2015.
18. Petrucci S, Ginevrino M, Trezzi I, et al. GBA-Related Parkinson's Disease: Dissection of Genotype–Phenotype Correlates in a Large Italian Cohort. *Movement Disorders*. 2020;35(11). doi:10.1002/mds.28195
19. Jesús S, Huertas I, Bernal-Bernal I, et al. GBA variants influence motor and non-motor features of Parkinson's disease. *PLoS One*. 2016;11(12). doi:10.1371/journal.pone.0167749
20. Olszewska DA, McCarthy A, Soto-Beasley AI, et al. Association Between Glucocerebrosidase Mutations and Parkinson's Disease in Ireland. *Front Neurol*. 2020;11. doi:10.3389/fneur.2020.00527
21. Duran R, Mencacci NE, Angeli A V., et al. The glucocerobrosidase E326K variant predisposes to Parkinson's disease, but does not cause Gaucher's disease. *Movement Disorders*. 2013;28(2):232-236. doi:10.1002/mds.25248
22. Ran C, Brodin L, Forsgren L, et al. Strong association between glucocerebrosidase mutations and Parkinson's disease in Sweden. *Neurobiol Aging*. 2016;45. doi:10.1016/j.neurobiolaging.2016.04.022
23. Davis MY, Johnson CO, Leverenz JB, et al. Association of GBA mutations and the E326K polymorphism with motor and cognitive progression in parkinson disease. *JAMA Neurol*. 2016;73(10):1217-1224. doi:10.1001/jamaneurol.2016.2245
24. Berge-Seidl V, Pihlstrøm L, Maple-Grødem J, et al. The GBA variant E326K is associated with Parkinson's disease and explains a genome-wide association signal. *Neurosci Lett*. 2017;658. doi:10.1016/j.neulet.2017.08.040
25. Picillo M, Petrucci S, Valente EM, et al. Progressive Supranuclear Palsy–Like Phenotype in a GBA E326K Mutation Carrier. *Mov Disord Clin Pract*. 2017;4(3):444-446. doi:10.1002/mdc3.12406
26. Blauwendraat C, Heilbron K, Vallerga CL, et al. Parkinson's disease age at onset genome-wide association study: Defining heritability, genetic loci, and α-synuclein mechanisms. *Mov Disord*. 2019;34(6):866-875. doi:10.1002/MDS.27659
27. Sidransky E, Nalls MA, Aasly JO, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med*. 2009;361(17):1651-1661. doi:10.1056/NEJMOA0901281
28. Brockmann K, Srulijes K, Hauser AK, et al. *GBA-Associated PD Presents with Nonmotor Characteristics*.; 2011.
29. Setó-Salvia N, Pagonabarraga J, Houlden H, et al. Glucocerebrosidase mutations confer a greater risk of dementia during Parkinson's disease course. *Movement Disorders*. 2012;27(3). doi:10.1002/mds.24045
30. Krohn L, Ruskey JA, Rudakou U, et al. GBA variants in REM sleep behavior disorder: a multicenter study. doi:10.1101/19010991
31. Brockmann K, Quadalti C, Lerche S, et al. Association between CSF alpha-synuclein seeding activity and genetic status in Parkinson's disease and dementia with Lewy bodies. *Acta Neuropathol Commun*. 2021;9(1). doi:10.1186/S40478-021-01276-6

32. Litvan I, Bhatia KP, Burn DJ, et al. SIC task force appraisal of clinical diagnostic criteria for parkinsonian disorders. *Movement Disorders*. 2003;18(5). doi:10.1002/mds.10459
33. Pavelka L, Rauschenberger A, Landoulsi Z, et al. ARTICLE Age at onset as stratifier in idiopathic Parkinson's disease-effect of ageing and polygenic risk score on clinical phenotypes. doi:10.1038/s41531-022-00342-7
34. Gustavsson EK, Trinh J, Mckenzie M, et al. Genetic Identification in Early Onset Parkinsonism among Norwegian Patients. Published online 2017. doi:10.1002/mdc3.12501
35. Purcell S, Neale B, Todd-Brown K, et al. PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81(3):559-575. doi:10.1086/519795
36. Leija-Salazar M, Sedlazeck FJ, Toffoli M, et al. Evaluation of the detection of GBA missense mutations and other variants using the Oxford Nanopore MinION. *Mol Genet Genomic Med*. 2019;7(3). doi:10.1002/mgg3.564
37. Rhoads A, Au KF. PacBio Sequencing and Its Applications. *Genomics Proteomics Bioinformatics*. 2015;13(5). doi:10.1016/j.gpb.2015.08.002
38. Li H. Minimap2: Pairwise alignment for nucleotide sequences. *Bioinformatics*. 2018;34(18):3094-3100. doi:10.1093/bioinformatics/bty191
39. Poplin R, Chang PC, Alexander D, et al. A universal snp and small-indel variant caller using deep neural networks. *Nat Biotechnol*. 2018;36(10):983. doi:10.1038/nbt.4235
40. Illumina. NovaSeq 6000 Sequencing System. *770-2016-025-H*. 2016;4(February).
41. Andrews S. FastQC. *Babraham Bioinformatics*. Published online 2010.
42. Miller NA, Farrow EG, Gibson M, et al. A 26-hour system of highly sensitive whole genome sequencing for emergency management of genetic diseases. *Genome Med*. 2015;7(1). doi:10.1186/s13073-015-0221-8
43. Illumina. Illumina DRAGEN Bio-IT Platorm. *User Guide*. 2019;(February).
44. Mark AD, Eric B, Ryan P, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Published online 2011. doi:10.1038/ng.806
45. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38(16). doi:10.1093/NAR/GKQ603
46. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans , Genome Aggregation Database Consortium. *434 | Nature |*. 2020;581:19. doi:10.1038/s41586-020-2308-7
47. Stenson PD, Ball E v, Mort M, et al. Human Gene Mutation Database (HGMD s): 2003 Update. *Hum Mutat*. 2003;21:577-581. doi:10.1002/humu.10212
48. Landrum MJ, Lee JM, Riley GR, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. doi:10.1093/nar/gkt1113
49. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res*. 2019;47. doi:10.1093/nar/gky1016
50. Tian Y, Pesaran T, Chamberlin A, et al. REVEL and BayesDel outperform other in silico meta-predictors for clinical variant classification. *Sci Rep*. 2019;9(1). doi:10.1038/s41598-019-49224-8

51. Ioannidis NM, Rothstein JH, Pejaver V, et al. REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. *Am J Hum Genet*. 2016;99(4):877-885. doi:10.1016/J.AJHG.2016.08.016
52. Jian X, Boerwinkle E, Liu X. In silico prediction of splice-altering single nucleotide variants in the human genome. *Nucleic Acids Res*. 2014;42(22):13534-13544. doi:10.1093/nar/gku1206
53. Sanger F, Nicklen S, Coulson AR. *DNA Sequencing with Chain-Terminating Inhibitors (DNA Polymerase/Nucleotide Sequences/Bacteriophage 4X174)*. Vol 74.; 1977. https://www.pnas.org
54. Liu X, Li C, Mou C, Dong Y, Tu Y. dbNSFP v4: a comprehensive database of transcript-specific functional predictions and annotations for human nonsynonymous and splice-site SNVs. *Genome Med*. 2020;12(1):1-8. doi:10.1186/S13073-020-00803-9/FIGURES/4
55. Johannesen KM, Liu Y, Koko M, et al. Genotype-phenotype correlations in SCN8A-related disorders reveal prognostic and therapeutic implications. *Brain*. 2022;145(9):2991-3009. doi:10.1093/BRAIN/AWAB321

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5. Data collection: A. Participation, B. Exportation, C. Curation

SP: 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B, 4B,5B, 5C
ZL: 1C, 2A , 2B, 2C,3A, 3B, 5C
LP: 1C, 2C, 3B, 5A, 5B, 5C
CS: 2C, 3B, 4A
EBA: 2C, 3B, 4A
CG: 2C, 3B, 4A
AKH: 2C, 3B, 4A
DRB: 2C, 3B
NC: 3B, 4A
PM: 1A, 1B, 1C, 2A, 2C, 3B, 4B, 5C
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The authors declare that there are no conflicts of interest relevant to this work.

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11. Tables

Table 1. Distribution of *GBA* variants in the Luxembourg Parkinson's study.

Subclassification	nucleotide - protein changes	Subjects	PD n=652	Parkinsonism Patients n=100	Healthy controls n=806
Severe	c.115+1G>A	2	1		1
	p.G234W	1	1		
	p.G241R	2	2		
	p.H294Q	1			1
	p.R398*	1	1		
	p.G416S	1	1		
	p.L483P	6	5		1
	p.R502H	1	1		
	p.N409S; p.L483P	1	1		
	p.L483P; p.A495P; p.V499V (*RecNciI*)	5	4		1
	p.K13R; p.L483P	1	1		
	p.F252I; p.T408M	1	1		
Mild	p.N409S	10	7		3
Risk	p.E365K	42	21+2[a]	1 DLB + 2 PSP	16
	p.T408M	28	15	1 DLB	12
	p.Y61H; p.T408M	1	1		
VUS	p.K13R	4	2		2
	p.Y61H	1	1		
	p.R78C	2			2
	p.A97G (new VUS)	1			1
	p.P161S	2	2		
	p.L213P	1	1		
	p.A215D (new VUS)	1	1		
	p.E427K	2	1		1
	p.R434C (new VUS)	1	1		
	p.H529R	1	1		
	p.R534C	1	1		
	p.A495P; p.V499V	3	1		2
	p.T408T	3	2		1

All variants were identified in the heterozygous state except in two individuals for p.E365K ([a] Homozygous state). Abbreviations: GBA, glucocerebrosidase gene; PD, Parkinson's Disease and Parkinson's Disease with Dementia; PSP, Progressive Supranuclear Palsy; DLB, Dementia with Lewy Body; VUS, Variants of unknown significance.

628 **Table 2. Frequency of GBA mutations in the Luxembourg Parkinson's study.**

Diagnosis	sub-classification of *GBA* mutations	Subjects	All GBA-Carrier (n)	Pathogenic GBA-Carrier	OR (95%CI)	*p*-values
PD		644	**77 (12%)**	**67 (10.4%)**	2.6 (1.6 to 4.1)	0.0001*
	severe			21 (3.2%)	11.4 (2.6 to 48.8)	0.0010*
	mild			7 (1.1%)	3.5 (0.7 to 17.2)	0.1137
	risk			39 (6%)	1.6 (1 to 2.8)	0.0470*
PSP	risk	59		2 (3.4%)	0.9 (0.2 to 3.9)	0.8941
DLB	risk	24		2 (8.3%)	2.2 (0.5 to 10)	0.2908
Healthy controls		678	34 (5%)	29 (4.3%)	-	-
	severe			2	-	-
	mild			2	-	-
	risk			25	-	-

629 After excluding the first-degree family members interrelated in the cohort and the healthy controls with
630 young age of assessment (< 60 AAA) with first degree PD relatives. GBA, glucocerebrosidase gene;
631 PD, Parkinson's Disease and Parkinson's Disease with Dementia; PSP, Progressive Supranuclear Palsy;
632 DLB, Dementia with Lewy Body. ORs are given with the 95% CI; Statistically significant results
633 highlighted in bold and red with * (p-value < 0.05).

Table 3. Demographic data for the PD patients in the Luxembourgish cohort separated by GBA mutation status.

Features	All pathogenic variants (n = 67)	Severe (n = 21)	Mild (n = 7)	Risk (n = 39)	Noncarriers (n = 561)
AAA, mean (SD)	66.5 (±10.2) [OR=0.37; p=0.4713]	65.1 (±10.2) [OR=0.1; p=0.3315]	67.1 (±15.6) [OR=0.68; p=0.9275]	67.1 (±9.2) [OR=0.67; p=0.8231]	67.5 (±10.9)
Sex, Male % (n)	40 (59.7%) [OR=0.71; p=0.1882]	13 (61.9%) [OR=0.77; p=0.5762]	5 (71.4%) [OR=1.19; p=0.8356]	22 (56.4%) [OR=0.62; p=0.149]	380 (67.7%)
AAO, mean (SD)	61.6 (±11.5) [OR=0.42; p=0.5668]	58.6 (±13.1) [OR=0.02; p=0.1383]	65.4 (±17.0) [OR=19.33; p=0.511]	62.5 (±9.3) [OR=1.07; p=0.9704]	62.5 (±11.8)
AAO ≤ 45	8 (11.9%) [OR=1.63; p=0.2301]	**5 (23.8%) [OR=3.76; p=0.0135*]**	2 (28.6%) [OR=4.82; p=0.0648]	1 (2.6%) [OR=0.32; p=0.2626]	43 (7.7%)
Disease Duration, mean (SD)	4.7 (±4.8) [OR=0.7; p=0.7074]	6.4 (±4.7) [OR=3.99; p=0.2338]	1.7 (±1.4) [OR=0.04; p=0.0979]	4.4 (±4.9) [OR=0.56; p=0.4966]	5 (±5.2)
Family History, N (%)	25 (37.3%) [OR=1.5; p=0.1137]	8 (38.1%) [OR=1.58; p=0.3167]	2 (28.6%) [OR=1.03; p=0.9726]	15 (38.5%) [OR=1.61; p=0.1651]	157 (28%)

After excluding the first-degree family members interrelated in the cohort, the healthy controls with young age of assessment (< 60 AAA) with first degree PD relatives and synonymous and VUS variants carriers. Data are given as mean (SD) or N (%). Significance level for comparison is $p < 0.05$. AAA, age at assessment in years ; AAO, Age at onset in years.

Table 4. Clinical characteristics of PD classified by GBA mutation status.

Type of data	Clinical characteristics and scales	PD *GBA* carrier														PD GBA non-carrier
		SEVERE					MILD					RISK					N=570
		PD_{GBA} (n=21)	missing values (%)	β (95%)	*p*-value	adj *p*-value	PD_{GBA} (n=7)	missing values (%)	β (95%)	*p*-value	adj *p*-value	PD_{GBA} (n=39)	missing values (%)	β (95%)	*p*-value	adj *p*-value	
Motor symptoms/scales	H&Y, mean (SD)	2.4 (±0.8)	3 (0.5%)	0.18 (-0.11 to 0.48)	0.2254	0.5635	2.0 (±0.6)	3 (0.5%)	-0.0 (-0.51 to 0.51)	0.9963	0.9992	2.2 (±0.8)	3 (0.5%)	0.07 (-0.16 to 0.29)	0.5503	0.9914	2.2 (±0.8)
	MDS-UPDRS II, mean (SD)	12.6 (±4.4)	13 (2.2%)	0.44 (-2.79 to 3.67)	0.7892	0.9441	10.1 (±6.4)	13 (2.3%)	0.86 (-4.72 to 6.44)	0.7629	0.9992	11.1 (±8.6)	13 (2.2%)	0.12 (-2.31 to 2.55)	0.9223	0.9914	11.4 (±8.3)
	MDS-UPDRS III, mean (SD)	34.8 (±15.7)	14 (2.4%)	-0.25 (-7.18 to 6.69)	0.9441	0.9441	26.2 (±9.7)	13 (2.3%)	-4.81 (-16.98 to 7.36)	0.4387	0.9992	33.3 (±17.7)	12 (2.0%)	-0.49 (-5.42 to 4.45)	0.8469	0.9914	34.6 (±16.2)
	MDS-UPDRS IV, mean (SD)	3.0 (±4.5)	7 (1.2%)	0.85 (-0.49 to 2.18)	0.214	0.5635	0.1 (±0.4)	7 (1.2%)	-0.64 (-2.88 to 1.6)	0.5737	0.9992	1.2 (±2.3)	7 (1.2%)	-0.43 (-1.4 to 0.53)	0.3779	0.8221	1.7 (±3.4)
	Dyskinesias, n (%)	5 (23.8%)	0	0.62 (-0.54 to 1.78)	0.2937	0.6425	0	-	-	-	-	4 (10.3%)	0	-0.01 (-1.15 to 1.13)	0.9878	0.9914	67 (11.9%)
	Falls, n (%)	5 (23.8%)	0	0.37 (-0.7 to 1.45)	0.4932	0.7193	0	-	-	-	-	7 (17.9%)	0	0.12 (-0.78 to 1.02)	0.7938	0.9914	98 (17.5%)
	Gait Disorder, n (%)	16 (76.2%)	0	0.9 (-0.13 to 1.93)	0.0873	0.5441	3 (42.9%)	0	-0.26 (-1.78 to 1.26)	0.7364	0.9992	18 (46.2%)	0	-0.32 (-1.0 to 0.35)	0.3503	0.8221	314 (56.0%)
	FOG, n (%)	8 (38.1%)	0	0.65 (-0.34 to 1.65)	0.1966	0.5635	0	-	-	-	-	7 (17.9%)	0	-0.2 (-1.14 to 0.73)	0.673	0.9914	126 (22.5%)
	Restless leg syndrome, n (%)	2 (9.5%)	0	0.06 (-1.44 to 1.56)	0.9413	0.9441	2 (28.6%)	0	1.62 (-0.07 to 3.31)	0.0605	0.8843	6 (15.4%)	0	0.71 (-0.22 to 1.64)	0.1324	0.8221	46 (8.2%)
	Motor fluctuation, n (%)	5 (23.8%)	0	0.12 (-1.03 to 1.27)	0.8427	0.9441	0	-	-	-	-	5 (12.8%)	0	-0.22 (-1.25 to 0.82)	0.6795	0.9914	95 (16.9%)
Non-motor symptoms/scales	BDI, mean (SD)	12.4 (±5.7)	28 (4.8%)	2.02 (-0.99 to 5.03)	0.1879	0.5635	8.0 (±3.5)	29 (5.1%)	-1.16 (-6.73 to 4.41)	0.6826	0.9992	8.0 (±5.7)	29 (4.8%)	-1.9 (-4.16 to 0.36)	0.0994	0.8221	9.9 (±7.1)
	MDS-UPDRS Part I, mean (SD)	**15.0 (±6.5)**	**15 (2.6%)**	**3.92 (0.99 to 6.86)**	**0.0088***	**0.2625**	8.6 (±2.4)	15 (2.6%)	-0.81 (-5.81 to 4.19)	0.7506	0.9992	9.5 (±6.8)	15 (2.5%)	-0.94 (-3.12 to 1.23)	0.3946	0.8221	**10.6 (±7.0)**
	PDSS, mean (SD)	98.3 (±20.9)	44 (7.6%)	-4.42 (-15.25 to 6.41)	0.424	0.6745	110.9 (±13.4)	43 (7.6%)	2.6 (-15.45 to 20.65)	0.778	0.9992	104.0 (±24.9)	45 (7.5%)	-1.3 (-9.38 to 6.78)	0.7527	0.9914	104.7 (±24.9)
	SCOPA-AUT, mean (SD)	17.1 (±8.0)	33 (5.7%)	1.67 (-1.76 to 5.1)	0.3393	0.6598	13.1 (±7.1)	32 (5.6%)	-0.26 (-5.98 to 5.46)	0.9284	0.9992	14.1 (±7.8)	33 (5.5%)	-0.67 (-3.2 to 1.86)	0.6038	0.9914	15.0 (±8.1)
	Sniffin's stick test, mean (SD)	**6.4 (±3.6)**	**7 (1.2%)**	**-1.56 (-3.05 to -0.07)**	**0.0403***	**0.4702**	6.6 (±2.1)	7 (1.2%)	-1.58 (-4.12 to 0.95)	0.2212	0.9992	7.4 (±4.0)	8 (1.3%)	-0.66 (-1.79 to 0.48)	0.2558	0.8221	**7.8 (±3.6)**
	SAS, mean (SD)	15.8 (±5.2)	34 (5.8%)	2.0 (-0.5 to 4.5)	0.1173	0.5441	12.6 (±3.8)	33 (5.8%)	-1.48 (-5.66 to 2.71)	0.4892	0.9992	13.1 (±6.3)	35 (5.8%)	-0.77 (-2.66 to 1.11)	0.4228	0.8221	14.0 (±5.7)
	MoCA, mean (SD)	24.0 (±4.7)	12 (2.1%)	-0.75 (-2.55 to 1.05)	0.4134	0.6745	25.6 (±3.7)	12 (2.1%)	0.89 (-2.15 to 3.92)	0.5664	0.9992	24.9 (±4.0)	14 (2.3%)	0.16 (-1.2 to 1.52)	0.8165	0.9914	24.4 (±4.5)
	Constipation, n (%)	10 (47.6%)	0	0.04 (-0.85 to 0.92)	0.9338	0.9441	5 (71.4%)	0	1.38 (-0.28 to 3.03)	0.1029	0.9004	14 (35.9%)	0	-0.33 (-1.02 to 0.36)	0.3518	0.8221	251 (44.7%)
	Dysphagia, n (%)	4 (19.0%)	0	-0.46 (-1.57 to 0.65)	0.4182	0.6745	1 (14.3%)	0	-0.53 (-2.66 to 1.6)	0.6273	0.9992	10 (25.6%)	0	0.0 (-0.75 to 0.76)	0.9914	0.9914	146 (26.0%)
	Insomnia, n (%)	6 (28.6%)	0	-0.06 (-1.04 to 0.92)	0.9072	0.9441	4 (57.1%)	0	1.42 (-0.11 to 2.95)	0.068	0.8843	7 (17.9%)	0	-0.56 (-1.41 to 0.28)	0.1898	0.8221	154 (27.5%)
	Orthostatism, n (%)	5 (23.8%)	0	-0.36 (-1.39 to 0.67)	0.4915	0.7193	4 (57.1%)	0	1.37 (-0.14 to 2.89)	0.0758	0.8843	15 (38.5%)	0	0.43 (-0.25 to 1.11)	0.2136	0.8221	166 (29.6%)
	Urinary incontinence, n (%)	6 (28.6%)	0	-0.1 (-1.08 to 0.88)	0.8443	0.9441	2 (28.6%)	0	0.14 (-1.54 to 1.82)	0.8729	0.9992	17 (43.6%)	0	0.63 (-0.04 to 1.31)	0.0666	0.8221	171 (30.5%)
	Hallucinations, n (%)	**8 (38.1%)**	**0**	**1.16 (0.23 to 2.09)**	**0.015***	**0.2625**	2 (28.6%)	0	1.18 (-0.49 to 2.85)	0.1664	0.9707	6 (15.4%)	0	0.05 (-0.87 to 0.97)	0.9088	0.9914	**87 (15.5%)**
	Excessive daytime sleepiness, n (%)	9 (42.9%)	0	0.45 (-0.45 to 1.35)	0.3302	0.6598	0	-	-	-	-	14 (35.9%)	0	0.3 (-0.4 to 0.99)	0.4041	0.8221	176 (31.4%)
	ICD, n (%)	2 (9.5%)	0	-0.26 (-1.79 to 1.27)	0.7402	0.9441	0	-	-	-	-	4 (10.3%)	0	0.21 (-0.89 to 1.31)	0.7051	0.9914	55 (9.8%)
	Syncope, n (%)	2 (9.5%)	0	0.84 (-0.7 to 2.38)	0.2842	0.6425	1 (14.3%)	0	1.68 (-0.54 to 3.91)	0.1377	0.9639	3 (7.7%)	0	0.55 (-0.71 to 1.82)	0.3891	0.8221	27 (4.8%)
	RBDSQ, mean (SD)	10 (47.6%)	42 (7.2%)	0.61 (-0.33 to 1.55)	0.2021	0.5635	1 (14.3%)	42 (7.4%)	-0.53 (-2.7 to 1.64)	0.6317	0.9992	14 (35.9%)	43 (7.2%)	0.38 (-0.35 to 1.12)	0.3052	0.8221	171 (30.5%)
Other clinical outcomes	LEDD (mg/day), mean (SD)	690.5 (±457.9)	19 (3.3%)	118.74 (-34.03 to 271.52)	0.1277	0.5441	324.7 (±224.7)	20 (3.5%)	-68.27 (-348.63 to 212.09)	0.6332	0.9992	496.6 (±443.2)	20 (3.3%)	8.54 (-106.24 to 123.32)	0.884	0.9914	514.7 (±404.7)
	PDQ-39, mean (SD)	52.0 (±26.3)	49 (8.4%)	9.17 (-1.69 to 20.04)	0.098	0.5441	26.1 (±17.7)	49 (8.6%)	-7.06 (-25.57 to 11.44)	0.4545	0.9992	34.9 (±27.2)	51 (8.5%)	-4.58 (-12.85 to 3.69)	0.278	0.8221	39.6 (±26.8)
	DBS, n (%)	3 (14.3%)	0	1.44 (-0.13 to 3.01)	0.0729	0.5441	0	0	-17.62 (-34180.02 to 34144.78)	0.9992	0.9992	1 (2.6%)	0	-0.06 (-2.23 to 2.12)	0.9588	0.9914	24 (4.3%)
Comorbidities	Diabetes, n (%)	2 (9.5%)	0	0.19 (-1.33 to 1.71)	0.8093	0.9441	1 (14.3%)	0	0.14 (-2.07 to 2.36)	0.8992	0.9992	5 (12.8%)	0	0.47 (-0.54 to 1.47)	0.3607	0.8221	55 (9.8%)
	Hypercholesterolemia, n (%)	6 (28.6%)	0	-0.43 (-1.41 to 0.55)	0.386	0.6745	2 (28.6%)	0	-0.66 (-2.35 to 1.04)	0.4466	0.9992	17 (43.6%)	0	0.15 (-0.52 to 0.81)	0.6631	0.9914	228 (40.6%)
	Cardiovascular disease, n (%)	1 (4.8%)	0	-1.55 (-3.6 to 0.51)	0.1399	0.5441	1 (14.3%)	0	-0.63 (-2.86 to 1.59)	0.577	0.9992	8 (20.5%)	0	0.12 (-0.72 to 0.95)	0.7863	0.9914	118 (21.0%)
	Arterial hypertension, n (%)	9 (42.9%)	0	0.11 (-0.81 to 1.03)	0.8192	0.9441	2 (28.6%)	0	-0.89 (-2.62 to 0.84)	0.314	0.9992	12 (30.8%)	0	-0.57 (-1.28 to 0.15)	0.1206	0.8221	250 (44.6%)
	Traumatic Brain Injury, n (%)	5 (23.8%)	0	0.15 (-0.88 to 1.18)	0.7798	0.9441	0	-	-	-	-	6 (15.4%)	0	-0.44 (-1.34 to 0.45)	0.3343	0.8221	124 (22.1%)

Comparison of each type (severe, mild, risk) of *GBA* mutations and its association with clinical characterization. We used regression models (linear and logistic). Data are given as mean and standard deviation (SD) for continuous clinical outcomes and as percentage for binary clinical outcomes. Models

adjusted for sex, age at assessment, and disease duration. Beta (β) regression coefficient are given with the 95% CI. Statistically significant results highlighted in bold with (*) sign and red (p-value < 0.05). Abbreviation : p-value, unadjusted p-value; adj p-value, corrected for multiple comparisons using FDR adjustment; AAO, age at onset; H&Y, Hoehn & Yahr; MDS-UPDRS, Movement Disorders Society - Unified Parkinson's Disease Rating Scale; FOG, freezing of gait; BDI, Beck Depression Inventory; PDSS, Panic Disorder Severity Scale; SCOPA-AUT, Scales for Outcomes in Parkinson's Disease-Autonomic questionnaire; SAS, Starkstein apathy scale; MoCA, Montreal Cognitive Assessment; ICD, impulse control disorder; RBDSQ, REM Sleep Behavior Disorder Screening Questionnaire; LEDD, L-dopa equivalent daily dose (mg/day); PDQ-39, Parkinson's Disease quality of life Questionnaire; DBS, Presence of treatment by Deep Brain Stimulation

Table 5. The deleterious impact of severe GBA-PD carriers in comparison with mild and risk and their clinical characteristics.

Type of data	Clinical characteristics and scales	PD_{GBA} carrier		missing values (%)	β (95%)	*p*-value	adj *p*-value
		severe (n=21)	mild + risk (N=46)				
Motor symptoms/scales	H&Y, mean (SD)	2.4 (±0.8)	2.2 (±0.8)	0	0.14 (-0.24 to 0.52)	0.4719	0.7549
	MDS-UPDRS II, mean (SD)	12.6 (±4.4)	10.9 (±8.2)	0	0.24 (-3.18 to 3.65)	0.8922	0.9706
	MDS-UPDRS III, mean (SD)	34.8 (±15.7)	32.4 (±16.9)	3 (4.5%)	0.65 (-7.69 to 8.98)	0.8793	0.9706
	MDS-UPDRS IV, mean (SD)	3.0 (±4.5)	1.0 (±2.2)	0	1.33 (-0.17 to 2.82)	0.082	0.574
	Dyskinesias, n (%)	5 (23.8%)	4 (8.7%)	0	0.65 (-1.09 to 2.39)	0.4621	0.7549
	Falls, n (%)	5 (23.8%)	7 (15.2%)	0	0.24 (-1.29 to 1.77)	0.7594	0.9601
	Gait Disorder, n (%)	**16 (76.2%)**	**21 (45.7%)**	**0**	**1.49 (0.25 to 2.73)**	**0.0188***	**0.2193**
	FOG, n (%)	8 (38.1%)	7 (15.2%)	0	0.79 (-0.73 to 2.32)	0.3091	0.7549
	Restless leg syndrome, n (%)	2 (9.5%)	8 (17.4%)	0	-0.98 (-2.81 to 0.85)	0.2952	0.7549
	Motor fluctuation, n (%)	5 (23.8%)	5 (10.9%)	0	0.28 (-1.36 to 1.92)	0.7348	0.9601
Non-motor symptoms/scales	**BDI, mean (SD)**	**12.4 (±5.7)**	**8.0 (±5.4)**	**2 (3.0%)**	**4.03 (1.08 to 6.98)**	**0.0074***	**0.1295**
	MDS-UPDRS Part I, mean (SD)	**15.0 (±6.5)**	**9.3 (±6.3)**	**0**	**4.91 (1.8 to 8.02)**	**0.0019***	**0.0665**
	PDSS, mean (SD)	98.3 (±20.9)	105.1 (±23.5)	3 (4.5%)	-2.79 (-14.9 to 9.33)	0.6521	0.9129
	SCOPA-AUT, mean (SD)	17.1 (±8.0)	13.9 (±7.6)	2 (3.0%)	2.61 (-1.62 to 6.85)	0.2269	0.7542
	Sniffin's stick test, mean (SD)	6.4 (±3.6)	7.3 (±3.7)	1 (1.5%)	-0.86 (-2.75 to 1.02)	0.3695	0.7549
	SAS, mean (SD)	15.8 (±5.2)	13.0 (±6.0)	3 (4.5%)	2.02 (-0.94 to 4.97)	0.1817	0.7542
	MoCA, mean (SD)	24.0 (±4.7)	25.0 (±3.9)	2 (3.0%)	-0.24 (-2.4 to 1.92)	0.8292	0.9706
	Constipation, n (%)	10 (47.6%)	19 (41.3%)	0	0.12 (-1.02 to 1.26)	0.8394	0.9706
	Dysphagia, n (%)	4 (19.0%)	11 (23.9%)	0	-0.44 (-1.83 to 0.95)	0.5348	0.8138
	Insomnia, n (%)	6 (28.6%)	11 (23.9%)	0	-0.02 (-1.27 to 1.23)	0.9767	0.9836
	Orthostatism, n (%)	5 (23.8%)	19 (41.3%)	0	-0.61 (-1.83 to 0.61)	0.327	0.7549
	Urinary incontinence, n (%)	6 (28.6%)	19 (41.3%)	0	-0.76 (-1.97 to 0.44)	0.2156	0.7542
	Hallucinations, n (%)	8 (38.1%)	8 (17.4%)	0	1.03 (-0.24 to 2.3)	0.1127	0.6485
	Excessive daytime sleepiness, n (%)	9 (42.9%)	14 (30.4%)	0	0.41 (-0.71 to 1.52)	0.4745	0.7549
	ICD, n (%)	2 (9.5%)	4 (8.7%)	0	-0.02 (-1.89 to 1.85)	0.9836	0.9836
	Syncope, n (%)	2 (9.5%)	4 (8.7%)	0	0.1 (-1.72 to 1.92)	0.9151	0.9706
	RBDSQ, mean (SD)	10 (47.6%)	15 (32.6%)	4 (6.0%)	0.48 (-0.68 to 1.64)	0.4197	0.7549
Other clinical outcomes	LEDD (mg/day), mean (SD)	690.5 (±457.9)	473.1 (±422.4)	2 (3.0%)	66.4 (-107.09 to 239.89)	0.4531	0.7549
	PDQ-39, mean (SD)	**52.0 (±26.3)**	**33.5 (±25.9)**	**2 (3.0%)**	**12.77 (0.45 to 25.09)**	**0.0422***	**0.3692**
	DBS, n (%)	3 (14.3%)	1 (2.2%)	0	0.76 (-2.17 to 3.69)	0.6122	0.8928
Comorbidities	Diabetes, n (%)	2 (9.5%)	6 (13.0%)	0	-0.26 (-2.0 to 1.48)	0.7681	0.9601
	Hypercholesterolemia, n (%)	6 (28.6%)	19 (41.3%)	0	-0.47 (-1.63 to 0.69)	0.4269	0.7549
	Cardiovascular disease, n (%)	1 (4.8%)	9 (19.6%)	0	-1.71 (-3.92 to 0.5)	0.1297	0.6485
	Arterial hypertension, n (%)	9 (42.9%)	14 (30.4%)	0	0.66 (-0.49 to 1.81)	0.2586	0.7542
	Traumatic Brain Injury, n (%)	5 (23.8%)	6 (13.0%)	0	0.82 (-0.55 to 2.2)	0.2385	0.7542

We used regression models (linear and logistic). Data are given as mean and standard deviation (SD) for continuous clinical outcomes and as percentage for binary clinical outcomes. Models adjusted for sex, age at assessment, and disease duration. Beta (β) regression coefficient are given with the 95% CI. Statistically significant results highlighted in bold with (*) sign and red (p-value < 0.05). Abbreviation : p-value, unadjusted p-value; adj p-value, corrected for multiple comparisons using FDR adjustment; AAO, age at onset; H&Y, Hoehn & Yahr; MDS-UPDRS, Movement Disorders Society - Unified Parkinson's Disease Rating Scale; FOG, freezing of gait; BDI, Beck Depression Inventory; PDSS, Panic Disorder Severity Scale; SCOPA-AUT, Scales for Outcomes in Parkinson's Disease-Autonomic questionnaire; SAS, Starkstein apathy scale; MoCA, Montreal Cognitive Assessment; ICD, impulse control disorder; RBDSQ, REM Sleep Behavior Disorder Screening Questionnaire; LEDD, L-dopa equivalent daily dose (mg/day); PDQ-39, Parkinson's Disease quality of life Questionnaire; DBS, Presence of treatment by Deep Brain Stimulation.

12. Figures

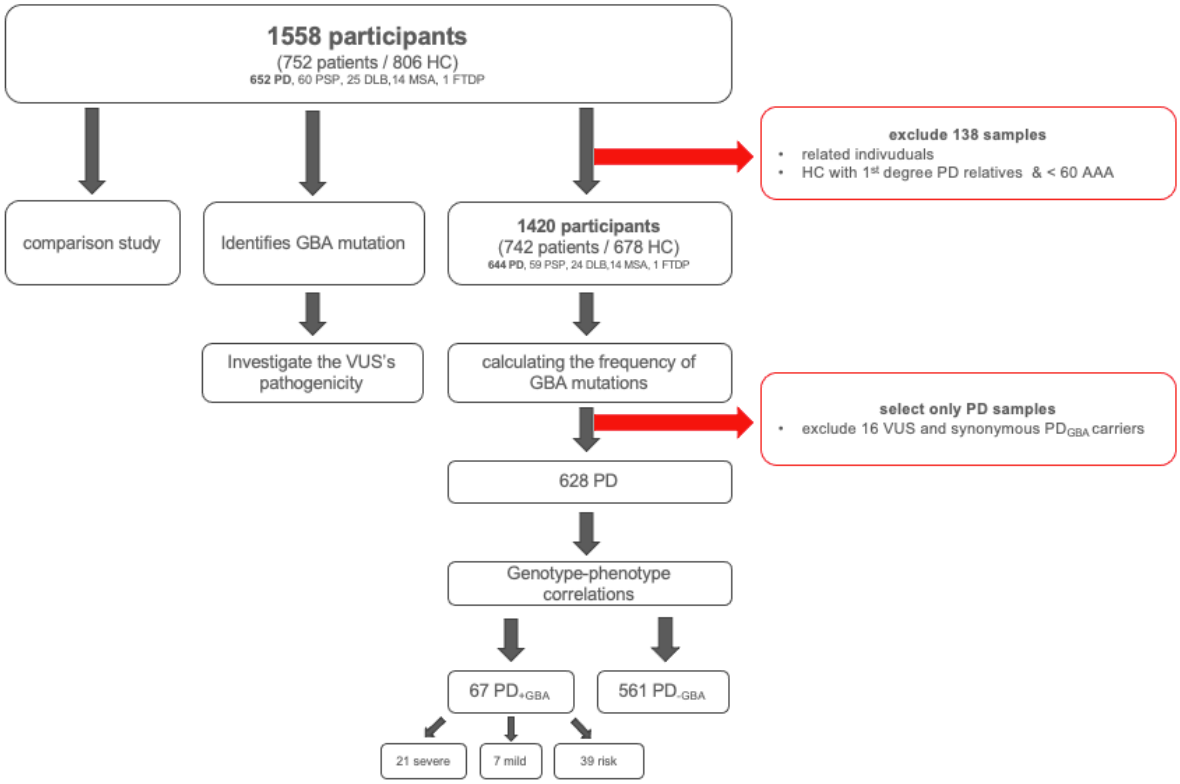


Figure 1: Description of the study dataset and methodology.

A.

dbSNP	protein change	Sample count	PacBio	WGS	NeuroChip
rs2230288	p.E365K	3	3 TP	3 TP	
rs75548401	p.T408M	1	TP	TP	TP
rs76763715	p.N409S	1	TP	TP	
rs77738682	p.N431S	1			FP
	p.A215D	1			FP
	*RecNcil**	1	TP		

B.

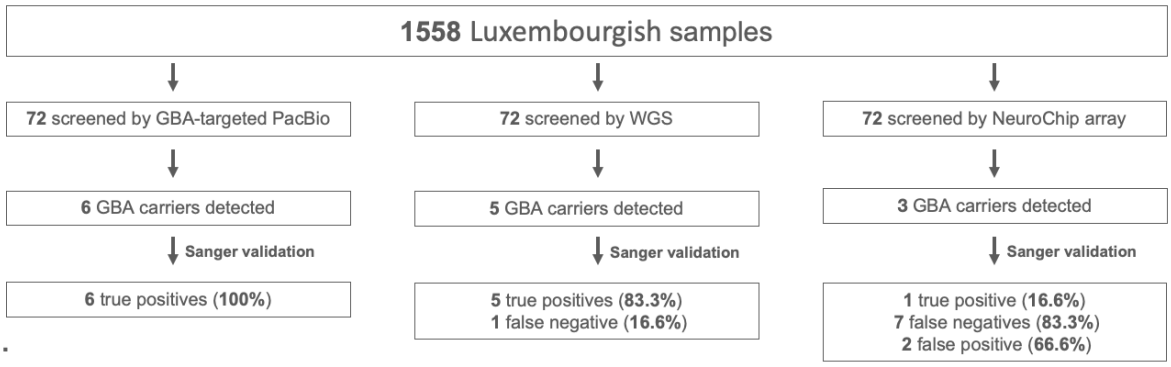
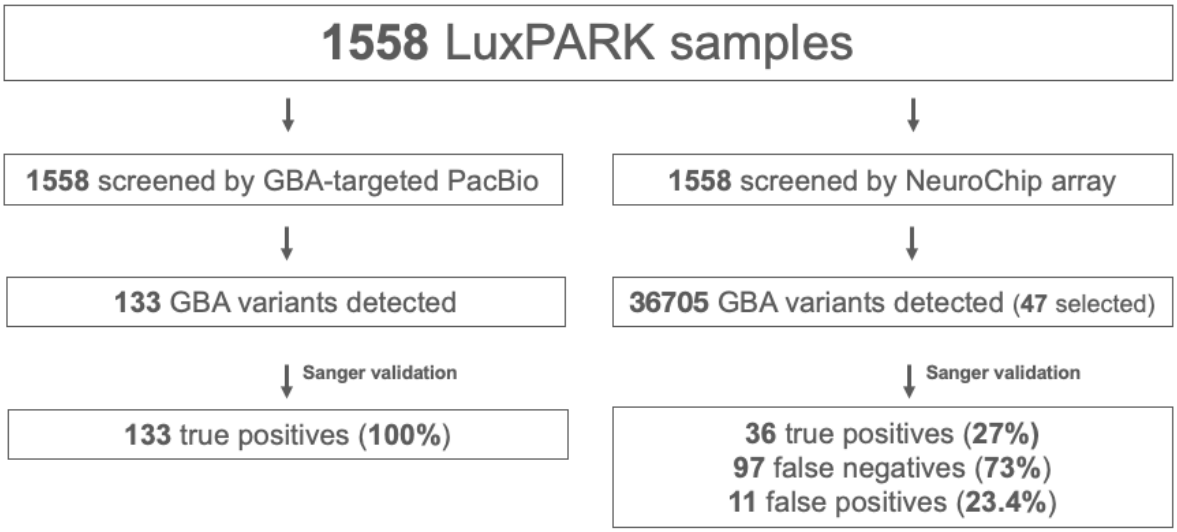


Figure 2. Comparison of variant calls from PacBio, WGS and NeuroChip genotyping data using 72 matched samples for the *GBA* gene and validated by Sanger sequencing.

A)**Rec*Ncil *(p.L483P; p.A495P; p.V499V)*; Sanger sequencing results : TP, true positive ; FP, false positive. Sample count gives total number of samples carrying the variant found by each method.

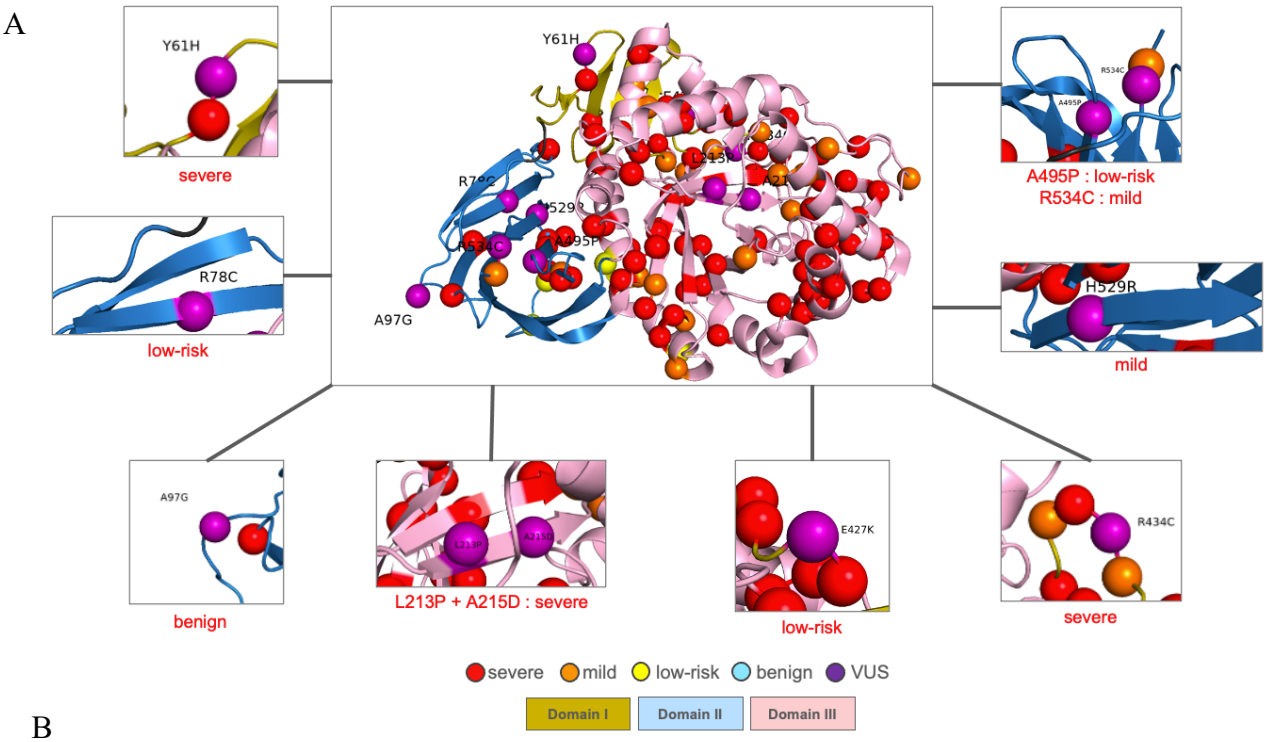
B) Comparative study of *GBA* variants detection by the GBA-targeted PacBio and NeuroChip array methods in the Luxembourg Parkinson's study. Due to overrepresented variants with the NeuroChip array, we applied for the detected variants a study-wide threshold of 1% in our cohort.



677

678 **Figure 3**: **Comparative study of GBA variants detection by the GBA-targeted PacBio and**
679 **NeuroChip array methods in the Luxembourg Parkinson's study.** Due to overrepresented
680 variants with the NeuroChip array, we applied for the detected variants a study-wide threshold
681 of 1% in our cohort.

A



B

Prediction	Subjects		protein change	nucleotide change	dbSNP	Höglinger et al.	ClinVar Significance	ClinVar	HGMD	HGMD interpretation	REVEL	CADD	GnomAD NFE	3D	domain
	PD (AA0)	HC (AAA)													
risk	2 (50/66)	3 (37/38/66)	K13R	c.A38G	rs150466109	VUS	Benign	GD	DM	GD	T	T	ultra-rare	/	/
severe	1 (38)		Y61H	c.T181C	rs1266341749	-	-	-	-	-	T	T	ultra-rare	coil-loop	I
risk		2 (56/58)	R78C	c.C232T	rs146774384	-	-	-	DM	PD susceptibility	T	D	ultra-rare	β-sheet	II
benign		1 (60)	A97G	c.C290G	-	-	-	-	-	-	T	T	-	coil-bend	II
severe	1 (56)		L213P	c.T638C	-	VUS	-	-	DM	PD	D	D	-	β-sheet	III
severe	1 (68)		A215D	c.C644A	-	-	-	-	DM	GD	D	D	-	β-sheet	III
risk	1 (65)	1 (70)	E427K	c.G1279A	rs149171124	VUS	Uncertain significance	Parkinsonism	DM	Reduced activity	T	T	ultra-rare	coil-turn	I
severe	1 (62)		R434C	c.C1300T	rs747284798	-	-	-	DM	GD 1	D	D	-	coil-loop	I
risk	1 (60)	2 (38/61)	A495P	c.G1483C	rs368060	-	Benign	GD	DM	-	T	T	ultra-rare	β-sheet	II
mild	1 (77)		H529R	c.A1586G	-	VUS	-	-	DM	PD	T	T	-	β-sheet	II
mild	1 (78)		R534C	c.C1600T	rs146519305	-	-	-	-	-	T	T	ultra-rare	coil-loop	II
risk	2 (61/64)	1 (61)	T408T	c.G1224A	rs138498426	VUS	Uncertain significance	GD	DM	PD	-	-	ultra-rare	buried residue	III

Figure 4. Sub-classification of VUS found in the Luxembourg Parkinson's study.
A) *GBA* missense and stop gain variants mapped onto the three-dimensional structure of GCase. Domain 1 is shown in dark yellow, domain 2 in blue, and domain 3 in pink. Variants classified as severe are coloured red, mild are coloured orange, risk in yellow and VUS are coloured purple. B) GBA, glucocerebrosidase gene; GD, Gaucher's disease; PD, Parkinson's disease. HGMD, The Human Gene Mutation Database; REVEL, Rare Exome Variant Ensemble Learner; CADD, Combined Annotation Dependent Depletion; gnomAD, The Genome Aggregation Database. DM, Disease causing mutation; D, Deleterious; T, Tolerate. Variants classified as severe are coloured red, mild are coloured orange, risk in yellow and VUS are coloured purple.