Utility and Implications of Exome Sequencing in Early-Onset Parkinson's Disease

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ABSTRACT: Background: Although the genetic load is high in early-onset Parkinson's disease, thorough investigation of the genetic diagnostic yield has yet to be established. The objectives of this study were to assess variants in known genes for PD and other movement disorders and to find new candidates in 50 patients with early-onset PD.

Methods: We searched for variants either within genes listed by the International Parkinson and Movement Disorder Society Task Force on Genetic Nomenclature or rare homozygous variants in novel candidate genes. Further, exome data from 1148 European PD patients (International Parkinson Disease Genomics Consortium) were used for association testing.

Results: Seven patients (14%) carried pathogenic or likely pathogenic variants in *Parkin, PLA2G6*, or *GBA*. In addition, rare missense variants in *DNAJC13*:p.R1830C and in *PPM1K*:p.Y352C were detected. *SPG7*:p.A510V and *PPM1K*:p.Y352C revealed significant association with PD risk ($P < 0.05$).

Conclusions: Although we identified pathogenic variants in 14% of our early-onset PD patients, the majority remain unexplained, and novel candidates need to be validated independently to better further evaluate their role in PD. © 2018 International Parkinson and Movement Disorder Society

Key Words: exome sequencing; early-onset Parkinson's disease; variants of uncertain significance

Although only a small minority of all Parkinson's disease (PD) cases have a known monogenic background, the role of pathogenic variants is more prominent in early-onset PD (EOPD),[1–4] stressing the utility of genetic testing in this PD subgroup. Whole-exome sequencing (WES) has increasingly been used as a clinical diagnostic tool in recent years and will likely play an important role in EOPD, as early-onset parkinsonian features can be caused by a plethora of gene mutations,[5–8] far exceeding the spectrum of PD genes typically featured on PD diagnostic gene panels. Although group-level WES data have been published for larger PD cohorts,[9–13] data assessing the diagnostic yield has currently been published for a total of 80 EOPD patients[14] only.

In an effort to provide a criterion-based list of genotype-phenotype relations, the International Parkinson and Movement Disorder Society (MDS) Task Force on Genetic Nomenclature in Movement Disorders recently compiled a list of confirmed genetically determined movement disorders.[15] We applied the published MDS Task Force gene list to 50 EOPD patients, in addition to searching for novel PD genes in a combined attempt to assess the utility and diagnostic (as well as research) potential of WES for PD.

Materials and Methods

We studied 50 mostly German patients with EOPD (average age, 50 years; range, 19-72 years; average onset age, 28 years; range, 6-40 years). A subset of patients (n = 21) had previously tested negative for variants in *LRRK2*, *Parkin*, and *PINK1*.[1,2,16] Local ethics approval was obtained from the Research Ethics Board of the University of Luebeck (Supplementary Methods).

Exome capture was carried out with Illumina (Illumina, San Diego, CA). We used a 3-step procedure to filter for candidate variants including screening for variants in (1) MDS Task Force genes[15] (Supplementary Table 2), (2) recently reported genes implicated in PD, and (3) rare protein-changing

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Relevant conflicts of interest/financial disclosures: The authors declare no conflict of interest.

Funding agencies: Funding has been obtained from the German Research Foundation (FOR 2488), the BMBF (MitoPD), the Hermann and Lilly Schilling Foundation, the European Community (SysMedPD), the Alexander Von Humboldt Foundation, CIHR fellowship, and the Joachim Herz Stiftung.

Received: 15 December 2017; **Revised:** 10 October 2018; **Accepted:** 10 October 2018

Published online 10 December 2018 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.27559

homozygous variants for potential novel EOPD genes (Fig. 1; Supplementary Methods). Homozygous variants were chosen, as there was not enough power to detect novel heterozygous variants, and there was no information on phase to detect compound heterozygous variants. All variants that survived filtering were validated by Sanger sequencing as previously described.[17] Multiplex ligation-dependent probe amplification (MLPA; MRC Holland, P051 probe mix) was used to assess *Parkin* copy number variants in heterozygous carriers of *Parkin* sequencing changes. The *PLA2G6* compound-heterozygous state of the 2 variants was verified by cloning (Supplementary Methods).

The association test with PD risk was assessed using the chi-square test in 1148 unrelated individuals with PD of European ancestry from the International Parkinson Disease Genomics Consortium (IPDGC)[18] compared with 63,369 non-Finnish Europeans of the publicly available GnomAD (genome aggregate database) as a control group (Supplementary Methods). To further evaluate the frequency of variants of uncertain significance (VUS), we assessed the number of VUS in 124 genes matched for the number of observed missense variants in ExAC but otherwise randomly chosen (Supplementary Methods).

Results

MDS Task Force Genes: Pathogenic/Likely Pathogenic Variants

WES yielded ~75,000 variants called per patient, with an overall mean coverage of greater than 150 × in the genes of interest (Supplementary Table 1). We found a total of 7 patients (14%) who carry pathogenic/likely pathogenic variants in known genes (Table 1). We identified 3 patients with biallelic and 2 patients with single heterozygous pathogenic variants in *Parkin* (Table 1). One of the latter 2 patients also carried a heterozygous *DJ-1* exon duplication (L-1888). It can be hypothesized that the heterozygous *Parkin* and *DJ-1* mutation in L-1888 cause the PD phenotype. However, digenic inheritance has not yet been convincingly demonstrated in PD, and no definite conclusions can be drawn based on this 1 case here. Of note, in all 3 biallelic carriers, the second variant comprised a heterozygous exon deletion/duplication that was detected by MLPA. The *Parkin* c.823C>T (p.R275W, NM_004562) pathogenic variant was recurrently found in 3 patients in the heterozygous state (Table 1, Supplementary Table 2). All patients with *Parkin* pathogenic variants presented with the typical clinical features of PD and an early onset including patients L-1888 and L-

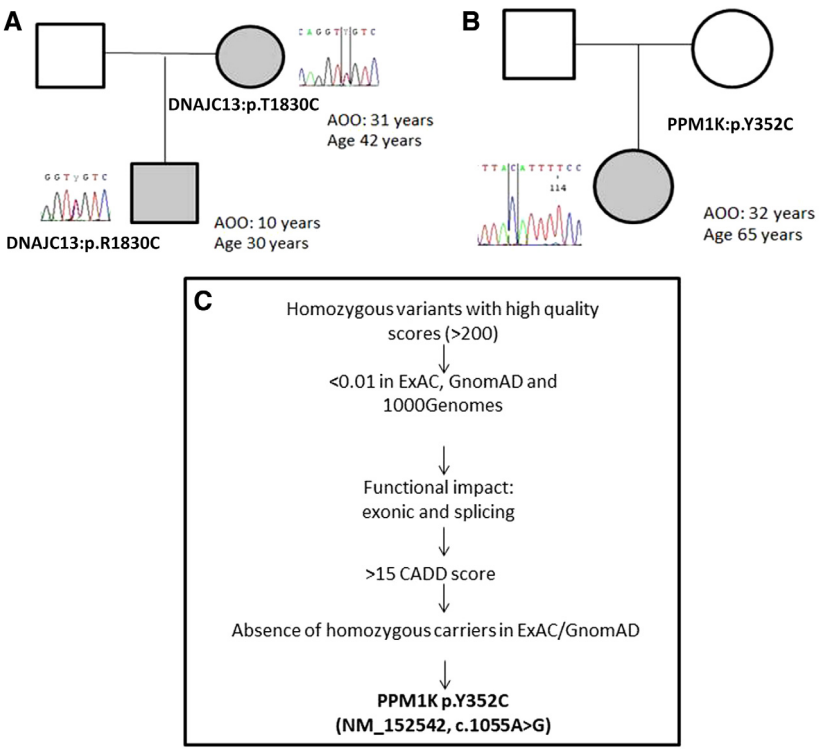

FIG. 1. Nominated novel pathogenic variants for EOPD. *DNAJC13* p.R1830C (NM_015268, c.5488C>T) variant presents in affected mother (L-1452) and son (L-1451). (B) *PPM1K* homozygous p.Y352C (NM_152542, c.1055A>G) variant in early-onset patient. (C) Filtering criteria to identify novel candidates. AOO, age of onset; age, age of patient; GnomAD, genome aggregate consortium; ExAC, exome aggregate consortium; CADD, combined annotation dependent. [Color figure can be viewed at wileyonlinelibrary.com] [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1. Detected rare pathogenic/likely pathogenic variants in the exome data in our 50 EOPD patients in genes listed in the tables of the MDS Task Force on Genetic Nomenclature in Movement Disorders[15]

Designation	Inh	OMIM	Variant identified	Chr:position (hg19)	Ref	Alt	ExAC	GnomAD	CADD	Patient ID	ACMG	Scoring
					Hereditary PD genes							
PARK-Parkin	AR	600116	*Parkin* het p.R275W	6:162206852	G	A	0.0021	0.0019	20.1	L-649, L-1888,[a] L-8599[a]	Path	PS3, PS4, PM3, PP1, PP3
			Parkin het p.Q34Rfs*5	6:162864411	CT	—	0.0000082	4.06×10^{-6}	34	L-513[a]	Path	PVS1, PS4, PM3, PM2, PP3
			Parkin het p.N52Mfs*29	6:162864358	T	—	NA	7.22×10^{-6}	34	L-3296[a]	Path	PVS1, PS4, PM3, PM2, PP3
NBIA/DYT/PARK-PLA2G6	AR	612953	*PLA2G6* het p.Y790*	22:38508219	A	C	0.000080	8.90×10^{-5}	37	L-1355	Path	PVS1, PS1, PS4, PM2, PP3
			PLA2G6 het p.A781T	22:38508248	C	T	0.0000096	1.45×10^{-5}	15.39	L-1355	Likely path	PS4, PM3, PP3
PARK-GBA	AD		GBA het p.R502C (p.R463C)[b]	1:155204987	G	A	0.000058	7.22×10^{-5}	19.35	L-1706	Likely path	PS4, PM1, PP2, PP3
			GBA het p.L483P (p.L444P)[b]	1:155205043	A	G	0.0031	0.0013	18.1	L-1927	Likely path	PS4, PM1, PP2, PP3
			GBA het p.F252I (p.F213I)[b]	1:155207932	A	T	0.000016	4.06×10^{-6}	15.74	L-865	Likely path	PS4, PM1, PP2, PP3

Inh, inheritance; OMIM, Online Mendelian Inheritance in Man; Chr:position, chromosome and position; Ref, reference; Alt, alternative; GnomAD, genome aggregate consortium; CADD, Combined Annotation Dependent Depletion; ID, identification; ACMG, American College of Medical Genetics; Het, heterozygous; Path, pathogenic; Likely path, likely pathogenic.

[a] See Supplementary Table 2 for additional *Parkin* (biallelic) or *DJ-1* (digenic) variant, and see Supplementary Table 3 for all VUS findings.

[b] Two nomenclatures are available as published research on GBA reports: common allelic nomenclature in parentheses.

649, who carried only 1 heterozygous *Parkin* mutation that may not necessarily be disease-causing. Another patient was validated to have compound-heterozygous variants in *PLA2G6* including a stop and missense change c.2370T>G (p.Y790X) and c.2341G>A (p.A781T) (NM_003560.2); see Supplementary Figure 1. Three likely pathogenic variants were identified within *GBA*: c.T754A (p.F252I [p.F213I]), c.T1448C (L483P [p.L444P]), c.C1504T (p.R502C [p.R463C]) (NM_001005742 [NM_000157.3]); see Table 1. One patient with a *GBA* variant responded well to deep brain stimulation, had cognitive decline (Montreal Cognitive Assessment of 21 points), and action tremor without myoclonus or cerebellar or dystonic signs (Supplementary Table 2). Detailed clinical information for the other 2 carriers of *GBA* variants was not available.

In addition to these (likely) pathogenic variants, 17 VUS were found in 15 patients (30%); see Supplementary Table 3 and Supplementary Results. Applying the same filtering criteria to randomly selected genes with matching missense variability (124 genes; Supplementary Table 4) in our 50 patients revealed 17 variants in 14 different genes in 17 patients (34%) that may be categorized as VUS (Supplementary Table 5). There was no difference in the number of detected VUS between the movement disorder genes and the randomly chosen genes in our EOPD patients ($P = 0.95$).

Novel Candidate Genes for Parkinson's Disease

Two patients (L-650, L-3036) carried the known pathogenic c.1529C>T (p.A510V) variant (NM_003119, rs61755320) in *SPG7* in the heterozygous state (biallelic *SPG7* mutations cause autosomal-recessive, pure, or complicated hereditary spastic paraplegia [HSP]). There are 599 carriers of this variant in GnomAD in 63,346 non-Finnish Europeans and 51 carriers among the 1148 IPDGC patients. An association analysis comparing these numbers revealed a significantly higher frequency of variant carriers among PD patients with an odds ratio of 4.87 (95% CI, 3.64-6.52; $P < 0.0001$).

In addition to the MDS Task Force genes, we also included a screening of recently identified, yet to be confirmed genes for PD. We detected 1 individual and his affected mother with a rare sequence alteration (c.5488C>T, p.R1830C, NM_015268) in *DNAJC13*. *DNAJC13*:p.R1830C has been found in GnomAD (MAF 0.00001807; carrier number, 5/138,329), is predicted to be damaging by MutationTaster/Polyphen2/LRT, has a CADD score of 21.3, and is highly conserved (GERP of 5.02).[19–22]

When searching for novel homozygous potentially pathogenic variants in our patients, we found 1 carrier (L-1523) with a rare homozygous variant in *PPM1K*

(c.1055A>G, p.Y352C, NM_152542). The *PPM1K*:p.Y352C variant was found in 1 additional patient (L-1661) in a heterozygous state from the 49 remaining EOPD patients. The variant is predicted to be damaging by MutationTaster/Polyphen2/LRT, has a CADD score of 19.2, and is highly conserved (GERP of 4.09). Although 4 of 1148 PD patients carried the p.Y352C variant in the heterozygous state, this variant was only found in 32 of the 55,800 sequenced non-Finnish Europeans sequenced within GnomAD. There was a significant difference that suggests this variant may confer risk for PD, with an odds ratio of 6.09 (95% CI, 2.15-17.25; $P = 0.005$). No additional homozygous or compound-heterozygous mutations in *PPM1K* were found among the 1148 PD patients by exome sequencing, and there were only 10 of 1148 patients with other rare single heterozygous protein-changing variants in *PPM1K* (variants listed in Supplementary Table 6).

Discussion

As WES for EOPD is starting to enter clinical practice, evaluating its diagnostic yield is crucial to improve our methods with respect to technical issues, variant interpretation, patient counseling, and the development of genetic testing guidelines.

Strictly speaking, the largest proportion of our EOPD patients remained "idiopathic" (43 of 50; 86%) or at least uncertain in origin. Our data suggest that 14% of EOPD patients may carry pathogenic/likely pathogenic variants. However, because we preselected for "mutation-free" individuals (n = 21), this may very well be an underestimate of the true diagnostic yield. For example, *Parkin* variants alone account for 20% of isolated EOPD.[3] We also found that patients with a family history of PD may have a higher diagnostic yield (73%) compared with patients with negative family history (48%); however, this was not significantly different because of the relatively small sample size (Supplementary Tables 8 and 9). Interestingly, we found 1 small family with a segregating rare variant in *DNAJC13*. The role of *DNAJC13* in PD is under debate, as both *DNAJC13* and *TMEM230* variants have been described in the same large multi-incident Mennonite family.[23–25] Our study identified an additional nuclear family that could support the role of *DNAJC13* in PD. Furthermore, we suggest that *PPM1K*:p.Y352C may be a risk factor for PD in the heterozygous state and even causative for EOPD when present on both alleles. *PPM1K* is highly expressed in the brain[26] and an exclusively mitochondria-targeted Ser/Thr phosphatase with an N-terminus signal to the mitochondria and exists as a soluble protein in the mitochondrial matrix, which may play a role in dopaminergic neuronal loss and parkinsonism.[27,28] However, one limitation of our study is the role of different sequencing platforms and sampling strategies covering different ethnicities. Based on the design of the comparison groups, it is impossible to include a PCA analysis for population stratification. Thus, we cannot completely rule out a population stratification impact on the discovered association. Thus, further validation of the 2 novel candidates is warranted in other large case and control datasets.

From the genetic testing perspective, it is of critical importance to realize and to discuss with the patients prior to initiating WES diagnostics that the most likely outcome of a WES genetic test will be "no genetic cause found" or an "uninterpretable result." On a more optimistic note, it is likely that reevaluation of VUS may improve the interpretation, as more data will become available in the future by follow-up studies, replications, or functional testing. Although these WES represent unprecedented opportunities with direct translational implications, it will be important to devise guidelines for diagnostic variant interpretation along with appropriate education of movement disorder clinicians to exploit WES data in the best possible fashion. In this context, it is important to realize that WES data can be considered a sustainable resource of potential diagnostic clues that — in case of initial equivocal results — can and should be revisited at appropriate intervals, possibly in conjunction with a (neuro) geneticist.

Author Contributions

Joanne Trinh — conception, organization, and execution of the research project; design and execution of the statistical analysis; writing of the first draft of the manuscript. Katja Lohmann — conception, organization, and execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Hauke Baumann — execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Alexander Balck — execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Max Borsche — execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Norbert Brüggemann — organization and execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Leon Dure — execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Marissa Dean — execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Jens

Volkmann — organization and execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Sinem Tunc — execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Jannik Prasuhn — execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Heike Pawlack — execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Sophie Imhoff — execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Christina M. Lill — organization of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Meike Kasten — execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Peter Bauer — execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Arndt Rolfs — execution of the research project; review and critique of the statistical analysis; review and critique of the manuscript. Christine Klein — conception, organization, and execution of the research project; review and critique of the statistical analysis; writing of the first draft and review and critique of the manuscript.

Acknowledgments: The authors thank the many patients and their families who volunteered and the longitudinal efforts of the many clinical teams involved. We thank Dr. Inken Wohlers for excellent bioinformatic assistance. We also thank the IPDGC members for sharing data (Supplementary text).

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Supporting Data

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