New endemic familial parkinsonism in south Moravia, Czech Republic and its genetic background

Tereza Bartoníková, MD, Katěrina Menšíková, MD, PhD, Kristýna Koláriková, MD, Radek Vodička, MD, PhD, Radek Vrtěl, MD, PhD, Pavel Otůba, MD, Michaela Kaiserová, MD, PhD, Miroslav Vaštík, MD, PhD, Lenka Mikulíčková, MD, Josef Ovečka, MD, Ludmila Sáčková, MD, František Dvořský, MD, Jiří Krša, MD, Petr Jugas, MD, Marek Godlava, MD, PhD, Martin Bareš, MD, PhD, Vladimír Janout, MD, PhD, Petr Hluštik, MD, PhD, Martin Procházká, MD, PhD, Petr Kanovský, MD, PhD

Abstract
An increased prevalence of familial neurodegenerative parkinsonism or cognitive deterioration was recently found in a small region of southeastern Moravia.

The aim of the study was to assess the genetic background of this familial disease.

Variants in the ADH1C, EIF4G1, FBXO7, GBA + GBA1P1, GIGYF2, HTRA2, LRRK2, MAPT, PRKN, DJ-1, PINK1, PLA2G6, SNCA, UCHL1, VPS35 genes were examined in 12 clinically positive probands of the pedigree in which familial atypical neurodegenerative parkinsonism was identified in previous epidemiological studies. Libraries were sequenced by massive parallel sequencing (MPS) on the Personal Genome Machine (PGM; Ion Torrent). Data were analyzed using Torrent Suite and IonReporter software. All variants were then verified and confirmed by Sanger sequencing.

We identified 31 rare heterozygous variants: 11 missense variants, 3 synonymous variants, 8 variants in the UTR region, and 9 intronic variants. Six variants (rs8101334, rs33995883, rs35507033, rs781737269, rs779760087, and rs63750072) were evaluated as pathogenic by at least one in-silico predictor.

No single “founder” pathogenic variant associated with parkinsonism has been found in any of the probands from researched pedigree. It may rather be assumed that the familial occurrence of this disease is caused by the combined influence of several “small-effect” genetic variants that accumulate in the population with long-lasting inbreeding behavior.

Abbreviations: ADH1C = alcohol-dehydrogenase 1C gene, also known as PARK 2, also known as PARK7, ATP13A3 = ATPase 13A3gene, DJ-1 = gene which encodes a protein of the peptidase C56 family, EIF4G1 = eukaryotic translation initiation factor 4 gamma 1 gene, FBXO7 = F-box only protein 7 gene, GBA = glucosylceramidase beta gene, GBA1P1 = glucosylceramidase beta pseudogene 1, GIGYF2 = GRB 10 interacting GYF protein 2, HTRA2 = HtrA serine peptidase 2 gene, LRRK2 = leucine rich-repeat kinase 2 gene, MAPT = microtubule associated protein tau gene, PARK1-PARK18 = Parkinson’s disease protein 1-18, PD = Parkinson’s disease, PDD = Parkinson disease dementia, PINK1 = putative kinase 1 gene, PLA2G6 = phospholipase A2 group VI gene, pPD = prodromal Parkinson disease, PRKN = gene for making a protein called parkin, PSP-P = progressive supranuclear palsy-parkinsonism, RSBD = REM sleep behavior disorder, SNCA = synuclein alpha gene, UCHL1 = ubiquitin C-terminal hydrolase L1 gene, VPS35 = vacuolar protein sorting 35 gene.

Keywords: familial neurodegenerative parkinsonism, molecular-genetic background, population with long-lasting inbreeding behavior

Editor: Xiong Kun.

The study was supported by grant from the Ministry of Health of the Czech Republic, grant Nr. 15–32715A, by grant from the Palacky University Medical School Internal Grant Agency—IGA LF 2018–009, and by grant from the Ministry of Health, Czech Republic—conceptual development of research organization—MH CZ—DPQ (FNOL, 00096682) 2017.

All authors declare no financial or other conflicts of interest. All authors have approved the final article.

*Department of Neurology, †Department of Medical Genetics, Faculty of Medicine and Dentistry, Palacký University, University Hospital, Olomouc, ‡General Practitioner, Lipov, ‡General Practitioner, Velká nad Veřňou, §General Practitioner, Břístnice pod Svatým Antonínkem, ¶Neurology Outpatient Clinic, Veselá nad Moravou, ¶ First Department of Neurology, Masaryk University Medical School, St. Anne University Hospital, Brno, † Department of Epidemiology and Public Health, Faculty of Medicine and Dentistry, Palacky University, University Hospital, Olomouc, Czech Republic.

Correspondence: Katěrina Menšíková, Department of Neurology, Palacky University Medical School and University Hospital, I. P. Pavlova 6, 77520 Olomouc, Czech Republic. (e-mail: katena.mensikova@fnol.cz).

Copyright © 2018 the Author(s). Published by Wolters Kluwer Health, Inc.

This is an open access article distributed under the Creative Commons Attribution License 4.0 (CCBY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Medicine (2018) 97:38(e12313)
Received: 2 May 2018 / Accepted: 14 August 2018
https://dx.doi.org/10.1097/MD.00000000000012313
1. Introduction

The results of epidemiological studies indicate that the prevalence of Parkinson disease (PD) and neurodegenerative parkinsonism in the general population is around 1.6% in Europe and approximately 1.5% in Asia and the Americas.\cite{1-8} In the population over 65 years of age, the prevalence of these diseases ranges from 1.1% to 2.2%\cite{1-11} and even higher prevalences have been repeatedly found in small, isolated populations.\cite{3,4,6,9}

We recently described such a phenomenon in 10 villages of a remote, small, rural, and isolated Moravian region of Czech Republic called “Hornacko” [Upper Lands]. The last census, conducted in 2011, determined the presence of 8664 inhabitants, 2927 of which were over 50 years of age. “Hornacko” people have their own specific customs and traditions, which are reflected in the folk art, crafts, architecture, costumes, songs, and dances; also, the local dialect is very different from that of the surrounding areas\cite{12,13}. In 2011 to 2015, we conducted an epidemiological study in this region which has found a significantly increased prevalence of neurodegenerative parkinsonism. The overall prevalence in the population over 50 years of age was 2.8% (95% confidence interval (CI), 2.2–3.4); the prevalence in the population between 50 and 64 years of age was 1.9% (95% CI, 1.2–2.5) and it was 4.06% (95% CI, 2.9–5.1) in the population over 65 years of age.\cite{14,15} Three large pedigrees with a familial occurrence of parkinsonism were found in which clinical geneticists
identified an autosomal-dominant inheritance pattern.\[^{16}\] Two of these pedigrees eventually compiled one large family tree spanning several generations from 1840 to the present.\[^{17}\] In this large pedigree, 12 probands with apparent clinical signs of parkinsonism were identified (Fig. 2). These probands were referred to a tertiary movement disorders center for detailed clinical and laboratory examinations including DNA sampling in order to obtain information about the clinical, biological, and genetic characteristics of this endemic and familial disorder.

The village from which these families originate (Javorník nad Velíčkou, PCN CZ-69674, elevation 412 m, population 720) is very specific even in this region: the population belongs almost entirely to the Lutheran denomination, unlike all neighboring villages, which are entirely Roman-Catholic. Marriages between the inhabitants of Javorník and residents of other villages were and still are quite exceptional, so the local population is practically isolated from a genetic point of view.\[^{16}\]

2. Methods

The study was approved by the ethics committee of Palacký University Medical School, and all patients and probands gave their informed consent.

All 12 probands who were evaluated as positive for parkinsonian symptoms were examined by a movement disorders specialist. The United Kingdom Parkinson’s Disease Society Brain Bank clinical diagnostic criteria were used for the diagnosis of parkinsonism; this was diagnosed when at least 2 of 4 Class I symptoms (resting tremor, rigidity, bradykinesia, and impaired postural reflexes) were present in a subject not receiving antiparkinsonian medication. Cognitive functions were assessed using the Mini Mental State Examination (MMSE) tool; cognitive deterioration was diagnosed when the MMSE score was \(\leq 24\). All positive probands were then examined in detail to exclude secondary forms of parkinsonism. The examination protocol included detailed neurological, psychological, and psychiatric examinations, examination of cerebrospinal fluid, magnetic resonance imaging of the brain; neurophysiological examinations were also performed (electroencephalographic recordings, electromyography, and assessments of visual, auditory, somatosensory, and motor evoked potentials).\[^{16,17}\] The clinical details of all probands are presented in Table 1.

DNA was isolated from peripheral blood by a DNA silica-gel membrane based isolation kit (QIAamp DNA Mini Kit - Qiagen; Strasse 1, 40724 Hilden, Germany). Each DNA sample was

---

Figure 2. Large pedigree with familial parkinsonism and variants that have been identified in 12 symptomatic probands. The numbers of probands (1; 2; 3; etc.) in pedigree correspond with the numbers of probands in Table 1. The names of genes and their mutations which have been identified in individual probands are in the figure assigned to their numbers.
diluted to a final concentration of 10 ng/µL. The ampiclon library was in silico designed by Ion AmpliSeq Designer (Thermo Fisher Scientific) for ADH1c, EIF4G1, FBXO7, GBA + GBA1, GIGYF2, HTR2A, LRRK2, MAPT, PRKN, DJ-1, PINK1, PLA2G6, SNCA, UCHL1, and VPS35 genes. In total, 617 amplicons covered 92.59% of Coding Sequence (Coding Region of a Gene) including 100 bp in the introns and Untranslated Regions of mRNA of the regions. Libraries were prepared using Ion AmpliSeq Library Kit 2.0 (Ion Torrent; Thermo Fisher Scientific, Waltham, Massachusetts, USA), with emulsion PCR done on the Ion OneTouch 2 Instrument (Ion Torrent) with Ion PGM Template OT2 200 Kit. Samples were barcoded to enable loading 8 samples on one Ion 316 Chip. The amplicons were sequenced by massively parallel sequencing (MPS) on the Personal Genome Machine (PGM; Ion Torrent) using Ion PGM Sequencing Kit (Ion Torrent) and Ion 316 Chip Kit v2. For the coverage control were the amplicons checked in Torrent Suite (Thermo Fisher Scientific, Waltham, Massachusetts, USA) using plugin Coverage Analysis. Data analysis and variant searching (Mapping, Variant calling, Annotation) was done by Torrent Suite and Ion Reporter software (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Data from PGM were collected and converted to Fastq and BAM formats using Ion Torrent Suite software. The Bam/Bai files were reloaded to Ion Reporter and variant calling workflow was in the first level relaxed to minimal amplicon coverage of 4 and a minimal ratio of alternative/common variant of 0.1. Variants were then filtered using the parameter of minor allele frequency (MAF) < 0.01. All variants were then verified and confirmed by Sanger sequencing. SIFT and PolyPhen-2 prediction software were used for missense variant evaluation. The PhyloP algorithm was used to assess the phylogenetical conservation.

### 3. Results

The variants identified in our 12 probands are shown in Table 2; 31 rare heterozygous variants were identified.

One variant was detected in the UTR-3 region in the SNCA (PARK1, PARK4) gene—c.*77G>T (5,8,9). In the PRKN (PARK2) gene, we found 2 variants, 1 in the UTR-3 region—c.*94T>C (6)—and 1 in exon—c.1180C>T (2,10). In the PINK1 (PARK6) gene was found 1 variant in the coding sequence—c.344A>T (8,10). In DJ-1 (PARK7) were found 2 variants in the noncoding region—c.*37C>T (10) and c.410–37C>T (10). Three intronic—c.4317+12delT (3,4), c.437–68T>C (5), and c.437–44T>G (8,9)—and 4 exonic variants—c.2167A>G (2,8), c.6241A>G (3,4), c.4541G>A (6,12), and c.1898C>T (6,12) in the LRRK2 (PARK8) gene were detected. In the GIGYF2 (PARK11) gene were found 3 variants in the noncoding sequence—c.*2030G>A (1), c.1593G>A (4,7), and c.380–66A>G (10) (this one has not been described before) and one in the coding sequence—c.3662C>T (4). In the PLA2G6 (PARK14) gene, one exonic variant—c.1027C>T (2,10) was found. In FBXO7/PARK15, 2 variants—c.540A>G (1) and c.1144+9C>T (7) were detected. In VPS35(PARK17), 3 variants in the noncoding sequence—c.283G>T (2,5), c.102+33C>T (1,2,10), and c.506+71T>C (2,4,5,9) were detected. Among 4 variants, 2 in the noncoding sequence—c.109G>A (2,3,9,11), c.1541–22T>C (4,7)—and 2 in the coding sequence—c.3706C>G (2,3,9,11), c.4272C>T (8,9)—were detected in the EIF4G1 (PARK18) gene. In the ADH1c gene was identified one variant located in the coding sequence—c.143C>T—in 2 patients (6,12). Three variants, 1 in the noncoding sequence—c.464G>A (6)—and 2 in the coding sequence—c.1915G>A (2,10) and c.689A>G (2)—were detected in the MAPT gene. Segregated variants were seen only in particular subpedigrees and are signed as * in Table 2. The most “interesting” variants (PhyloP score ≥ 2 and at least 1 prediction tool evaluating it as pathogenic) are further emphasized in the Discussion section.

### 4. Discussion

Some past studies have suggested that people living in rural areas could be more exposed to putative environmental, mainly agricultural, influences and would be more likely to develop parkinsonism than those living in urban areas. However, the area surveyed in our study is surrounded by a much larger rural area, in which the agricultural and environmental conditions are quite similar; to date, no signs of higher parkinsonism than those living in urban areas.[19] However, the molecular-genetic DNA analysis performed in 12 clinically positive probands in our pedigree has found several new variants in the loci associated with familial parkinsonism in the past:

**Table 1**

| Nr. | Gr. | Age | A/O | Phenotype | Symptoms |
|-----|-----|-----|-----|-----------|----------|
| 1   | M   | 79  | 66  | PSP-P     | Bradykinesia, rigidity, postural instability, apraxia of lid opening, vertical gaze palsy, progressive speech apraxia, cognitive disorder (dementia) |
| 2   | F   | 79  | 71  | PDD       | Bradykinesia, rigidity, postural instability, rest tremor |
| 3   | F   | 83  | 68  | PD        | Bradykinesia, hypomimia, postural instability |
| 4   | M   | 77  | 62  | PD        | Bradykinesia, rigidity, rest tremor bilaterally, freezing of gait and fluctuations |
| 5   | F   | 80  | 60  | PD        | Increased postural reflexes, rest tremor of both hands |
| 6   | F   | 74  | 57  | PSP-P     | Bradykinesia, rigidity, rest tremor of both hands, postural instability, vertical gaze palsy, later dementia |
| 7   | F   | 56  | 52  | PD        | Bilateral rigidity, impaired postural reflexes, rest tremor of both hands |
| 8   | F   | 51  | 46  | PD        | Slight bradykinesia, impaired postural reflexes |
| 9   | M   | 56  | 54  | PD        | Rest tremor of both hands, impaired postural reflexes, hypomimia |
| 10  | F   | 55  | 50  | PD        | Rest tremor of right hand, hypomimia, impaired postural reflexes |
| 11  | F   | 54  | 45  | PD        | Slight bradykinesia, impaired postural reflexes |
| 12  | F   | 54  | 48  | PD        | Anosmia, RSBD |

A/O = age at disease onset; Gr. = gender; Nr. = patient number; PD = Parkinson disease; PDD = Parkinson disease dementia; pPD = prodromal Parkinson disease; PSP-P = progressive supranuclear palsy—parkinsonism; RSBD = REM sleep behavior disorder.
4.1. *NM_004562.2*: c.1180 C>T *PRKN (PARK2)*

The missense substitution was evaluated as pathogenic using prediction programs (in SIFT pathogenic; in PolyPhen-2 probably pathogenic). It leads to an exchange of an acidic for an uncharged polar amino acid. Rare occurrence and also a higher degree of evolutionary conservation of the site has been reported. Moura et al. identified the variant in 9 (6.8%) of his patients; it was evaluated as non-pathogenic polymorphism. Lücking et al. reported a statistically significant difference between patients and controls; the variant was found in 10/194 patients and in 12/125 controls. In another recent study, this variant was found in similar proportions in both patients and controls. ²²

4.2. *NM_198578.3*: c.6241A>G *LRRK2 (PARK8)*

The substitution is located in an evolutionarily conserved region, which could, according to Polyphen-2, have a pathogenic character. However, SIFT evaluated this variant as benign. Its benign role is supported by the recent finding of Benitez et al., who found this variant in 17 of 478 PD patients and 15 of 337 controls. In another study, Foo et al. found the variant in none of the patients and in 1 control (0.25%).

4.3. *NM_198578.3*: c.4541G>A *LRRK2 (PARK8)*

This missense variant in the coding region leads to the exchange of an acidic for an uncharged polar amino acid. It was evaluated as pathogenic using prediction programs (SIFT pathogenic; in PolyPhen-2 probably pathogenic). This variant was found in 9 (6.8%) of his patients; it was evaluated as non-pathogenic polymorphism. Lücking et al. reported a statistically significant difference between patients and controls; the variant was found in 10/194 patients and in 12/125 controls. In another recent study, this variant was found in similar proportions in both patients and controls.
association between the variant and the level of tau protein was described in the cerebrospinal fluid. The variant has not yet been functionally analyzed.

The detailed study of the presence of mutual variants in the researched pedigree indicates that the genetic background of this endemic neurodegenerative disease has a clearly heterogeneous character: 31 different variants were found in 12 genes. When we tracked the variants within the pedigree, we found a dominant trait in only 3 branches of the family and only for some variants. In patients 3 and 11, we found similar variants of the ELF4G1 gene. In contrast, LRRK2 variants were present in the maternal proband only. In patients 2 and 10, similar variants of ADH1C, LRRK2, and PRKN; MAPT variants were present in the maternal proband only. In patients 2 and 10, similar genetic background and apparently heterogeneous traits. In this molecular genetic study of the core pedigree, we have not found one “founder” pathogenic variant associated with parkinsonism in all or the most PD patients of this pedigree. Therefore, it could rather be assumed that the familial occurrence of this disease is caused by the combined influence of several “small-effect” genetic variants that accumulate in the population with long-lasting inbreeding behavior. Whether there is any link to the VPS35 positive familial parkinsonism, which has been discovered in the relatively near region of Upper Austria by Zimprich et al. and Struhal et al. remains open for further research. For the planned functional analyses, we have so far collected only one brain specimen from the research pedigree. Therefore, our future study will also utilize proper cell lines with combinations of targeted mutagenesis to assess the effects of particular rare variants.

5. Conclusion

Our study has provided an evidence that the recently described endemic neurodegenerative parkinsonism, which can manifest in different phenotypes (see Table 1), has also different genetic background and apparently heterogeneous traits. In this molecular–genetic study of the core pedigree, we have not found one “founder” pathogenic variant associated with parkinsonism in all or the most PD patients of this pedigree. Therefore, it could rather be assumed that the familial occurrence of this disease is caused by the combined influence of several “small-effect” genetic variants that accumulate in the population with long-lasting inbreeding behavior. Whether there is any link to the VPS35 positive familial parkinsonism, which has been discovered in the relatively near region of Upper Austria by Zimprich et al. and Struhal et al. remains open for further research. For the planned functional analyses, we have so far collected only one brain specimen from the research pedigree. Therefore, our future study will also utilize proper cell lines with combinations of targeted mutagenesis to assess the effects of particular rare variants.

Acknowledgments

The authors are grateful to Jan Pavlik, MD, native of Kuzelov and Hornacko researcher, for his substantial contribution to the manuscript by providing ethnographic data and references and to Jarmila Tomesova, the nurse of the general practitioner’s office for her contribution to organizing research in the examined region.

Author contributions

Conceptualization: Katerina Mensikova, Radek Vodicka, Radek Vrtel, Marek Godava, Martin Bares, Vladimir Janout, Petr Kanovsky.

Supervision: Radek Vrtel, Martin Prochazka, Petr Kanovsky.

Writing – original draft: Tereza Bartonikova, Kristyna Kolarikova, Radek Vodka.

Writing – review & editing: Katerina Mensikova, Petr Hlustik, Martin Prochazka, Petr Kanovsky.

References

[1] de Rijk MC, Tzourio C, Breteler MM, et al. Prevalence of parkinsonism and Parkinson’s disease in Europe: EUROPARKINSON Collaborative Study. J Neurol Neurosurg Psychiatry 1997;62:10–5.
[2] de Rijk MC, Breteler MM, Graveland GA, et al. Prevalence of Parkinson’s disease in the elderly: the Rotterdam Study. Neurology 1995;45:2143–6.
[3] Kis B, Schrag A, Ben-Shlomo Y, et al. Novel three-stage ascertainment method: prevalence of PD and parkinsonism in South Tyrol, Italy. Neurology 2002;58:1820–5.
[4] Benito-Leo J, Bermejo-Pareja F, Rodriguez J, et al. Prevalence of PD and other types of parkinsonism in three elderly populations of central Spain. Mov Disord 2003;18:267–74.
[5] Zhang ZX, Roman GC. Worldwide occurrence of Parkinson’s disease: an updated Review. Neuroepidemiology 1993;12:195–208.
[6] Barbosa MT, Caramelli P, Maima DP, et al. Parkinsonism and Parkinson’s disease in the elderly: a community based survey in Brazil (the Bambuí study). Mov Disord 2006;21:800–8.
[7] Das SK, Misra AK, Ray BK, et al. Epidemiology of Parkinson disease in the city of Kolkata, India. Neurology 2010;75:1362–9.
[8] Winkler AS, Tutuncu E, Trenadališová A, et al. Parkinsonism in a population of northern Tanzania: a community-based door-to-door study in combination with a prospective hospital-based evaluation. J Neurol 2010;257:799–805.
[9] Wermuth L, Joensen P, Bürger N, et al. High prevalence of Parkinson’s disease in the Faroe Islands. Neurology 1997;49:426–32.
[10] Bergačeké A, De la Puenta E, Lopez de Munain A, et al. Prevalence of Parkinson’s disease and other types of Parkinsonism. A door-to-door survey in Bidasoa, Spain. J Neurol 2004;251:340–5.
[11] Claveria LE, Duarte J, Sevillano MD, et al. Prevalence of Parkinson’s disease in Cantalope, Spain: a door-to-door survey. Mov Disord 2002;17:242–9.
[12] Pavlovičová M. Folk Culture and its Historic and Social Reflexes. Masaryk University Press, Brno 2007.
[13] Valčk M. Social and Cultural Changes in the Village: Moravian Countryside at the Turn of Third Millennium. Masaryk University Press, Brno 2011.
[14] Mensikova K, Kanovsky P, Kaiserova M, et al. Prevalence of neurodegenerative parkinsonism in an isolated population in south-eastern Moravia, Czech Republic. Czech Slov Neurol N 2014;77:714–20.
[15] Mensikova K, Godava M, Kanovsky P, et al. Familial autosomal-dominant neurodegenerative parkinsonism with cognitive deterioration spanning five generations in a genetically isolated population of south-eastern Moravia, Czech Republic. Biomed Pap 2016;160:158–60.
[16] Frolic V, Holý D, Jiřábké R. Hornácko. The Life and Culture of the People from Moravian-Slovakian borderlands (In Czech). Blok, Brno 1996, ISBN 80-86720-40-1.11.
[17] Koller W, Vetere-Overfield B, Gray C, et al. Environmental risk factors in Parkinson’s disease. Neurology 1999;40:4218–21.
[18] Moura KC, Campos Junior M, DeRosso AL, et al. Genetic analysis of PARK2 and PINK1 genes in Brazilian patients with early-onset Parkinson’s disease. Dis Markers 2013;35:181–5.
[19] Lücking CB, Cheseau V, Lohmann E, et al. Coding polymorphisms in the parkin gene and susceptibility to Parkinson disease. Arch Neurol 2003;60:1253–6.
[20] Fiola O, Zahoraková D, Pospsilova L, et al. Parkin (PARK2) mutations are rare in Czech patients with early-onset Parkinson’s disease. PLoS One 2014;9:e107358.
[21] Benitez BA, Davis AA, Jin SC, et al. Resequencing analysis of five Mendelian genes and the top genes from genome-wide association studies in Parkinson’s Disease. Mol Neurodegener 2016;11:29.

[22] Foo JN, Tan LC, Liany H, et al. Analysis of non-synonymous-coding variants of Parkinson’s disease-related pathogenic and susceptibility genes in East Asian populations. Hum Mol Gen 2014;23:3891–7.

[23] Nichols WC, Marek DK, Pauciulo MW, et al. R1514Q substitution in Lrrk2 is not a pathogenic Parkinson’s disease mutation. Mov Disord 2007;22:234–7.

[24] Toft M, Mata IF, Ross OA, et al. Pathogenicity of the Lrrk2 R1514Q substitution in Parkinson’s disease. Mov Disord 2007;22:389–92.

[25] Jin SC, Pastor P, Cooper B, et al. Pooled-DNA sequencing identifies novel causative variants in PSEN1, GRN and MAPT in a clinical early-onset and familial Alzheimer’s disease Ibero-American cohort. Alzheimers Res Ther 2012;4:34.

[26] Kauwe JS, Cruchada C, Mayo K, et al. Variation in MAPT is associated with cerebrospinal fluid tau levels in the presence of amyloid-beta deposition. Proc Natl Acad Sci USA 2008;105:8050–4.

[27] Zimprich A, Benet-Pagés A, Struhal W, et al. A mutation on VPS35, encoding a subunit of the retromer complex, causes late onset Parkinson disease. Am J Hum Genet 2011;89:168–75.

[28] Struhal W, Presslauer S, Spielberger S, et al. VPS35 Parkinson’s disease phenotype resembles the sporadic disease. J Neural Transm 2014;21:755–9.

[29] Bartonikova T, Mensikova K, Mikulicova L, et al. Familial atypical parkinsonism with rare variant in VPS35 and FBXO7 genes. Medicine (Baltimore) 2016;95:e5398.