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LOSS-OF-FUNCTION MUTATIONS IN *RAB39B* ARE ASSOCIATED WITH TYPICAL EARLY-ONSET PARKINSON DISEASE

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Rab proteins are small molecular weight guanosine triphosphatases involved in the regulation of vesicular trafficking.¹ Three of 4 X-linked *RAB* genes are specific to the brain, including *RAB39B*. Recently, Wilson et al.² reported that mutations in *RAB39B* cause X-linked intellectual disability (ID) and pathologically confirmed Parkinson disease (PD). They identified a ~45-kb deletion resulting in the complete loss of *RAB39B* in an Australian kindred and a missense mutation in a large Wisconsin kindred. Here, we report an additional affected man with typical PD and mild mental retardation harboring a new truncating mutation in *RAB39B*.

Methods. We looked for coding and splice site mutations in *RAB39B* (RefSeq accession number NM_171998.2) using data mining in exomes from a cohort of 1,348 unrelated patients with PD (60% men, mean age at onset 41.7 ± 11.0 years) and 530 controls (65% men, mean age at examination 45.1 ± 10.7 years), mostly of European ancestry, recruited through the International Parkinson's Disease Genomics Consortium. Whole exome was sequenced using, for the most part, Illumina TruSeq chemistry and Illumina HiSeq 2000 instrument, using 100 bp paired-end reads, according to the manufacturer's protocols. A mean of 75% of reads had coverage of at least 30-fold in both patients with PD and controls.

The human reference genome UCSC hg19 was used for sequence alignment and variant calling with the Burrows-Wheeler Aligner (<http://bio-bwa.sourceforge.net/>) and the Genome Analysis Toolkit (<https://www.broadinstitute.org/gatk/>). PCR duplicates were removed prior to variant calling using Picard software (<http://broadinstitute.github.io/picard/>). Variants were annotated with ANNOVAR software (<http://www.openbioinformatics.org/annovar/>).

Variants of interest were then verified by bidirectional Sanger sequencing using an ABI 3730 automated sequencer (Applied Biosystems, Life Technologies, Carlsbad, CA) with SeqScape v2.6 software (Applied Biosystems). Additional screening of the 2 *RAB39B*

exons by direct sequencing was performed in a cohort of 192 unrelated French men with early-onset (EO) parkinsonism (mean age at onset 34.5 ± 7.6 years) recruited through the French network for the study of Parkinson disease genetics and 392 unrelated UK PD cases (mean age at onset 49.8 ± 14.2 years), including 48% men (mean age at onset 49.1 ± 14.4 years).

Standard protocol approvals, registrations, and patient consents. Informed consent was obtained from all participants and the genetic studies were approved by local ethics committees.

Results. Among the 1,348 patients with PD, we identified a single man of French origin with parkinsonism who harbored a novel nonsense mutation (c.557G>A in exon 2 [p.Trp186stop] of *RAB39B*) that was not found in 530 unrelated control individuals of European origin, public databases (dbSNP137) (<http://www.ncbi.nlm.nih.gov/SNP/>), or in an additional cohort of 61,486 unrelated individuals from the publicly available Exome Aggregation Consortium (<http://exac.broadinstitute.org/>). In addition, this patient was excluded for mutations in all other known PD genes in data from exome analyses. Subsequent screening of *RAB39B* in 380 additional men with EO parkinsonism failed to identify any additional variants, suggesting that *RAB39B* is a very rare cause of parkinsonism.

The patient with the *RAB39B* p.Trp186stop mutation had early disease onset (39 years) and typical parkinsonism with asymmetric rest tremor, an akineto-rigid syndrome, and a good response to levodopa. He had mild mental retardation, which required sheltered employment. No other clinical abnormalities, such as pyramidal, cerebellar, or ocular disorders, were detected. No family history of PD was reported. Brain MRI performed twice was normal.

Eight years after disease onset, the patient developed treatment-related complications, including motor fluctuations, dyskinesia, and limb dystonia, and was thoroughly evaluated in view of possible deep brain stimulation. A neuropsychological examination showed good global cognitive performance (Mattis 138/144) but difficulty concentrating and subcortico-frontal signs. A psychiatric interview revealed dysthymic disorder with impulsiveness, explaining why surgery was ultimately

not performed. Apomorphine treatment was initiated at age 55. The patient died the same year.

Discussion. Previous studies showed *RAB39B* to be a rare cause of X-linked ID,^{3–6} which may be associated with autism spectrum disorder, epileptic seizures, and macrocephaly, but not parkinsonian signs. A recent study reported 2 families with loss-of-function mutations in *RAB39B*.² All affected men from the 2 kindreds presented similar clinical phenotypes with variable degrees of ID in their childhood, including developmental delay, cognitive impairment, macrocephaly, and, in some, seizures; EO parkinsonism with tremor appeared subsequently as the presenting symptom. Neuropathologic examination was consistent with α -synuclein pathology. Although we could not exclude the presence of large and complex rearrangements undetectable by the exome sequencing method, we identified an *RAB39B* p.Trp186stop mutation carried by a 39-year-old man with parkinsonism. The case presented here extends the phenotype caused by loss-of-function *RAB39B* mutations to include X-linked typical EO parkinsonism with mental retardation that is mild enough to allow autonomous living. *RAB39B* plays a role in vesicular trafficking pathway, possibly affecting α -synuclein pathology, as recently reported for another PD-associated gene, *VPS35*.⁷

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Author contributions: Dr. Lesage: drafting the manuscript, analysis and interpretation of data, and statistical analysis. Dr. Bras: acquisition, analysis, and interpretation of data. Dr. Cormier-Dequaire: acquisition of clinical data. Mrs. Condroyer: acquisition of genetic data. Dr. Nicolas: bioinformatics analyses. Mrs. Darwent, Dr. Guerreiro, Dr. Majounie, and Mrs. Federoff: exome data acquisition and analysis. Dr. Heutink, Dr. Wood, Dr. Gasser, and Dr. Hardy: acquisition and interpretation of data. Dr. Tison: acquisition of clinical data. Dr. Singleton: supervising the exome data acquisition, analyses and interpretation, and obtaining funding. Dr. Brice: drafting/revising the manuscript, study concept or design, study supervision, and obtaining funding. All authors critically reviewed and approved the final manuscript.

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