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SHORT COMMUNICATION

GIGYF2 mutation in late-onset Parkinson's disease with cognitive impairment

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Although in the last two decades there has been considerable progress in understanding the genetic basis of Parkinson's disease (PD), the majority of PD is sporadic and its genetic causes are largely unknown. In an attempt to identify novel genetic causes of PD, whole-exome sequencing and subsequent analyses were performed in a family featuring late-onset PD with cognitive impairment. A novel genetic variant (p.Arg610Gly) in the *GIGYF2* gene, previously known to be associated with PD, was identified as potential disease-causing mutation. The *GIGYF2* p.Arg610Gly mutation situated in the GYF domain of the encoding protein was predicted to be pathogenic and to disrupt the GYF's ligand-binding abilities. Although further research is still required, this finding may shed light on the GIGYF2-associated mechanisms that lead to PD and suggests insulin dysregulation as a disease-specific mechanism for both PD and cognitive dysfunction.
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Parkinson's disease (PD) is the second most common neurodegenerative disease behind Alzheimer disease and affects more than four million people worldwide. Although in the last two decades there has been considerable progress in understanding the genetic basis of PD, its pathogenic causes are largely unknown.[1] In this study, we aimed to identify novel genes causing PD by performing whole-exome sequencing and subsequent analyses in a Spanish family featuring a late-onset form of PD (Figure 1). The age at onset of our family ranged from 78 to 88 years old and the clinical phenotype was characterized by the presence of a mild motor parkinsonism with an unilateral tremor in one member, a rigid-akinetic unilateral syndrome in a second member, and a jaw tremor in a third member. Even though jaw tremor has been considered a symptom of essential tremor, when it appears in essential tremor is probably a marker for subsequent conversion to PD.[2] Cognitive impairment also occurred in all affected individuals. See Supplementary Online Material for more clinical details.

Whole-exome sequencing was performed in two affected siblings by using the SureSelectXT Human All exon 50 Mb exon-capture kit (Agilent Technologies Inc., Santa Clara, CA, USA) and HiSeq 2000 following the manufacturer's instructions for paired-end 150-bp reads (Illumina Inc., San Diego, CA, USA). Whole-exome sequencing data were then processed and analyzed through a computational pipeline following the general workflow adopted by the 1000 genomes project.[3] Ninety-one percent of the target exome was captured at 30-fold coverage or higher in both patients. Common genetic variation (frequency $>3\%$) observed in the latest dbSNP137 build, 1000 Genomes Project Phase 1, other public databases, such as the Exome Variant Server of the National Heart, Lung and Blood Institute (NHLBI) Exome Sequencing Project,[4] and exomes generated in house[3] were removed from further analyses. Sanger sequencing was used for single-nucleotide variant (SNV) call validation and disease-segregation analyses. Although four novel SNVs were identified present in the three affected individuals and absent in large number of control individuals ($>10\,000$), including 188 ethnicity-matched control chromosomes, only one SNV, located in the *GIGYF2* gene and not present in the Exome Aggregation Consortium, was predicted to be pathogenic (Table 1). While the pathogenic role of *GIGYF2* in PD remains controversial,[5] the *GIGYF2* p.Arg610Gly mutation, which is situated in the GYF domain of the protein that is thought to possess ligand-binding properties,[6] was shown to be highly conserved across different species in both GIGYF1 and GIGYF2 proteins (Figure 1), and was predicted to disrupt the binding between the protein's GYF domain and its interacting ligands (data not shown). The exon containing the p.Arg610Gly mutation was then sequenced in 107 Spanish PD patients, yielding no additional pathogenic mutation

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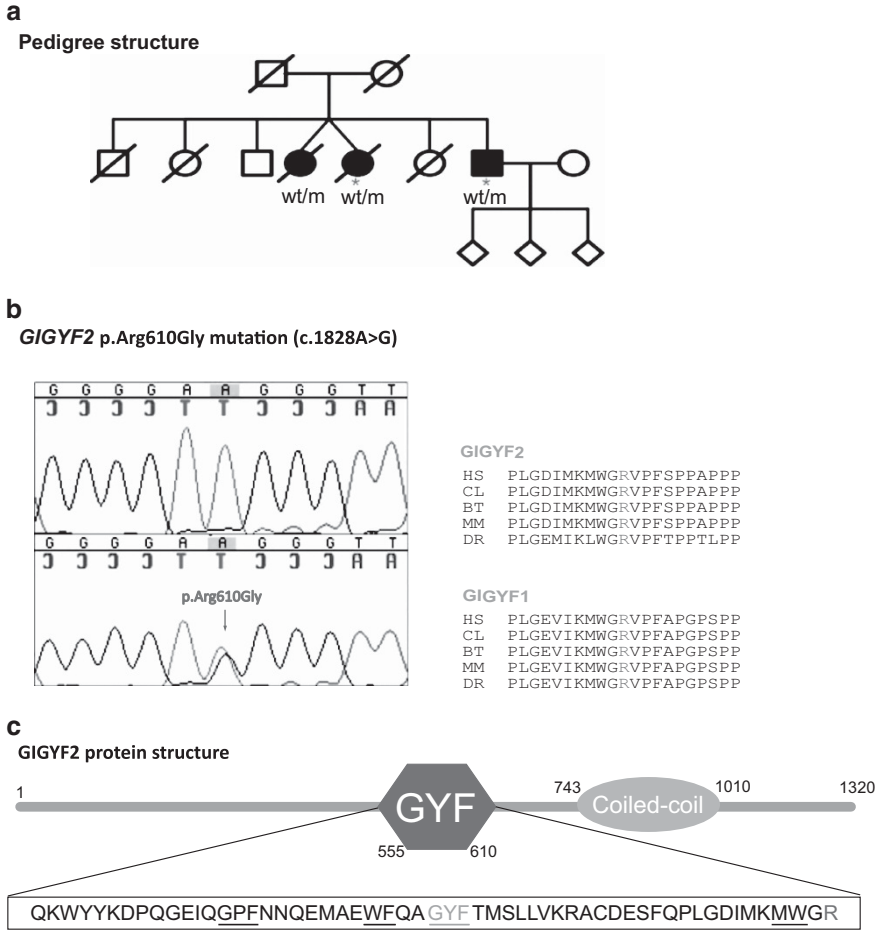

Figure 1 *GIGYF2* mutation in LOPD. Owing to the late-onset of the disease presentation, ranging from 78 to 88 years, and the early death of apparently unaffected parents (age of death: 72 and 43 years for father and mother, respectively), the pattern of inheritance in this family remains unknown. (**a**) Pedigree structure of the family analyzed in this study. wt/m: heterozygous mutation carrier. WES was performed in individuals highlighted with a blue asterisk. (**b**) GIGYF2 p.Arg610Gly mutation: chromatogram sequences of wild-type (top) and mutant (bottom) sequences are shown on the right side, whereas conservation of the *GIGYF2* p.Arg610Gly mutation in different species and GIGYF1 protein is shown on the left side. HS: *Homo sapiens*; CL: *Canis lupus*; BT: *Bos taurus*; MM: *Mus musculus*; DR: *Danio rerio*. (**c**) GIGYF2 protein structure predicted by SMART (http://smart.embl-heidelberg.de/). The GYF domain consists of highly conserved glycine–tyrosine–phenylanine residues following the 'GP[YF]xxxx[MV]xxWxxx[GN]YF' motif containing 60 amino acids. The R610 amino acid (highlighted in red) is the last residue of the GYF domain. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 1 WES results: SNVs identified in a Spanish family featuring late-onset PD and cognitive impairment

						Pathogenecity's prediction					
Chr	*Position (bp)*	*Ref> mutant allele*	*Gene*	*Nucleotide change*	*Protein change*	*MutPred*	*PolyPhen*	*SIFT*	*Brain expression/ conservation*	*ExAc browser*	*Associated disease*
2	119915372	C>G	C1QL2	c.474G>C	p.Lys158Asn	0.480	Possibly damaging	Tolerated	No/yes	11/35 424	None
2	216274821	C>T	FN1	c.1958G>A	p.Arg653His	0.578	Benign	Tolerated	High/yes	2/61 050	GFND
2	**233671326**	**A>G**	**GIGYF2**	**c.1828A>G**	**p.Arg610Gly**	**0.626**	**Probably Damaging**	**Deleterious**	**High/yes**	**Not present**	**PD (AD)**
6	151336773	G>T	MTHFD1L	c.2530G>T	p.Ala844Ser	0.531	Probably Damaging	Tolerated	High/No	1/66 696	LOAD and NTDs

Abbreviations: GFND, glomerulopathy with fibronectin deposits; LOAD, late-onset Alzheimer disease; NTDs, neural tube defects; PD, Parkinson's disease. The only mutation predicted to be pathogenic by three computational methods and not present in the ExAc browser is highlighted in bold. ExAc browser refers to the Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org) (April 2015). The ExAC contains sequencing data of over 60 705 unrelated individuals of various disease-specific and population genetic studies. The ExAc data presented are the data identified in the European population.

carriers; and the entire coding region of *GIGYF2* was sequenced in 45 Spanish PD patients (age at onset ranged from 61 to 81 years), leading to the identification of one novel mutation, p.Lys1006Gln_insQ, which is probably non-pathogenic as it lies within a highly polymorphic polyglutamine repeat, along with other already-reported, non-pathogenic mutations. All mutations identified were later tested in neurologically normal individuals and excluded from being risk factors for PD (Table 2a–c).

Among the other SNVs identified, we suspected that only the mutation within *MTHFD1L*, which has been associated with an increased risk of late-onset Alzheimer disease[7] and neural tube defects,[8] may have role in the phenotypic expression of our family. The *MTHFD1L* p.Ala844Ser mutation was then investigated in 107 Spanish PD patients and 105 Spanish patients with late-onset Alzheimer disease. No additional mutation carrier was identified, suggesting that it has no implications in the pathophysiology of PD and AD. The remaining SNVs identified were localized in *FN1* and *C1QL2* genes, respectively, and as such are unlikely to have a role in the pathogenesis of PD: *FN1* encoding for fibronectin is responsible for glomerulopathy with fibronectin deposits in humans[9] and has been involved in cell adhesion and migration processes, including embryogenesis, wound healing, blood coagulation, host defense and metastasis;[10] *C1QL2,* which is not expressed in brain tissues,[11] belongs to a large family of multimeric secreted glycoproteins.

Given the physiological role of GIGYF2 in the regulation of vesicular transport and insulin/insulin like growth factor 1 (IGF-1) signaling in the central nervous system,[12,13] the role of insulin in the regulation of brain dopaminergic activity[14] and the identification of elevated levels of IGF-1 and IGF-binding proteins in the serum and cerebrospinal fluid of patients with PD,[15] we hypothesize that aberrations in proteins involved in the insulin/IGF-1 signaling pathway, including GIGYF2, may be the key players in the pathogenesis of LOPD. The fact that most biological functions of IGF-1, which acts as a homeostatic modulator for normal brain functionality and synaptic plasticity, are mediated by the IGF-1 receptor[16] and that GIGYF2 has been shown to play role in the regulation of IGF-1 receptor trafficking in specific, mammalian, neuronal populations, including hippocampal pyramidal neurons also supports this hypothesis.[17] Even though high prevalence of insulin resistance has been reported in patients with PD[18] and different studies have revealed an important role of insulin in normal memory function and learning ability,[19] a possible role of the *MTHFD1L* gene in the cognitive dysfunction of our reported family cannot be ruled out. However, this coupled with the fact that overexpression of *GIGYF2* has not only been shown to correlate with an increased neuronal apoptosis but also to diminish cognitive function[17] may suggest that the cognitive impairment seen in our family may be due to a possible insulin dysregulation caused by *GIGYF2* genetic variability identified in this study. Because dysregulation of insulin may predispose neurodegenerative disease late in life,[19] *GIGYF2* mutation carriers may not develop the full parkinsonian symptoms until an advanced age as occurred in our reported family.

In summary, our study and others suggest that *GIGYF2* genetic variability may be, although rare, a cause of LOPD. Although there are still many challenges to be met, this study adds insights into the contribution of *GIGYF2* to the pathogenesis of PD and suggests insulin dysregulation as a disease-specific mechanism for both PD and cognitive dysfunction.

Table 2 *GIGYF2* genetic variability identified in this study

Sample	*A. O*	*DNA change*	*Protein change*	*Spanish PD population (Fqcy)*	*Spanish control population (Fqcy)*
2a) Novel GIGYF2 mutations identified in this study					
Family I (3 patients)	**82–88**	**c.1828A>G**	**p.Arg610Gly**	**0.014**[a]	**0.000**
Family II (1 patient)	64	c.3016_3018insCAG	p.Lys1006Gln_insQ	0.001	0.000
2b) Previously described GIGYF2 mutations identified in this study					
Sporadic II, III, IV	64, 81, 71	c.3689_3709del21	p.1230_1236delLPQQQQQ	0.033	0.074
Sporadic V	76	c.3712insCAGCAG	p.1237insQQ	0.001	0.005
Family II (1 patient)	64	c.3736_3747del12	p.1246_Q1249delPQQQ	0.001	0.016

			Spanish PD population (Fqcy)		*Pilot_3_CEU exon_capture panel/HapMap-CEU (Fqcy)*	
SNPs (Major allele)	*DNA change*	*Protein change*	*Major allele*	*Minor allele*	*Major allele*	*Minor allele*
2c) Normal GIGYF2 genetic variation identified in this study						
Rs11555646 (A)	c.-4A>C	N.A	0.739	0.260	0.712	0.288
Rs2289912 (C)	c.1441C>A	p.Pro481Thr	0.990	0.010	0.986	0.014
Rs2305138 (G)	c.1617G>A	p.Glu539=	0.950	0.050	0.955	0.045
Rs3816334 (G)	c.3003G>A	p.Gln1001=	0.739	0.260	0.708	0.292
Rs10555297 (delACA)	c.3693_3695delACA	p.Q1232delQ	0.739	0.260	0.745[b]	0.265[b]
Rs12328151 (G)	c.3714G>A	p.Pro1238=	0.836	0.163	0.812 (0.728[b])	0.149 (0.272[b])
Rs6437074 (A)	c.3747+15A>G	N.A	0.772	0.228	0.708 (0.867[b])	0.292 (0.133[b])
Rs3217558 (–)	c.3747+43insA	N.A	0.978	0.022	0.962[b]	0.038[b]

Abbreviation: Fqcy, allelic frequencies.
GIGYF2 SNP allelic frequencies in Spanish PD population and control population are listed. GIGYF2 SNP allelic frequencies in the Pilot_3_CEU exon_capture and HapMap-CEU panels available at NCBI database (http://www.ncbi.nlm.nih.gov/projects/SNP/) are listed. The only pathogenic mutation identified is highlighted in bold.
[a]Tested in 107 Spanish PD patients.
[b]Allelic frequencies from the Spanish control population since no data were found in NCBI database. The *GIGYF2* allelic frequencies in PD and control population were found to be almost identical.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)