

## Mutations in the *GIGYF2* (*TNRC15*) Gene at the PARK11 Locus in Familial Parkinson Disease

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The genetic basis for association of the PARK11 region of chromosome 2 with familial Parkinson disease (PD) is unknown. This study examined the *GIGYF2* (Grb10-Interacting GYF Protein-2) (*TNRC15*) gene, which contains the PARK11 microsatellite marker with the highest linkage score (D2S206, LOD 5.14). The 27 coding exons of the *GIGYF2* gene were sequenced in 123 Italian and 126 French patients with familial PD, plus 131 Italian and 96 French controls. A total of seven different *GIGYF2* missense mutations resulting in single amino acid substitutions were present in 12 unrelated PD index patients (4.8%) and not in controls. Three amino acid insertions or deletions were found in four other index patients and absent in controls. Specific exon sequencing showed that these ten sequence changes were absent from a further 91 controls. In four families with amino acid substitutions in which at least one other PD case was available, the *GIGYF2* mutations (Asn56Ser, Thr112Ala, and Asp606Glu) segregated with PD. There were, however, two unaffected carriers in one family, suggesting age-dependent or incomplete penetrance. One index case (PD onset age 33) inherited a *GIGYF2* mutation (Ile278Val) from her affected father (PD onset age 66) and a previously described PD-linked mutation in the *LRRK2* gene (Ile1371Val) from her affected mother (PD onset age 61). The earlier onset and severe clinical course in the index patient suggest additive effects of the *GIGYF2* and *LRRK2* mutations. These data strongly support *GIGYF2* as a PARK11 gene with a causal role in familial PD.

### Introduction

Parkinson disease (PD [MIM 168600]) is a neurodegenerative disorder affecting 1%–2% of the population above age 60.<sup>1</sup> The cause of dopaminergic neuronal loss in the nigro-striatal pathway and consequent bradykinesia, resting tremor, muscular rigidity, and postural instability that characterize PD is largely unknown. Although only 10%–30% of PD is familial,<sup>2</sup> there is strong interest in identifying genes that contribute to familial PD, because these genes are expected to define mechanisms and new therapeutic approaches that apply to more common, sporadic forms of the disease.

Family-based whole-genome linkage scans have identified 13 chromosomal loci (PARK1 to PARK13) that show linkage to PD.<sup>3–14</sup> Candidate-gene analysis at these genomic loci linked to PD has identified eight causative genes in PD (*SNCA* [MIM 163890], *PARK2* [*Parkin*] [MIM 602544], *UCHL1* [MIM 191342], *PINK1* [MIM 608309], *PARK7* [*DJ1*] [MIM 602533], *LRRK2* [MIM 609007], *ATP13A2* [MIM 610513], and *HTRA2* [MIM 606441]).<sup>12,15–26</sup> The mechanisms through which these genes contribute to the development of PD have not yet been clearly established. Autosomal-dominant transmission is associated with mutations in the *SNCA*, *LRRK2*, and, probably, *UCHL1* and *HTRA2* genes, whereas the four other genes are autosomal recessive. These genes only account for a small proportion of PD cases, except in specific clinical subgroups or populations. This is the case for the G2019S mutation in the *LRRK2* gene found in up to

18% of Ashkenazi Jews and 39% of North African Arabs with PD but in only 1 to 3% of their corresponding controls.<sup>27,28</sup>

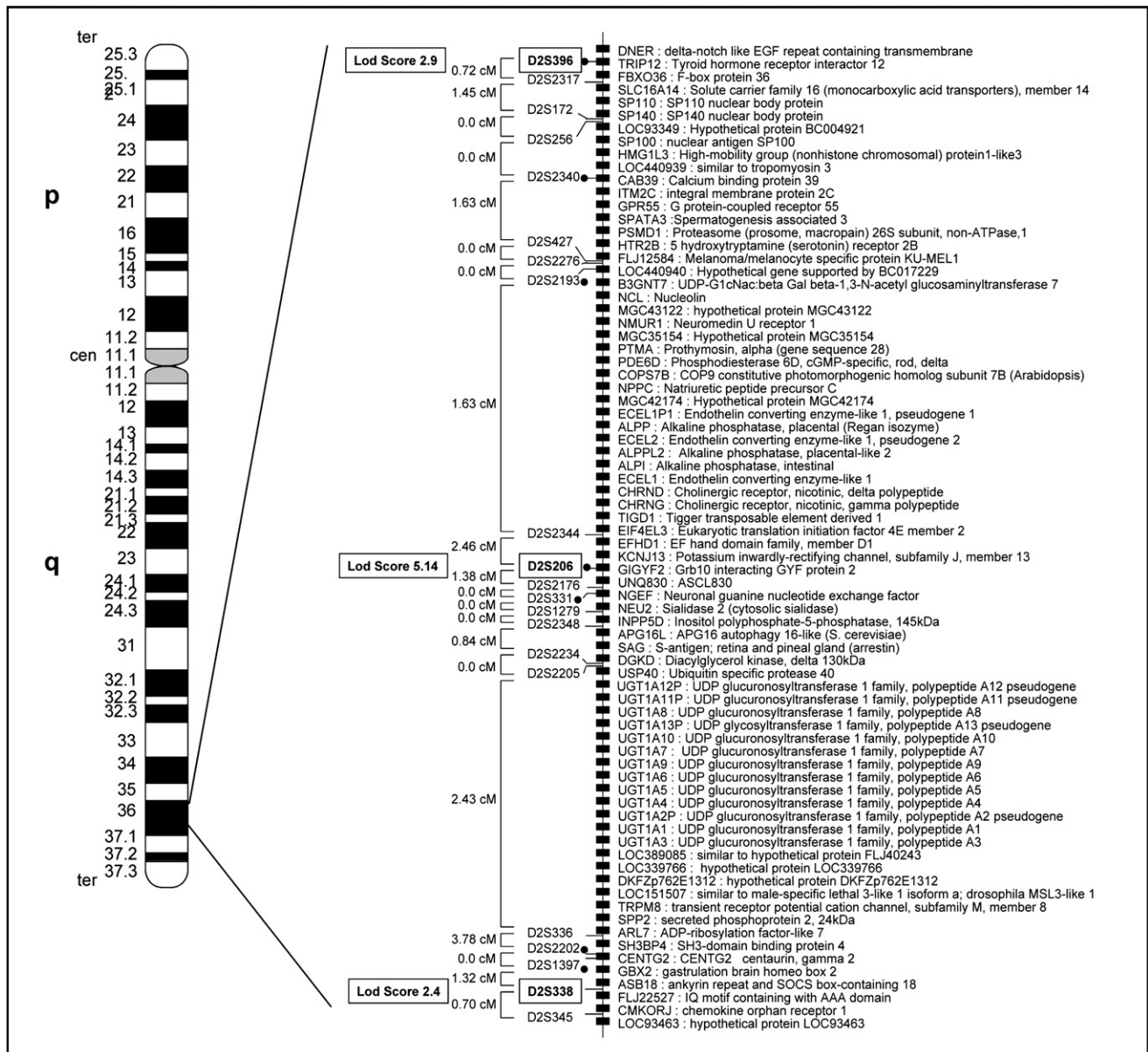
The PARK11 locus on chromosome 2q36-37 was initially identified by whole-genome linkage analysis in a population of PD patients with at least one first-degree affected relative.<sup>8,29,30</sup> This linkage region was not confirmed in another PD population.<sup>31</sup> However, an earlier association analysis,<sup>32</sup> which represents a more powerful method for identifying risk-factor genes,<sup>33</sup> also revealed significant association between markers in the PARK11 region and PD.

The PARK11 locus corresponds to an 18 cM interval between microsatellite markers D2S396 and D2S338, with LOD scores of 2.9 and 2.4, respectively,<sup>8,29,30</sup> that contains 73 potential candidate genes (Figure 1). The PARK11 microsatellite marker D2S206, which has the highest LOD score of 5.14, is contained within intron 21 of a 27-exon gene encoding 1299 amino acids, which has been alternately designated *GIGYF2* (Grb10-Interacting GYF Protein 2)<sup>34</sup> or *TNRC15* (Trinucleotide Repeat-Containing 15). We previously identified *GIGYF2* by yeast two-hybrid screening as one of two homologous proteins, *GIGYF1* (or *PERQ1*) and *GIGYF2*, interacting through their GYF domain with the Grb10 adaptor protein.<sup>34</sup> Grb10 and the *GIGYF* proteins are of interest for their potential involvement in insulin-like growth factor (IGF) and insulin signaling.<sup>34–39</sup> Because the IGFs and insulin have important effects in the central nervous system<sup>40,41</sup> and are potentially associated with PD,<sup>42–45</sup> we investigated the involvement of the *GIGYF2* (*TNRC15*) gene in PD.

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**Figure 1. Genetic Map of the PARK11 Locus, Extending from Markers D2S396 to D2S338**

Microsatellite markers enclosed within boxes have been significantly linked to Parkinson disease.<sup>29</sup> Dots indicate markers localized within the respective genes. Gene-mapping data are from the National Center for Biotechnology Information (NCBI) website.

## Methods and Materials

### Study Population

Index cases with familial Parkinson disease (PD) ( $n = 123$ ), defined by the presence of at least one first-degree affected relative (parent, child, or sibling), and healthy controls ( $n = 131$ ) were obtained from an Italian DNA bank assembled by the Parkinson Institute, Istituto Clinico di Perfezionamento (Milan, Italy) (Table 1). DNA from a second set of patients with familial PD compatible with dominant transmission ( $n = 126$ ) and controls ( $n = 96$ ) was obtained from the French Parkinson Disease Genetics Study Group (Paris, France) (Table 1). The clinical diagnosis of PD in both populations was established according to widely accepted criteria.<sup>46,47</sup> This required the presence of bradykinesia and at least one of the following: resting

tremor, rigidity, and postural instability; a positive response to dopaminergic therapy; the absence of atypical features or other causes of parkinsonism. Control DNAs were from unrelated individuals from the same populations. All controls were free of symptoms suggestive of PD,  $\geq 45$  years old, and with a negative family history for movement disorders at least in their first-degree relatives. The project was approved by the local ethical authorities, and written informed consent was obtained from all subjects.

The study population was analyzed for the *LRK2* G2019S mutation. Six of the 123 Italian patients with familial PD (4.8%) carried the G2019S mutation, which was absent from the 131 Italian controls.<sup>48</sup> In the French population, only two of the 126 familial-PD patients (1.6%) and one of the 96 controls (1.0%) carried the G2019S mutation.<sup>27,49</sup>

**Table 1. Population Phenotype Descriptions**

	Italian Population		French Population	
	PD n = 123	Control n = 131	PD n = 126	Control n = 96
Parkinson age of onset, Control age of study entry (Mean $\pm$ SD)	53 $\pm$ 12	69 $\pm$ 9	48 $\pm$ 12	64 $\pm$ 9
Young onset, $\leq$ 21 years	0		4	
Early onset, $\leq$ 45 years	28		50	
Late onset, $>$ 45 years	95		72	
At least one first-degree relative affected (%)	100		100	
Only father affected (%)	25.6		33.9	
Only mother affected (%)	24.0		32.2	
Both parents affected (%)	0.8		0.9	
Only siblings affected (%)	44.6		1.7	
Only progeny affected (%)	0		6.0	
At least one parent and one sibling affected (%)	5.0		18.3	
At least one parent and one progeny affected (%)	0		4.4	
At least one parent, one sibling and one progeny affected (%)	0		1.7	
At least one sibling and one progeny affected (%)	0		0.9	
Consanguinity (%)	8	ND	0	ND
Sex ratio (F/M)	54/69	75/56	63/63	61/35
Resting tremor (%)	80	0	78.3	0
Bradykinesia (%)	99	0	93.0	0
Rigidity (%)	78	0	88.9	0
Dementia (%)	5.9	0	11.7	0
Disease duration (mean year $\pm$ SD)	14.0 $\pm$ 6.4	0	10.2 $\pm$ 9.4	0
Positive L-Dopa response (% of patients)	98	0	94	0
L-Dopa treatment duration (mean year $\pm$ SD)	11.5 $\pm$ 6.6	0	8.25 $\pm$ 6.25	0

The L-dopa response was considered positive when at least a 30% improvement was observed on the basis of clinical assessment.

### GIGYF2 Gene Sequencing and Mutation Analysis

Genomic-primer design for all exons was performed with Vector NTI-Suite 6.0 (InforMax, Invitrogen). Each exon and at least 50 bp of flanking intronic sequence was PCR amplified from genomic DNA with the primer pairs listed in Table 2. PCR amplification was carried out with Taq DNA polymerase (FastStart Taq, 5U/ $\mu$ l, Roche) and 25 pmol of forward and reverse primers. PCR was performed in 50  $\mu$ l volumes with conditions of 95°C for 2 min followed by 35 cycles of 95°C for 30 s, annealing temperature for 30 s (58°C for exons 1,3,4,6,8–10,13–15,17,18,21,23–27; 53°C for exons 2,22; 47°C for exons 5,19; 59°C for exon 7; 56°C for exons 11,12,16; and 61°C for exon 20), and 68°C for 1 min, with a final 68°C hold for 5 min. Exons 9 and 10 plus the intervening intron were small enough to be amplified in a single PCR reaction. The PCR products were purified via Montage PCR<sub>96</sub> Plate vacuum filtration (Millipore) and sequenced with either the forward or reverse primer designed for the PCR amplification, except for several with a sequencing primer indicated by “seq” after the primer name in Table 2. Sequencing products were analyzed on an ABI 3730xl or ABI 3700 sequencer with Sequence Analysis 3.3 software (Applied Biosystems). All 27 coding exons of the *GIGYF2* gene were sequenced in the 249 familial-PD cases and 227 controls. All missense mutations, deletions, and insertions were subcloned for confirmation. Purified PCR-amplification products were vector ligated with the TOPO-TA cloning sequencing kit (Invitrogen). At least six recombinant colonies were selected at random for each variation and sequenced.

After the completion of these analyses, the eight exons found to contain mutations (exons 2, 4, 8, 9, 11, 14, 25, and 26) were PCR amplified and sequenced in 91 additional DNA samples obtained from the French Parkinson Disease Genetics Study Group.

### Statistical Analysis

Data are presented as mean  $\pm$  standard deviation. A database including genetic and phenotypic data for each PD patient and control was constructed in Statview 5.1 (SAS Institute, Cary, NC), and all statistical analyses were performed with this program. Allelic frequency (q) of *GIGYF2* gene variation was expressed as relative frequency in 2n chromosomes (with “n” as the number of subjects). Statistical significance was determined by Chi square testing ( $p < 0.05$ ). Hardy-Weinberg equilibrium was considered for a Hardy-Weinberg Chi square test  $< 3.84$ .

### Results

*GIGYF2* was screened for mutations as a candidate gene at the PARK11 locus by complete sequencing of all 27 coding exons in the index cases of families with PD from Caucasian populations (n = 249). This included 123 unrelated Italian PD patients and 126 unrelated French PD patients with at least one affected first-degree relative. For comparison, sequencing of the 27 coding exons of *GIGYF2* was also performed in 131 Italian and 96 French unrelated controls. The general characteristics of PD index cases and controls are shown in Table 1. Although most patients developed PD after age 45 (n = 167), there were also cases with early (n = 78) and juvenile onset (n = 4). Most patients had only one affected first-degree relative.

We identified seven different heterozygous amino acid changes in the *GIGYF2* gene in 12 unrelated index cases with PD ( $P_{mutX}$ ) (Table 3). None of these seven *GIGYF2*

**Table 2. GIGYF2 Gene Primers Used for PCR and Sequencing Analysis**

Primer Code	Sequence	PCR	Sequencing	Primer Size (bp)	PCR Size (bp)
GG2-E1F	GTCTGTCTCTACCTCTCTCACT	PCR		22	485 (E1F-E1R)
GG2-E1F seq	ACAGGTTTCTTCACATA		SEQ	17	
GG2-E1R	GTTTCAGCGAGGCACATAA	PCR	SEQ	20	
GG2-E2F	CTGATACTTTGAGATAG	PCR	SEQ	17	643 (E2F-E2R)
GG2-E2R	GCTCCTCTGTGGCTCTTT	PCR	SEQ	20	
GG2-E3F	TGCTAAGGACACATACAGTTGC	PCR	SEQ	22	735(E3F-E3R)
GG2-E3R	TGATTTTCTCCCTCCCAA	PCR	SEQ	20	
GG2-E4F	GTGTTATTTAGATGGTTGCT	PCR	SEQ	20	728 (E4F-E4R)
GG2-E4R	AAGTTCTCTATCCTCCTCCT	PCR	SEQ	20	
GG2-E5F	ATTTCACTATCAACTGT	PCR		18	910 (E5F-E5R)
GG2-E5F seq	TTTATGGTGGTGAATGTGTGC		SEQ	21	
GG2-E5R	CAAAAACACCTTAATGCTCA	PCR		21	
GG2-E5R seq	GATGTTCAATACTTA		SEQ	17	
GG2-E6F	TAGAAAATGGGAAAGTGAGTGTC	PCR	SEQ	24	807(E6F-E6R)
GG2-E6R	TGGCATTATTTTCTCCTCAG	PCR		20	
GG2-E6R seq	CTGATACAAAAGCAAAGAT		SEQ	19	
GG2-E7F	GCGCTTTGTGTGTGGATGAGG	PCR	SEQ	23	625 (E7F-E7R)
GG2-E7R	CTTCCCTTATTTTCAGTCC	PCR	SEQ	19	
GG2-E8F	CTTTAGAGTATGTTGCTGG	PCR	SEQ	20	662 (E8F-E8R)
GG2-E8R	TCTTATCGGGTCTTTGGC	PCR	SEQ	19	
GG2-E9-10F	TTAGAGGATGCTGAAGAAG	PCR	SEQ	19	678 (E9-10F - E9-10R)
GG2-E9-10R	GCATAAGCAGAAGCCTAACT	PCR	SEQ	20	
GG2-E11F	GTAGTTAGCCTTCACAGA	PCR	SEQ	18	296 (E11F-E11R)
GG2-E11R	TAAGAGGAGACAAAAGAAT	PCR	SEQ	20	
GG2-E12F	ATACCATTAGAGTTGACCA	PCR		20	873 (E12F-E12R)
GG2-E12F seq	TGGCATTGTGGGAGAAGTTAC		SEQ	22	
GG2-E12R	GGGATGCTCAACTGGTATGT	PCR	SEQ	20	
GG2-E13F	TTGACAGATGCCACCTCG	PCR	SEQ	18	321 (E13F-E13R)
GG2-E13R	GTGTTCCGTGACTAATA	PCR	SEQ	18	
GG2-E14F	GATGTCTGAGTTTTTATTC	PCR	SEQ	19	325 (E14F-E14R)
GG2-E14R	AGGTGAGACTTGAGAGCAGA	PCR	SEQ	20	
GG2-E15F	CTGAATAGACCCTTTGC	PCR		18	557 (E15F-E15R)
GG2-E15F seq	TTTCTAATAATGTCTCCTGGG		SEQ	21	
GG2-E15R	CGCTTATCTCCACCTGAAAT	PCR	SEQ	20	
GG2-E16F	CAAATAACCTGGAGAACCTGC	PCR		21	443 (E16F-E16R)
GG2-E16F seq	GCAAATGACAAGAACAAG		SEQ	18	
GG2-E16R	ATAAAAGAGATGCTGACT	PCR	SEQ	18	
GG2-E17F	GCTCATTTAGGCACAGGGA	PCR	SEQ	19	634 (E17F-E17R)
GG2-E17R	CGGGCTTTTGGCTGCTACG	PCR		20	
GG2-E17R seq	AGCAATGTTTATGTCTCTG		SEQ	18	
GG2-E18F	GAGTGTTCCTGTGCTGGTT	PCR	SEQ	20	239 (E18F-E18R)
GG2-E18R	GGTCAGAGTCCAGATACA	PCR	SEQ	20	
GG2-E19F	ATACTTTTTACTCCAGAT	PCR	SEQ	18	538 (E19F-E19R)
GG2-E19R	TGAGCAAAAACCAGAAAGAT	PCR	SEQ	20	
GG2-E20F	GCTTCTCTGTCATCTCTAT	PCR	SEQ	20	516 (E20F-E20R)
GG2-E20R	CTGGGTGATGGGAGTGAGAC	PCR	SEQ	20	
GG2-E21F	TGGAGTTGAGAGAAATGC	PCR	SEQ	18	308 (E21F-E21R)
GG2-E21R	GTAACCACTAACCATAAT	PCR	SEQ	19	
GG2-E22F	TATCTAAAAACAGGAATC	PCR	SEQ	18	329 (E22F-E22R)
GG2-E22R	TAAAAAGATAAGAAGACAGT	PCR	SEQ	20	
GG2-E23F	CGCCAGCAGAGGGAGTTGAT	PCR		20	550 (E23F-E23R)
GG2-E23F seq	AGTATTATCTTTCTGAC		SEQ	18	
GG2-E23R	CATAGGTGTGGTCATTTTG	PCR	SEQ	19	
GG2-E24F	CATCCATCCAGTATTGCCA	PCR	SEQ	19	305 (E24F-E24R)
GG2-E24R	GTCTGCTCTGTCAATCCAC	PCR	SEQ	20	
GG2-E25F	ATGTGGTATGGATGTTGTGATTAT	PCR		24	616 (E25F-E25R)
GG2-E25F seq	TTACCAAGTGTCTGCATTA		SEQ	20	
GG2-E25R	TGGGAGATACACAAAGGT	PCR		18	
GG2-E25R seq	CATTCTTGGGTTACTCA		SEQ	18	
GG2-E26F	CTGAGTGGGATGCTTGGTCTA	PCR		22	395 (E26F-E26R)
GG2-E26F seq	AGGGTGAGCCAAGTCTGAATCTGC		SEQ	24	
GG2-E26R	GCACTCTCCAACACTCA	PCR	SEQ	18	
GG2-E27F	ACAGACTTACATTCCAGC	PCR	SEQ	18	246 (E27F-E27R)
GG2-E27R	AAGAGTAAATGGTGAGTAAGTGT	PCR	SEQ	23	

**Table 3. GIGYF2 Mutations and Gene-Sequence Variation Identified in PD and Control Populations**

Exon	Variation Reference	Nucleotide Change	Amino Acid Change	Distance (bp)	Major Alleles	Allele Frequency (q)		
						PD 498 Chr.	Control 454 Chr.	p Value
<b>GIGYF2 Mutations</b>								
Ex2	PD <sub>mut</sub> -1	c.167A → G	Asn56Ser (AAC-AGC)	16776	A	0.008	0.000	0.056
Ex4	PD <sub>mut</sub> -2	c.334A → G	Thr112Ala (ACA-GCA)	25326	A	0.002	0.000	0.339
Ex8	PD <sub>mut</sub> -3	c.832A → G	Ile278Val (ATA-GTA)	59866	A	0.002	0.000	0.339
Ex9	PD <sub>mut</sub> -4	c.1003T → A	Ser335Thr (TCT-ACT)	60129	T	0.002	0.000	0.339
Ex11	PD <sub>mut</sub> -5	c.1370A → C	Asn457Thr (AAT-ACT)	63886	A	0.006	0.000	0.098
Ex14	PD <sub>mut</sub> -6	c.1818C → G	Asp606Glu (GAC-GAG)	78790	C	0.002	0.000	0.339
Ex25	PD <sub>mut</sub> -7	c.3625_3648del24	Del LPQQQQQQ 1209-1216	116588		0.004	0.000	0.184
Ex25	PD <sub>mut</sub> -8	c.3648_3649insCAACAG	Ins QQ 1217	116611		0.002	0.000	0.357
Ex25	PD <sub>mut</sub> -9	c.3661_3672del12	Del PPQQ 1221-1224	116624		0.002	0.000	0.357
Ex26	PD <sub>mut</sub> -10	c.3724G → A	Val1242Ile (GTA-ATA)	119378	G	0.002	0.000	0.339
Ex26	Ctrl <sub>mut</sub> -1	C.3512A → G	His1171Arg (CAT-CGT)	116475	A	0.000	0.002	0.286
<b>GIGYF2 Variants</b>								
Ex11	Variant-rs2289912	c.1378C → A	Pro469Thr (CCT-ACT)	63894	C	0.018	0.020	0.843
Ex25	Variant-1	c.3613_3633del21	Del QQQQLPQ 1205-1211	116576		0.056	0.057	0.924
Ex25	Variant-rs10555297	c.3630_3632del3	Del Q 1210	116593	DelACA	0.464	0.457	0.843
Ex25	Variant-2	c.3633_3634insCAG	Ins Q 1212	116596		0.016	0.021	0.555
Ex25	Variant-3	c.3673_3684del12	Del PQQQ 1225-1228	116636		0.018	0.007	0.144
<b>GIGYF2 SNPs</b>								
Ex2	SNP-1	c.69T → C	Ser23 (AGT-AGC)	16678	T	0.002	0.002	0.948
Ex8	SNP-2	c.828G → C	Gly276 (GGG-GGC)	59862	G	0.002	0.002	0.948
Ex9	SNP-3	c.1047T → C	Asp349 (GAT-GAC)	60173	T	0.002	0.000	0.407
Ex12	SNP-rs2305138	c.1554G → A	Glu518 (GAG-GAA)	65187	G	0.054	0.035	0.159
Ex13	SNP-4	c.1716G → T	Ala572 (GCG-GCT)	75626	G	0.006	0.000	0.150
Ex21	SNP-5	c.2835G → T	Ser945 (TCG-TCT)	108989	G	0.004	0.000	0.241
Ex22	SNP-rs3816334	c.2940G → A	Gln980 (CAG-CAA)	113169	G	0.281	0.319	0.198
Ex25	SNP-rs7563724	c.3630A → G	Pro1210 (CCA-CCG)	116593	A	0.014	0.021	0.445
Ex25	SNP-rs12328151	c.3651G → A	Pro1217 (CCG-CCA)	116614	G	0.227	0.225	0.948
Ex27	SNP-6	c.3855G → A	Ser1285 (TCG-TCA)	125899	G	0.006	0.009	0.615

mutations found in PD patients were observed in 227 controls. They consist of seven single-nucleotide changes resulting in amino acid substitutions in exon 2 (Asn56Ser), exon 4 (Thr112Ala), exon 8 (Ile278Val), exon 9 (Ser335Thr), exon 11 (Asn457Thr), exon 14 (Asp606Glu), and exon 26 (Val1242Ile) (Table 3, Figure 2). The Asn56Ser substitution was found in four unrelated PD patients (one Italian and three French), and the Asn457Thr substitution was present in three unrelated PD patients (two Italian and one French). All other amino acid substitutions were observed only in a single PD patient. In contrast, sequencing of all coding exons in 227 unrelated control individuals detected a single 71-year-old healthy Italian woman without a family history of PD, with an amino acid substitution in exon 25 (His1171Arg, Ctrl<sub>mut</sub>-1) (Table 3, Figure 2), which was not found in the PD population or other control subjects. The eight exons shown to contain mutations in the PD patients and the single control (exons 2, 4, 8, 9, 11, 14, 25, 26) then were sequenced in a further 91 control DNA samples from the French Parkinson Disease Genetics Study Group. In this set of additional controls, none of the identified mutations and no new variations were found.

Most of the seven single amino acid substitutions identified in GIGYF2 in patients represent conservative amino acid sequence changes, but alignment of the human GIGYF2 protein sequence with 12 other species demonstrates

that they occur within highly conserved amino acid blocks and involve residues that are highly, although not absolutely, conserved across species (Figure 3). In addition, these amino acid substitutions are significantly more frequent in PD patients (12 of 249) than in controls (1 of 227) ( $p < 0.003$ ).

Among the 12 PD probands with GIGYF2 mutations, eight had at least one parent affected, one had both parents affected, and three had a single affected sibling (Figure 4). PD was present by medical history across three successive generations in three of the families and across two generations in five of the families (Figure 4). Among the probands of families with GIGYF2 missense mutations, there were a total of six females and six males with the diagnosis of PD, and the occurrence of PD in two or three successive generations in most families is consistent with autosomal-dominant inheritance. DNA samples were available from at least one affected relative in four families. In family 068-004, PD was present in three successive generations and the affected mother of the male proband was shown to have the same Asp606Glu mutation (Figure 4). The proband was diagnosed with PD at age 42, his mother at age 83. In family 057-009, the male index case inherited the Asn56Ser mutation from his affected mother. The age of PD onset was 41 in the proband and 73 in the mother. Among eight siblings of the proband, three were



**Figure 2. Chromatograms Illustrating *GIGYF2* Gene Mutations**

All show analysis by forward sequencing, except for the exon 4 (Thr112Ala) mutation, which was sequenced in reverse, and insertion (Ins QQ 1217) and deletions (Del LPQQQQQ 1209-1216 and Del PPQQ 1221-1224), for which individual alleles were sequenced after subcloning.

	Asn56Ser	Thr112Asp	Ile278Val	Ser335Thr	Asn457Thr	Asp606Glu	His171Arg	Val1242Ile
Homo sapiens	LFLKDNKIPS	GGGTVVGAPR	GSGSIDDDRD	GEECSDSEGS	PPVFNPSPTL	PHMGELDQER	ESPYEVHDIYI	LHSVVFQTN
Pan troglodytes	LFLKDNKIPS	GGGTVVGAPR	GSGSIDDDRD	GEECSDSEGS	PPVFNPSPTL	PHMGELDQER	ESPYEVHDIYI	LHSVVFQTN
Macaca mulatta	LFLKDNKIPS	GGGTVVGAPR	GSGSIDDDRD	GEECSDSEGS	PPVFNPSPTL	PHMGELDQER	ESPYEVHDIYI	LHSVVFQTN
Canis familiaris	LFLKDNKIPS	GGGTVVGAPR	GSGSIDDDRD	GEECSDSESS	PPVFNPSPAL	PHMGELDQER	ESPYEVHDIYI	LHSVVFQTN
Bos taurus	LFLKDNKIPS	GGGTVVGAPR	GSGSIDDDRD	GEECSDSEGS	PPVFNPSPAL	PHMGELDQER	ESPYEVHDIYI	LHSVVFQTN
Equus caballus	LFLKDNKIPS	GGGTVVGAPR	GSGSIDDDRD	GEECSDSESS	PPVFNPSPAL	PHMGELDQER	ESPYEVHDIYI	LHSVVFQTN
Rattus norvegicus	LFLKDKIPF	GGGTVVGAPR	GSGSIDDDRD	GEERSDSDSS	PPLPTPSSAS	PHMGELDQER	ESPYEVHDIYI	LHSVVFQTN
Mus musculus	LFLKDKIPF	GGGTVVGAPR	GSGSIDDDRD	GEERSDSDSS	PPVFNPSPAL	PHMGELDQER	ESPYEVHDIYI	LHSVVFQTN
Gallus gallus	LFVKDNKIPS	GG-TVVGAPR	GSGSIDDDRD	GEERSDSEGS	PPVNSNPAL	PHMGELDQER	ESPYEVHDIYI	LHSVVFQTN
Monodelphis domestica	LFLKDNKIPS	GGGTAVGAPR	GSGSIDDDRD	GEERSDSEGS	PPVASAGPAL	PHMGELDQER	ESPYEVHDIYI	LHSVVFQTN
Danio rerio	LYVKDNMIPV	GGGTAVGAPR	GSGSAEERD	-----	-----	PILGDADQER	DSPEVHDIYI	-----
Xenopus laevis	LFVKDNKIPS	GGGTAVGAPR	GSGSAEERD	GADLSDSDGS	-----	PRLGDLDPER	ESPYEVHDIYI	-----
Ornithorhynchus anatinus	LFLKDNKIPS	GGGTAVGAPR	-----	-----	-----	-----	ESPYEVHDIYI	LHSVVFQTN

**Figure 3. Alignment of GIGYF2-Mutation Protein-Sequence Regions in Multiple Species**

Overall identity of available amino acid sequences of various species with *Homo sapiens* (gi 42476299) is: *Pan troglodytes* (gi 114583902) 99%, *Macaca mulatta* (gi 109101490) 99%, *Canis familiaris* (gi 73993973) 97%, *Bos taurus* (gi 156120403) 95%, *Equus caballus* (gi 149711637) 95%, *Rattus norvegicus* (gi 149016387) 94%, *Mus musculus* (gi 34365797) 93%, *Gallus gallus* (gi 118094814) 86%, *Monodelphis domestica* (gi 126314299) 86%, *Danio rerio* (gi 71834468) 56%, *Xenopus laevis* (gi 148223517) 56%, and *Ornithorhynchus anatinus* (gi 149599305) 39%. The dotted lines correspond to sequence regions not yet available or sequence regions of known sequence but nonhomology.

genotyped. Two of these, a dizygotic female twin and a brother, bear the same mutation but do not have clinically apparent PD. Their ages at the time of most-recent medical history were 61 and 73 years. Follow-up will be required to determine whether they ultimately develop PD. Another unaffected brother, age 67, does not carry the Asn56Ser mutation. In family 28-V-0056, the male index case had an affected brother, and both carried the Thr112Ala mutation. Although both brothers had onset at age 43, neither their mother, who died at age 72, nor their father, who died at age 52, had known PD (Figure 4).

In the Italian family 1034-D-0385, the female index case inherited the Ile278Val candidate mutation from her father. Sequence analysis of a second PD-associated gene, *LRRK2*, in this family revealed the previously described *LRRK2* Ile1371Val mutation in the index case and her mother.<sup>50</sup> The father had PD onset at age 66, the mother at age 61, and the index case bearing the two different gene mutations had PD onset at the much earlier age of 31 years. She has had progressive, severe PD with marked fluctuations in clinical course in spite of initial response to L-dopa therapy. At 50 years of age, she currently is partially compensated on intraduodenal L-dopa infusions.

Three other nucleotide variations were found in four index cases with PD and none of the controls. They consisted of a deletion of eight (Del LPQQQQQQ 1209–1216) and four codons (Del PPQQ 1221–1224) and an insertion of two codons (Ins QQ 1217) in exon 25 (Table 3). The Del LPQQQQQQ 1209–1216 deletion was detected in two unrelated French PD patients. The InsQQ1217 insertion was associated with the Asn457Thr missense mutation in a patient with onset at age 43. These deletion and insertion sequences are in a region of Gln (Q) and Pro (P) repeats in exon 25 that is not conserved across species. Furthermore, four other insertions and deletions have been observed in this region, with similar frequencies in PD patients and controls. One was previously described in dbSNP (rs10555297, Table 3), and three are new (Variant-1, -2, and -3, Table 3). We therefore speculate that the rare deletion and insertion

sequences identified in PD patients could represent less-frequent variants without functional significance.

In addition, we found one amino acid substitution already described in dbSNP (rs2289912) (Table 3), as well as ten nonsynonymous SNPs, including four previously described in dbSNP (SNP-rsxxxx) (Table 3), and six new SNPs (SNP-X) (Table 3). All these variants appear to be polymorphisms on the basis of their similar frequencies in PD cases and controls.

## Discussion

Our study has examined *GIGYF2* (*TNRC15*) as a candidate gene for PARK11. This was accomplished by complete sequencing of all 27 coding exons of the *GIGYF2* gene in two different populations of Caucasian patients with familial PD and in population-matched controls (123 Italian PD patients and 131 controls, and 126 French PD patients and 96 controls). We found seven different missense mutations in the *GIGYF2* gene in 12 index patients with PD from Italy (n = 7) and France (n = 5). The following evidence supports the pathogenic role of these amino acid changes: i) they are not found in geographically matched controls (a single amino acid change was detected in only one of 127 controls) (p < 0.003); ii) the seven PD missense mutations (Asn56Ser, Thr112Ala, Ile278Val, Ser335Thr, Asn457Thr, Asp606Glu, and Val1242Ile) and the control mutation (His171Arg) were absent from an additional 91 controls; iii) they involve amino acids that are relatively conserved across 12 nonhuman species; iv) when available affected relatives were sampled, they were also found to carry the same missense mutation (except for one of the parents in the case with bilineal transmission of PD). These data provide strong support for a role of mutations in the *GIGYF2* gene as a frequent cause of familial PD, at least in Italy and France, and as a major contributing factor at the PARK11 locus. The other sequence variants found in patients are probably not causative, because they affect a nonconserved region containing highly

polymorphic repeats rich in Q and P residues. They are more likely rare polymorphisms.

The 12 index cases with PD and *GIGYF2* missense mutations include six women and six men with a mean age at onset of  $48.7 \pm 10.2$  years (range 33–68). The clinical features of these patients did not differ from those of patients with typical idiopathic PD (Table 4). Unilateral tremor and bradykinesia appeared at onset in most of the mutation carriers, all of whom responded to L-dopa and developed dyskinesia and/or motor fluctuations as the disease progressed.

The *GIGYF2* missense mutations are all found in the heterozygous state compatible with autosomal-dominant transmission. This inheritance pattern is also supported by the observation of parent-child transmission in 11 of the 12 pedigrees. However, age-dependent penetrance or reduced penetrance is likely. In family 20-V-0056, the parents of the two affected siblings died without being diagnosed with PD, and in family 757-028, two siblings with the mutation are still healthy even though they are older than their affected brother. It will be important to precisely evaluate the penetrance of *GIGYF2* mutations and to determine their frequency in idiopathic PD.

It has been suggested that interactions between multiple genetic factors could have a role in the development of PD.<sup>52</sup> In the first report on the *PARK11* locus, the LOD score of 5.14 for the entire study population was noted to decrease to 4.12 when 11 of 65 families with known *Parkin* mutations were excluded.<sup>30</sup> In the present study, we provide further evidence for gene interaction involving the *PARK11* locus in an individual with co-occurrence of Ile278Val *GIGYF2* and Ile1371Val *LRRK2* mutations, who had a much earlier onset of PD than did either parent with single gene mutations. Given that *GIGYF2* and *LRRK2* mutations have each been found in approximately 5% and 6% of familial PD patients, respectively, it should be possible to identify additional patients with both mutations to further test their potential interaction. Although the functional role of the *LRRK2* protein is not known, it contains a mixed-function kinase-like sequence, and the most common *LRRK2* mutations (G2019S and Arg1441Cys) enhance the kinase activity.<sup>53</sup> Since *GIGYF2* was initially identified through its binding to the Grb10 adaptor protein<sup>34</sup> and Grb10 is thought to function by linking proteins to tyrosine kinases,<sup>36</sup> it will be important in future studies to determine whether *GIGYF2* interacts with the *LRRK2* kinase.

The function of the *GIGYF2* protein is presently unknown. Consistent with the development of a neurodegenerative disease in humans with *GIGYF2* mutations, *GIGYF2* mRNA is strongly expressed in multiple regions of the central nervous system (Novartis Gene Expression Atlas). Together with the homologous *GIGYF1* protein, *GIGYF2* was cloned from a mouse-cDNA-expression library by yeast 2-hybrid screening with an N-terminal fragment of the Grb10 adaptor protein.<sup>34</sup> The Grb10 N-terminus contains a proline-rich region, and both *GIGYF* proteins

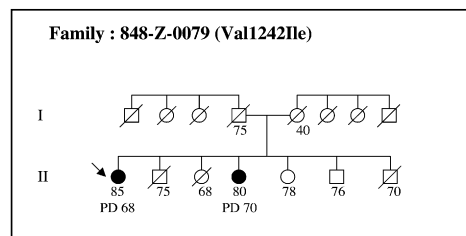
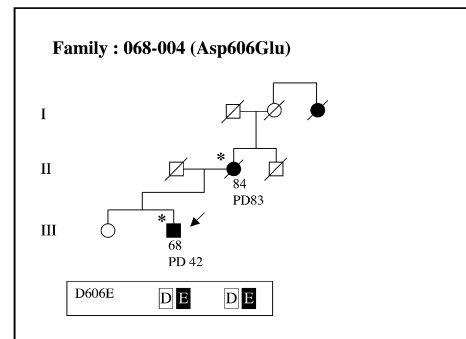
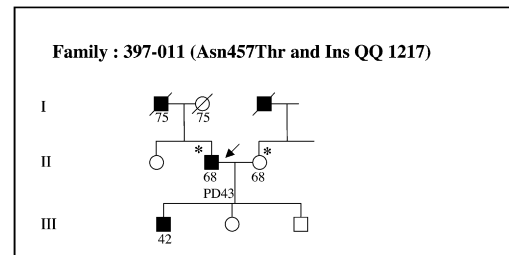
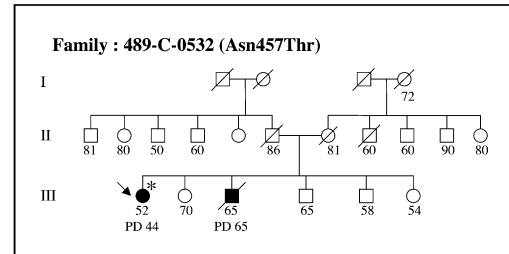
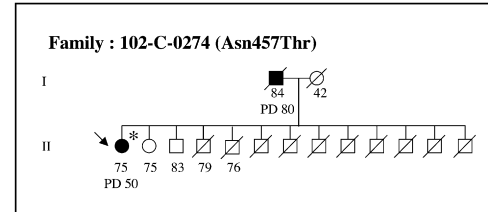
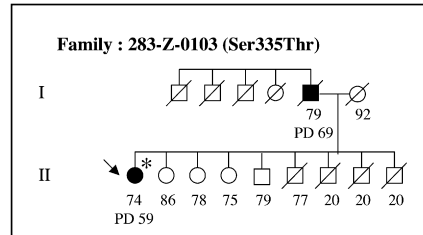
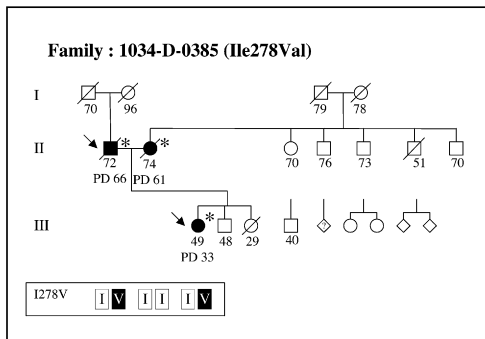
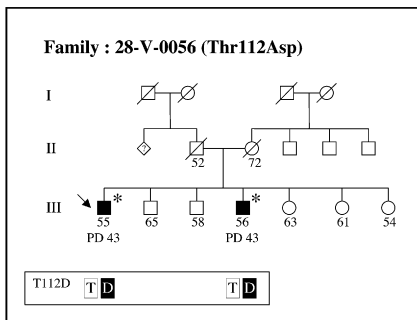
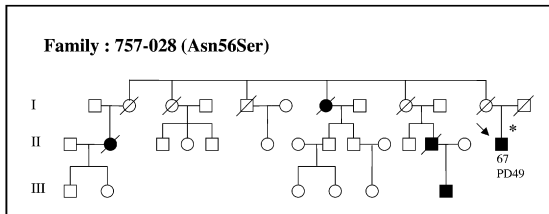
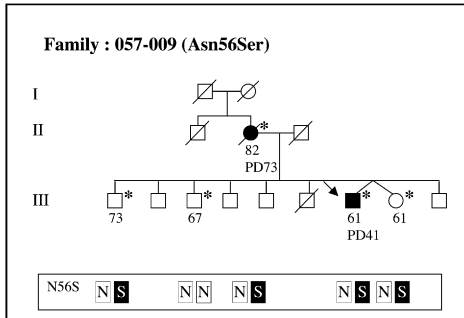
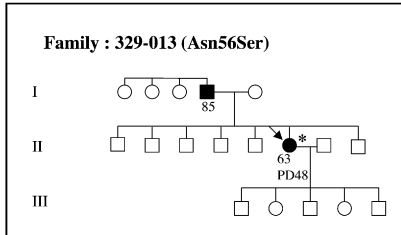
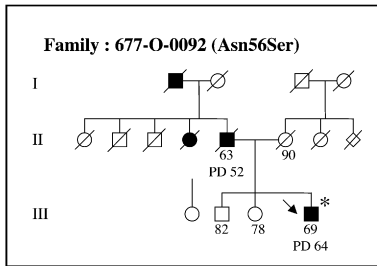
contain a GYF motif, which had previously been shown to bind proline-rich sequences.<sup>54</sup> With the yeast 2-hybrid method, the GYF domains of *GIGYF1* and *GIGYF2* were confirmed to interact with the Grb10 proline-rich region.<sup>34</sup> The GYF domain is named for a Gly-Tyr-Phe triad and forms a bulge-helix-bulge structure important for binding to proline sequences.<sup>54</sup> On the basis of its flanking amino acid residues, the GYF domain of *GIGYF2* can be classified as a member of the SMY2 subtype.<sup>55</sup> There is evidence that this class of GYF domains has a preferred pro-rich binding motif that is present in several proteins with roles in mRNA splicing. GYF-domain-containing proteins in general are hypothesized to function in mRNA splicing and/or other aspects of mRNA processing, such as nuclear export.

The interaction between the *GIGYF* proteins and the Grb10 adaptor protein evident in the yeast 2-hybrid system has been confirmed in mammalian cells expressing endogenous levels of Grb10 and a myc-tagged GYF-domain-containing fragment of the *GIGYF1* protein.<sup>34</sup> Further studies demonstrated that this fragment of *GIGYF1* was recruited to activated IGF-I receptors, presumably via the Grb10 adaptor. Of interest, overexpression of the *GIGYF1* fragment resulted in augmented IGF-I receptor signaling. Similar studies with full-length *GIGYF1* or *GIGYF2* have not been possible, because the full-length proteins form toxic inclusion bodies in cells when overexpressed, presumably as a consequence of protein self-aggregation.<sup>34</sup> In future experiments at *GIGYF2* cellular concentrations below the threshold for inclusion-body formation, it will be important to determine whether the association of *GIGYF2* with PD involves its interaction with Grb10 and the IGF and/or insulin signaling pathways. It will also be important to determine whether the mutations that we have identified lead to the development of PD by altering *GIGYF2* interactions with Grb10 and the IGF and/or insulin pathways or other functions of the *GIGYF2* protein.

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**Table 4. Phenotype of Patients with GIGYF2 Mutations**

Patient Number	677-0-0092	057-009	329-013	757-028	28-V-0056	1034-D-0385	283-Z-0103	102-C-0274	489-C-0532	397-011	068-004	848-Z-0079
GIGYF2 Mutation	Asn56Ser				Thr112Asp	Ile278Val	Ser335Thr	Asn457Thr			Asp606Glu	Val1242Ile
Sex (M/F)	M	M	F	M	M	F	F	F	F	M	M	F
Origin (ITA/FRA)	ITA	FR	FR	FR	ITA	ITA	ITA	ITA	ITA	FR	FR	ITA
Age of Onset (yr)	64	41	48	49	43	33	59	50	44	43	42	68
Disease Duration at Examination (yr)	7	9	10	15	14	19	15	25	8	25	15	17
Resting Tremor	–	ND	+	–	+	+	+	+	+	+	+	+
Bradykinesia	+	ND	+	+	+	+	+	+	+	+	+	+
Rigidity	+	ND	+	+	+	+	+	+	+	+	+	+
Dementia	–	ND	–	–	–	–	–	–	–	–	–	–
L-Dopa Response	+	ND	+	+	+	+	+	+	+	+	+	+
L-Dopa Duration (yr)	7	ND	8	10	10	15	13	21	3	25	7	17
UPDRS (ON)	14	ND	ND	9	19	24	22	26	13	35	9	18
UPDRS (OFF)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	16	37
H & Y (ON)	2.5	ND	ND	1.5	2.5	3	3	3	2	2.5	2.5	2.5
H & Y (OFF)	ND	ND	ND	ND	ND	4	3	ND	ND	ND	ND	3

"ND" denotes "not determined."

"–" denotes "phenotype absent."

"+" denotes "phenotype present."

"ITA" denotes "Italy."

"FRA" denotes "France."

"UPDRS" denotes "Unified Parkinson's Disease Rating Scale."<sup>51</sup>

"H & Y" denotes "modified Hoehn and Yahr staging."<sup>51</sup>

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## Web Resources

The URLs for data presented herein are as follows:

Novartis Gene Expression Atlas, <http://expression.gnf.org>

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>

Parkinson Institute DNA Bank, <http://www.parkinson.it/dnabank.html>

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## Figure 4. Pedigrees of Patients with GIGYF2 Missense Mutations

Arrows indicate the index cases. Patients with PD are in black, with current age or age at death indicated if known. Age of onset is the number adjacent to PD. Numbers under other family members correspond to the best-available estimate of current age or the age at death based on information provided by the index cases. Asterisks indicate individuals from whom DNA was obtained. When information on mutation segregation was available, the genotypes are indicated in the box below the pedigree.

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