Variation in *GIGYF2* is not associated with Parkinson disease

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ABSTRACT

Objective: A recent study reported that mutations in a gene on chromosome 2q36-37, *GIGYF2,* result in Parkinson disease (PD). We have previously reported linkage to this chromosomal region in a sample of multiplex PD families, with the strongest evidence of linkage obtained using the subset of the sample having the strongest family history of disease and meeting the strictest diagnostic criteria. We have tested whether mutations in *GIGYF2* may account for the previously observed linkage finding.

Methods: We sequenced the *GIGYF2* coding region in 96 unrelated patients with PD used in our original study that contributed to the chromosome 2q36-37 linkage signal. Subsequently, we genotyped the entire sample of 566 multiplex PD kindreds as well as 1,447 controls to test whether variants in *GIGYF2* are causative or increase susceptibility for PD.

Results: We detected three novel variants as well as one of the previously reported seven variants in a total of five multiple PD families; however, there was no consistent evidence that these variants segregated with PD in these families. We also did not find a significant increase in risk for PD among those inheriting variants in *GIGYF2* ($p = 0.28$).

Conclusions: We believe that variation in a gene other than *GIGYF2* accounts for the previously reported linkage finding on chromosome 2q36-37. *Neurology*® 2009;72:1886–1892

GLOSSARY

GDS = Geriatric Depression Scale; **MMSE** = Mini-Mental State Examination; **NCRAD** = National Cell Repository for Alzheimer's Disease; **PD** = Parkinson disease; **PSG** = Parkinson Study Group; **UPDRS** = Unified Parkinson's Disease Rating Scale.

Parkinson disease (PD) is the second most common neurodegenerative disorder, affecting 3% of the population above age 75.[1] Mutations in five genes can result in autosomal dominant or autosomal recessive forms of PD.[2] Previously, we reported linkage to an 18 cM region on chromosome 2q36-37 in a sample of 194 multiplex PD kindreds.[3] Subsequently, we demonstrated that the evidence of linkage in this region was even greater when the dataset was limited to the subset of pedigrees having a stronger family history of PD, typically consistent with autosomal dominant inheritance.[3,4]

Within the 18-cM region identified in our linkage study, the gene for Grb10-Interacting GYF protein 2 (*GIGYF2*) was identified by Giovannone and colleagues[5] using yeast two-hybrid screening in a study of novel proteins linked to insulin-like growth factor receptors by the Grb10 adapter. GIGYF2 is hypothesized to modulate IGF-I signaling.[5] As the IGFs and insulin have important effects in the CNS and are potentially associated with PD,[6-11] the

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Medical Devices: 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA).

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Table 1	Description of 96 sequenced patients with Parkinson disease (PD)
Characteristics	**Values**
Age at onset, y, mean ± SD	62.0 ± 10.5
Average no. of other reported family members with PD	3.7
Parent reported with PD (%)	39.6
Verified PD (%)	100
Autopsy confirmation of PD (%)	7.3

potential involvement of *GIGYF2* (also known as *TNRC15*) in PD was recently investigated.[12] The identification of seven missense mutations in *GIGYF2* in 12 of 249 unrelated patients with PD was reported. These mutations were not observed in 227 controls.

Owing to the small size of many of the pedigrees and the sampling of only some of the affected individuals, there were very limited data to suggest that these mutations segregate within these families. The goals of this study were to characterize sequence variation within *GIGYF2* in a select subset of our large sample of patients with familial PD and to test in our entire sample whether any identified sequence variants increased the risk for PD.

METHODS

Subjects. As part of an ongoing study designed to identify genes contributing to PD susceptibility (PROGENI Study), subjects with PD were recruited through the Parkinson Study Group (PSG), a network of 65 participating clinical centers located throughout North America. The inclusion criterion was a sibling pair, both of whom were reported to have a diagnosis of PD or were showing signs of PD. Subjects were seen in person by a movement disorder specialist who completed the Unified Parkinson's Disease Rating Scale (UPDRS) Parts II and III,[13,14] the Mini-Mental State Examination (MMSE),[15] the Geriatric Depression Scale (GDS),[16] and the Blessed Functional Activity Scale.[17] In addition, a Diagnostic Checklist was completed, which consists of inclusion criteria associated with autopsy confirmed PD as well as exclusion criteria corresponding to features associated with other non-PD pathologic diagnoses.[18] Responses on the Diagnostic Checklist were used to classify each subject with PD as either verified PD (VPD, n = 871), with all findings consistent with PD, or nonverified PD (NVPD, n = 453), with the subject failing to meet at least one inclusion criterion or meeting one exclusion criterion. Peripheral blood was obtained after completion of appropriate written informed consent approved by each individual institution's institutional review board.

The control sample consisted of 1,447 neurologically normal non-Hispanic Caucasians who provided appropriate written informed consent. The control samples were obtained from three different sources: the National Cell Repository for Alzheimer's Disease (NCRAD), the National Institute of Neurological Disorders and Stroke Human Genetics Resource Center at the Coriell Cell Repositories (Camden, NJ; DNA), and controls recruited as part of an ongoing PD study at Indiana University (PROGENI-CARES).[19]

Molecular methods. PCR and sequencing primers were designed using the chromosome 2 genomic contig sequence NC_000002.10 enabling PCR/sequencing of all 27 coding exons and intron/exon boundaries of *GIGYF2* (table e-1 on the *Neurology*® Web site at www.neurology.org). PCR products were purified and sequenced as previously described.[19]

TaqMan allelic-discrimination assays (Applied Biosystems, Foster City, CA) were developed to screen for the novel missense variants identified in the 96 sequenced samples as well as the seven point mutations (N56S, T112A, I278V, S335T, N457T, D606E, V1242I) previously reported.[12] These 10 assays were used to genotype our complete sample of 566 PD families (with 1,497 family members, 1,324 reported to have PD), as previously described,[19,20] as well as the 1447 neurologically normal non-Hispanic Caucasian control subjects (tables 1 and 2).

PCR primers were designed flanking the polyglutamine repeat region in exon 25. The forward primer 5′-GGAGTTTGCCAAGCAGTCC-3′ and the reverse primer 5′-TACCGCATACACCACACTAC-3′ were used to amplify DNA from ~200 PD and ~100 control subjects. The PCR products were analyzed by electrophoresis through 4% composite agarose and visualized by ethidium bromide staining. Based on the results of the gel electrophoresis, exon 25 PCR products corresponding to at least six different patterns were cloned using the TOPO Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA). DNA sequence analysis was performed on miniprep DNA from 15 different clones from each of the cloned patterns. To determine the frequency of each of these different alleles in all 1,497 family members (1,324 PD subjects) and 1,447 controls, the forward primer used to PCR amplify the polyglutamine re-

Table 2 Description of 1,324 genotyped patients with Parkinson disease (PD) and 369 controls

Source	Type	No.	Mean (range) age at onset*/examination,† y	% Male
Patients with PD	Cases	1,324	60.9 (18-89)	57.8
Verified patients with PD	Cases	1,175	60.4 (18-84)	59.1
NINDS Human Genetics Resource Center (Coriell)	Controls	871	58.0 (19-90)	42.3
PROGENI-CARES	Controls	228	65.5 (28-83)	49.8
National Cell Repository for Alzheimer's Disease	Controls	44	76.9 (58-92)	43.2

*Age at onset of patients with PD.
†Age at examination of controls.
NINDS = National Institute of Neurological Disorders and Stroke.

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Table 3 *GIGYF2* screening

Exon	Nucleotide change	Amino acid change	No. of PD subjects (no. of families)	No. of controls
2	c.167A→G	N56S	4 (2)	0
4	c.334A→G	T112A	0	0
7	c.684T→A*	D228E	2 (1)	0
8	c.832A→G	I278V	0	0
9	c.1003T→A	S335T	0	0
10	c.1219A→G*	K407E	1 (1)	0
11	c.1370A→C	N457T	0†(1)	0
14	c.1818C→G	D606E	0	0
25	c.3583C→T*	R1195C	1 (1)	0
26	c.3724G→A	V1242I	0	1

*Novel variant identified in sequencing 96 PD subjects from the families with the strongest evidence of linkage to chromosome 2q.
†This individual did not have PD.
PD = Parkinson disease.

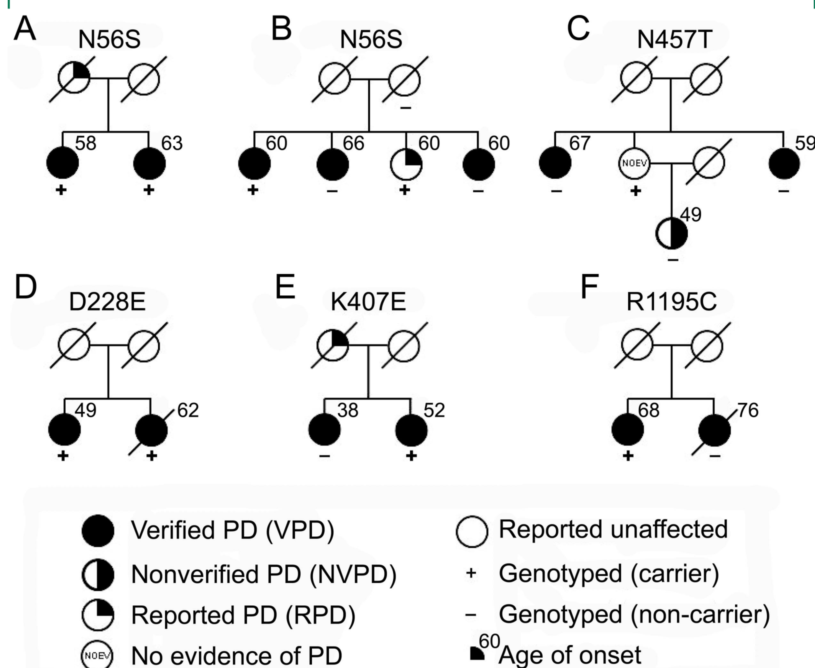
gion was labeled with 6-FAM and fluorescent genotyping was performed on a 3730xl DNA Analyzer (Applied Biosystems) and analyzed using GeneMapper 4.0 (Applied Biosystems). Allele counts were permuted using only one individual per family, and χ^2 and odds ratios were calculated separately for each allele using these counts. Genetic association analyses were only performed using non-Hispanic Caucasian samples (cases and controls).

RESULTS To investigate the frequency of *GIGYF2* variants in familial PD, all 27 exons of *GIGYF2* were sequenced in one PD case from each of 96 different multiplex PD families (table 1). These PD cases were specifically selected because their families provide evidence of linkage to chromosome 2q.[3,4] Sequencing in the 96 index PD cases identified one subject heterozygous for the previously reported N56S variant in exon 2.[12] We also identified one subject with each of the following novel variants: D228E in exon 7, K407E in exon 10, and R1195C in exon 25 (table 3). In addition, seven substitutions previously reported as polymorphisms[12] were identified in 78 of the 96 index cases: P460T (rs2289912): 1 patient (reported incorrectly as P469T[12]); E518E (rs2305138): 12 patients; S945S: one patient; Q980Q (rs3816334): 65 patients; delQ1210 (rs10555297): 77 patients; P1217P (rs12328151): 41 patients; S1285S: 5 patients (table 3).

Our entire sample of 566 multiplex PD families, consisting of 1,497 members, including 1,324 reported to have PD, were genotyped for the seven *GIGYF2* point mutations previously reported[12] as well as the three novel missense variants we identified through sequencing. We identified several additional subjects carrying *GIGYF2* variants (figure 1). Among the seven previously reported *GIGYF2* variants, the N56S variant identified in one of the 96 sequenced patients with PD was identified in three additional subjects: one from the same family as the subject identified by sequencing and two in an additional family. In family A, both siblings with verified PD carried the N56S variant. In family B, four siblings were initially reported to have symptoms of PD. Following evaluation, three of the siblings met criteria for verified PD, while the fourth sibling did not complete a study visit, but did provide a blood sample. Among these four siblings, two carried the N56S variant while two did not. The mother of these four siblings provided a blood sample but was not evaluated in person. She did not report any symptoms of PD before death at age 90 and did not carry the N56S variant. The father of these siblings died at the age of 39 and was not reported to have any symptoms of PD. In this larger family, the N56S does not segregate completely with disease. Combining our results with those of the previous 249 subjects with PD studied,[12] the N56S variant has been identified in 0.9% (3 of 345) of unrelated PD subjects and 0.4% (6 of 1573) of all PD subjects studied.

One individual was shown to be heterozygous for the previously reported N457T variant (family C). This individual was evaluated at age 81 and found to have no evidence of PD. This individual has two siblings with verified PD, neither of whom carries the variant. In addition, the individual carrying the variant also had a daughter with PD; however, she did not carry the variant. Thus, there is no evidence that

Figure 1 Segregation of *GIGYF2* variants in pedigrees



The *GIGYF2* variant identified in each family is indicated above the pedigree. To maintain the anonymity of the pedigree, the gender of all subjects is denoted as female. PD = Parkinson disease.

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Table 4 *GIGYF2* exon 25 screening

Alleles	Allele size (bp)	Frequency (VPD), %*	Frequency (controls), %	Odds ratio (*p* value)
Normal†	160	34.2	34.9	0.97 (0.77)
Del Q 1210	157	56.1	54.6	1.06 (0.68)
Del PPQQ 1221-1224	148	0.0‡	0.3	§
Del Q 1210 + Del PQQQ 1225-1228	145	1.4	1.1	1.33 (0.40)
Del QQQQLP 1205-1210	142	0.0‡	0.0‡	§
Del QQQQLPQ 1205-1211	139	5.8	6.1	0.96 (0.81)
Del LPQQQQQQ 1209-1216	136	0.7	0.8	§
Del Q 1210 + Ins QQ 1217	163	1.8	2.2	0.81 (0.47)

*Analyses were limited to non-Hispanic Caucasian individuals and only included verified Parkinson disease cases without a causative mutation in *PRKN* or *LRRK2* .
†The normal allele is based on the *GIGYF2* sequence from chromosome 2 genomic contig sequence NC_000002.10.
‡No non-Hispanic, Caucasian individual harbored this allele.
§Odds ratio not computed when both groups had allele frequencies less than 1%.
VPD = verified Parkinson disease.

the N457T variant is segregating with disease in this family.

We did not identify any members of our PD families who carried the T112A, I278V, S335T, D606E, or V1242I variants previously identified.[12] While positive controls were not available for these variants for use in the TaqMan allelic discrimination assays, there was 100% correlation between the sequencing results of the 96 subjects with PD and the TaqMan assay results for these same subjects for these five variants.

We also genotyped our full sample for the three novel variants identified in the 96 sequenced patients with PD (D228E, K407E, and R1195C). The D228E variant was found in one additional patient with PD, the sibling of the individual in whom the variant was first identified (family D). The affected sibling of the individual identified by sequence analysis carrying the novel K407E variant does not carry this variant and thus this variant does not segregate with disease (family E). Similarly, the affected sibling of the individual carrying the novel R1195C variant does not carry this variant so this variant does not seem to be segregating with PD either (family F). While previous molecular screening has identified causative mutations in *PRKN* and *LRRK2* in 128 patients with PD in our patient cohort, none of the patients with *GIGYF2* variants in this report were shown to carry either a *PRKN* or *LRRK2* mutation.[19,21,22]

The TaqMan allelic-discrimination assays for the 10 variants previously reported[12] or identified in the sequencing of patients with PD as part of this report were also genotyped in 1,447 neurologically normal non-Hispanic Caucasian controls.[19] Only one of the 10 variants genotyped (V1242I) was identified in one control sample (table 3).

Previously, deletions and insertions in exon 25 of *GIGYF2* were reported.[12] To determine how many different alleles might be represented in our study samples, we used a combination of agarose gel electrophoresis, cloning and sequencing of individual alleles, and fluorescent genotyping. In our analysis of 1,497 family members from 566 PD families and 1,447 neurologically normal controls, a total of eight different alleles was observed, with six of these corresponding to the six different patterns initially observed by gel electrophoresis (table 4). These six alleles ranged from a deletion of eight amino acids (Del LPQQQQQQ 1209-1216) to an insertion of two amino acids (Ins QQ 1217). Each of these alleles was also previously reported.[12] Exon 25 PCR products from two individuals each heterozygous for one of the two new alleles identified by the fluorescent genotyping (142 and 148 bp) were subcloned and sequenced to determine the corresponding change at the DNA/amino acid level. Sequence analysis identified one novel allele (Del QQQQLP 1205-1210) and one allele previously reported (Del PPQQ 1221-1224).[12] Figure 2 shows the DNA sequence of the eight alleles detected in our study. The Del QQQQLP 1205-1210 (142 bp) allele was identified in two siblings from a single family but not in any controls while the Del PPQQ 1221-1224 (148 bp) allele was detected in three controls but in none of the PD families. Each of the other six alleles was identified at similar frequencies in both patients with PD and controls and there was no evidence that any of these *GIGYF2* insertion/deletions increased the risk for PD (table 4).

DISCUSSION The goal of this study was to test whether variants in *GIGYF2* could account for the previous evidence of linkage to chromosome

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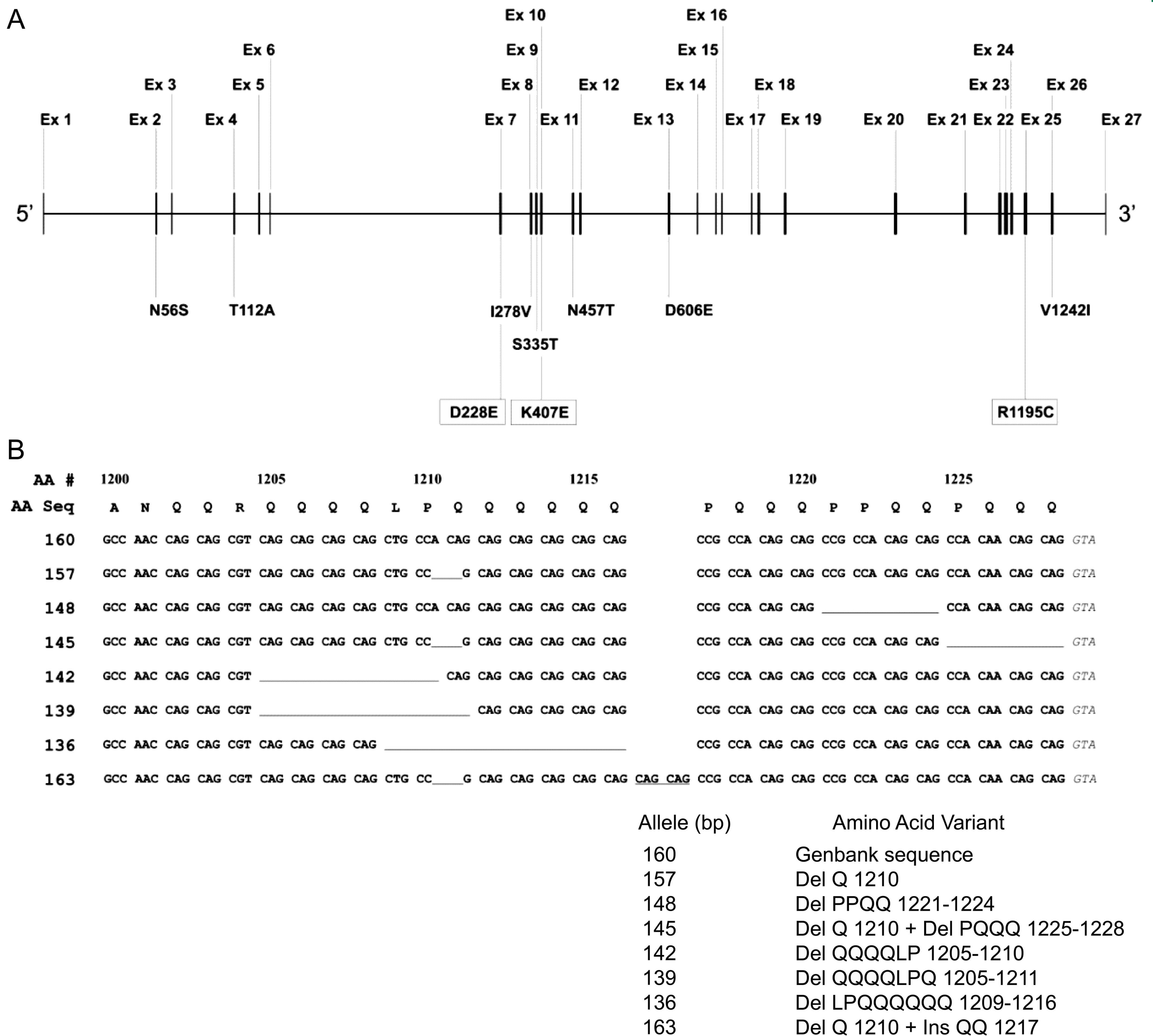
Figure 2 Schematic of *GIGYF2* gene (A) and amino acid/DNA sequence of *GIGYF2* polymorphic exon 25 alleles (B)



(A) Schematic of *GIGYF2* gene showing location of identified variants. The *GIGYF2* gene structure is depicted approximately to scale with the exons numbered above the gene. Below the gene are shown variants identified in patients with PD. Those in boxes represent the three novel variants of this report while the remaining seven were previously published. (B) Amino acid/DNA sequence of *GIGYF2* polymorphic exon 25 alleles. At top is shown the amino acid sequence corresponding to the 3′ portion of exon 25 encoding residues 1200 to 1228 (numbers above amino acid sequence). Shown directly below the amino acid sequence are the eight different alleles identified in our study sample. Numbers at left designate allele sizes (bp) by fluorescent genotyping. The 160 bp allele is the normal reference allele based on the GenBank sequence. All other alleles are shown relative to the normal 160 bp allele. Missing residues are depicted as underlined gaps in the sequence. The QQ insertion at residue 1217 in allele 163 is indicated by the underlined CAG codons occurring between codons 1216 and 1217 in the normal sequence.

2q36-37 reported in our collection of multiplex PD families.[4,23] Our previous study found that a subset of our families, in particular those with the strongest family history of disease, provided the greatest evidence of linkage to this region. Direct sequence analysis of the *GIGYF2* coding region in one subject with PD from each of 96 unrelated PD families identified four variants (4.2%) not identified in controls (figure 2). This is similar to the 4.8% frequency previously reported.[12] However, our screening of 10 variants (7 previously reported and 3 novel from this study) in 566 families yielded only 6 families with known variants (1.1%). We did not find a significant increase in risk for PD among those inheriting variants in *GIGYF2* as compared to controls ($p = 0.28$). With the identification of so few potential mutations in *GIGYF2* in these families, it is very unlikely that these few variants, observed in only six families (and segregating with disease in only two families) (see figure 1), could have accounted for the substantial

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linkage evidence (lod = 5.1) reported in our sample.[4] Therefore, we do not believe that variation in *GIGYF2* accounts for the previously reported linkage finding on chromosome 2q36-37.

Of the three novel variants identified in this study (table 3), two of them (K407E in exon 10 and R1195C in exon 25) represent nonconservative amino acid substitutions which could potentially alter either structure or function of GIGYF2. The third novel variant (D228E in exon 7) would not be predicted to interfere in protein function. Alignment of the human GIGYF2 protein sequence with 17 other species indicate that the three variants occur within conserved amino acid blocks and involve residues that are highly conserved across species.

While we did not detect sequence variants that appeared consistent with a causative effect on PD, we also explored the possibility that variation in exon 25 in *GIGYF2* might increase the susceptibility or risk of PD. The analysis of exon 25 represented a challenge due to the large number of glutamine residues encoded in this exon. Nineteen of the final 27 codons in exon 25 encode glutamine primarily using the CAG codon (18/19) (figure 2). The repeated CAGs result in several different alleles that vary due to insertions/deletions in this region and were also observed in a previous report.[12] Many of the 96 samples sequenced were heterozygous for these insertional/deletional events, preventing an accurate determination of the sequence using conventional direct sequence analysis of PCR products as was used for the other exons. Several different sequence patterns were noted on the chromatograms. However, following careful delineation of all insertions and deletions in exon 25 using a combination of cloning/sequencing of the individual alleles and fluorescent genotyping, we did not detect evidence that any of the eight observed alleles were found at higher frequency in subjects with PD as compared with controls (table 4). Our analysis of *GIGYF2* was limited to coding sequence alterations and would not identify dosage changes as have been identified in the genes for parkin and α-synuclein in some subjects with PD.[2]

Despite careful examination of *GIGYF2* to identify all sequence variation, we did not detect significant or even suggestive evidence that variation in *GIGYF2* can cause or increase the risk of PD even when using a cohort of samples providing evidence of linkage to chromosome 2q36-37. Therefore, we hypothesize that there is another gene within this chromosome 2q region that when mutated results in familial PD. Studies are ongoing to identify this gene or genes. Given the increased interest in genetic testing for PD, it is imperative that the pathogenicity of any newly identified genetic variant be determined before it is included in any panel for diagnostic testing. These data should be available to the clinician to enable proper genetic counseling, especially to those undergoing presymptomatic testing. We urge caution in the implementation of *GIGYF2* genetic testing to ensure minimization of the risks of misinterpretation.

AUTHOR CONTRIBUTIONS

Statistical analyses were conducted by Drs. Pankratz and Foroud.

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APPENDIX

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