EIF4G1 in familial Parkinson's disease: pathogenic mutations or rare benign variants?

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Abstract

Mutations in the *eukaryotic translation initiation factor 4-gamma* (*EIF4G1*) gene, encoding a component of the eIF4F translation initiation complex, were recently reported as a possible cause for autosomal dominant (AD) form of Parkinson's disease (PD). Here, we describe the screening of all 31 *EIF4G1* coding exons in a series of 251 index cases with AD PD, mostly of French origin and in 236 European control subjects. We identified 12 rare coding variants (either non-synonymous amino-acid substitutions or in frame deletions/ insertions), including six variants present only in cases and three in controls. Segregation was possible only for one variant (p.E462delInsGK) that was found in two affected siblings. In addition, we found two previously reported pathogenic variants in two isolated patients (p.G686C) and in a control subject (p.R1197W). These data do not support the pathogenicity of several *EIF4G1* variants in PD, at least in the French population.

Keywords: Parkinson's disease; Genetics; EIF4G1; Mutation analyses

1. Introduction

The first causal mutation for Parkinson's disease (PD) was identified in 1997, in the gene *SNCA*/PARK1. Two other genes [*Leucine-rich repeat kinase 2* (*LRRK2*)/PARK8 and *vacuolar protein sorting 35 ortholog* (*VPS35*)] were since conclusively associated with autosomal dominant (AD) and four [*parkin*/PARK2, *PTEN-induced kinase 1* (*PINK1*)/PARK6, *DJ-1*/PARK7, *ATP13A2*/PARK9] with early-onset autosomal recessive (AR) PD (Corti et al., 2011; Vilariño-Güell et al., 2011; Zimprich et al., 2011). Recently, mutations in *eukaryotic translation initiation factor 4-gamma* (*EIF4G1*)/PARK18 was reported as a probable cause of AD late-onset PD (Chartier-Harlin et al., 2011). Here, to

determine the frequency and pathogenicity of *EIF4G1* variants, we screened all 31 *EIF4G1* coding exons in a series of AD PD patients and matched controls.

2. Methods

2.1 Subjects

We selected 251 index cases (>90% French) with dominant inheritance [≥2 affected individuals in 2 successive generations identified by examination of secondary cases (n=101) or family history (n=150)]. Most (>73%) had definite PD (Hughes et al., 1992): mean age at onset in 140 male and 111 female index patients was 50.2±12.0 years (range 14-86); age at examination, 58.2±11.8 years (range 24-87). Control subjects, 236 Europeans (134 males, 102 females, mainly spouses) without family histories of PD, were examined at age 58.1±11.8 (range 31-85). Patients with *SNCA* multiplications, *SCA2* CAG repeat expansions and *VPS35* mutations were excluded. Since digenic parkinsonism has been observed (Dächsel et al., 2006), subjects were screened for *LRRK2* p.G2019S (most already reported, Lesage et al., 2009).

2.2 Molecular methods

The local ethics committee approved the study. Peripheral blood was collected, with written informed consent, and DNA extracted from leukocytes by standard procedures. The 31 *EIF4G1* coding exons and exon-intron junctions were sequenced as reported, with modifications (Chartier-Harlin et al., 2011). Variant frequencies were compared in patients and controls with the chi-square test (significant at $p<0.05$). Mutation nomenclature follows HGVS recommendations: +1 is A of ATG initiation codon in NCBI Reference Sequences (RefSeq) NM_198241.2. Mutation taster (http://www.mutationtaster.org), Sorting Intolerant from Tolerant (SIFT) (http://sift.jcvi.org/) or Polymorphism Phenotyping (PolyPhen-2) were used for *in silico* analyses.

3. Results

Sixty sequence variants (35 intronic, 25 exonic) were found (Tables 1, 2). Twelve coding variants, including six novel, were synonymous (five only detected in controls). We also identified four novel, rare non-synonymous amino acid substitutions or in-frame insertions/deletions (indels) (p.A433V/c.1298C>T, p.E465del/c.1384_1386delGAA, p.E462delInsGK/c.1384_1386insGAA, p.P446H/1337C>A) (Table 1); none affected protein function *in silico*. Segregation analysis was not possible except for p.E462delInsGK, found in two affected siblings. Of the 12 rare missense or indels variants, six were present exclusively in cases and three in controls, including two reportedly putative disease-causing mutations (Chartier-Harlin et al., 2011): p.G686C/c.2056G>C found in two isolated patients and p.R1197W/c.3589C>T in a healthy 46 year-old control (Table 1).

Male patient FPD-832-1 carried *LRRK2* p.G2019S in addition to *EIF4G1* p.G686C. Diagnosed at age 58, his Hoehn and Yahr score, at age 68, was 2/5; his UPDRS III motor score, 41/132 "off" and 24 "on," reflected a good L-dopa response; he had no cognitive impairment [Mini Mental State Examination (MMSE) =28/30]. Female patient FPD-252-13, with *EIF4G1* p.G686C, had PD according to UK Brain Bank criteria (Hughes et al., 1992), onset at age 52, a Hoehn and Yahr score of 2/5, at age 67, excellent L-dopa response, peak-dose dyskinesias, but no cognitive impairment (MMSE=29/30).

Variants with minor allele frequencies (MAF) ≥0.05 (n=9) were tested for Hardy-Weinberg equilibrium (HWE) and association with PD in patients and controls (Table 2). Two of them (rs939317 and rs1879244) deviated from equilibrium in the patient group with p-values of 0.01 and 0.02, respectively versus 0.31and 0.35 in the healthy control group. None of the polymorphic variants showed an association with PD in the case-control study, except for the rs9846954 variant (genotype, p=0.03; alleles, p=0.007; Table 2) that was not significant after Bonferroni correction.

4. Discussion

Linkage and candidate gene analysis identified the p.R1205H mutation in *EIF4G1*, encoding a component of the eIF4F translation initiation complex that regulates cell survival in response to stressors, in a large French family with AD late-onset PD and seven smaller families of various origins, probably resulting from an ancestral founder (Chartier-Harlin et al., 2011). Screening additional patients with parkinsonism and Lewy body disease identified four less frequent putatively disease-causing mutations, p.A502V, p.G686C, p.S1164R and p.R1197W, absent from ~4,000 controls, but their involvement in disease pathogenesis remains inconclusive, in absence of segregation analyses. The most frequently reported *EIF4G1* mutation, p.R1205H, was not present in the 487 individuals tested here, nor p.A502V, but the rare p.G686C variant was found in two PD families, one with *LRRK2* p.G2019S, suggesting that *EIF4G1* mutations are not a common cause of PD at least in our population; the p.R1197W variant however found in a control is probably a rare benign polymorphism. In our study, we identified three novel rare missense and indels variations, present only in cases but their pathogenicity remains to be proven in the absence of segregation data and deleterious effects predicted *in silico*. This result highlights the difficulties of interpreting the occurrence of rare missense variations in an isolated sporadic patient or in families that are too small to be informative in segregation analyses, even if they are not found in a large number of controls.

As previously reported (Chartier-Harlin et al., 2011), no significant association with PD was found. Our cases with p.G686C mutations had idiopathic PD, good responses to L-DOPA and no dementia, consistent with the late-onset idiopathic Lewy body parkinsonism previously reported in *EIF4G1* patient carriers.

In conclusion, we do not provide conclusive evidence that the variants identified are deleterious. In Chartier-Harlin and colleagues' study and in ours, *EIF4G1* variants seem to be

very rare in the PD population. Large multi-center studies, as performed for the *LRRK2* gene (Ross et al., 2011) are needed to determine the frequency and the pathogenicity of *EIF4G1* in PD in diverse populations worldwide. So far, the evidence of its role remains inconclusive.

Disclosure statement

Dr. Lesage, Ms. Condroyer, Dr. Lohmann, Dr. Anheim, Ms. Honoré, Dr. Durif, Dr. Damier, Dr. Viallet, Dr. Bonnet, Dr. Ouvrard-Hernandez and Dr. Vidhaillet report no disclosures. Dr. Klebe was founded from the Deutsche Forschungsgemeinschaft (DFG) (DFG Kl 1433/2-1) and received royalties from Thieme publishers. Dr. Tison has received speaker honoraria from UCB, Novartis, Lundbeck; GSK, Boehringer Ingelheim and Aguettant has served on the scientific advisory board for Novartis and Addex pharmaceuticals, has received a research grant from Novartis and received travel expenses from Novartis, UCB and Lunbeck. Dr. Dürr is a site investigator for the Track HD study. Dr. Brice received honoraria from the Wolfson Foundation for reviewing the scientific project, and received research support for the French Agency for Research.

The study was approved by the local ethnics committees (INSERM, CCPPRB du Groupe Hospitalier Pitié-Salpêtrière, Paris). Patients and healthy control individuals provided written informed consent.

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encoding a subunit of the retromer complex, causes late-onset Parkinson disease. Am. J. Hum. Genet. 89, 168-175.

Table 1. Rare coding *EIF4G1* gene variants (MAF<5%) detected in the 251AD PD index cases and 236 healthy controls.

Location	Accession N°.	Nucleotide change[a]	Protein change	Cases (frequency, %) (n=251)	Healthy controls (frequency, %) (n=236)
Exon10		**c.720C>T**	**p.I240I**	0	1 HTZ (0.4)
Exon10	rs16858632	c.932A>G	p.Y311C	1 HTZ (0.4)	0
Exon10		**c.1298C>T**	**p.A433V**	1 HTZ (0.4)	0
Exon10		**c.1337C>A**	**p.P446H**	0	1 HTZ (0.4)
Exon10		**c.1384_1386delGAA**	**p.E465del**	1 HTZ (0.4)	0
Exon10		**c.1384_1386insGAA**	**p.E462delInsGK**	1 HTZ (0.4)	0
Exon10	rs111659103	c.1413_1421del	p.G472_A474del	9 HTZ (3.6)	9 HTZ (3.8)
Exon12	rs111924994	c.1648G>C	p.A550P	0	1 HTZ (0.4)
Exon14		**c.2028A>G**	**p.T676T**	0	1 HTZ (0.4)
Exon14	rs112019125	c.2056G>T	p.G686C	2 HTZ (0.8)	0
Exon15	rs111396765	c.2149G>C	p.A717P	1 HTZ (0.4)	0
Exon15		**c.2211C>T**	**p.S737S**	0	1 HTZ (0.4)
Exon 20	rs112420733	c.2976A>G	p.P992P	5 HTZ (2.0)	2 HTZ (0.8)
Exon 24	rs113388242	c.3589C>T	p.R1197W	0	1 HTZ (0.4)

Exon 25	rs35629949	c.3685C>G	p.P1229A	2 HTZ (0.8)	4 HTZ (1.7)
Exon 25	rs2230570	c.3698T>C	p.L1233P	7 HTZ (2.8)	7 HTZ (3)
Exon 29		**c.4179G>T**	**p.G1393G**	0	1 HTZ (0.4)
Exon 29	rs76779558	c.4251C>T	p.V1417V	13 HTZ (5.2)	9 HTZ (3.8)
Exon 30	rs111921843	c.4383C>T	p.F1461F	2 HTZ (0.8)	6 HTZ (2.5)
Exon 30	rs112718796	c.4386C>T	p.D1462D	6 HTZ, 1 HMZ (2.8)	6 HTZ (2.5)
Exon 32	rs11559218	c.4551C>T	p.D1517D	0	6 HTZ (2.5)
Exon 32		**c.4572G>T**	**p.A1524A**	1 HTZ (0.4)	0
Exon 33		**c.4740A>G**	**p.K1580K**	0	1 HTZ (0.4)

[a]Human *EIF4G1* cDNA sequence (RefSeq Accession number NM_198241.2) was used as the reference sequence.

The A of the ATG translation initiation start codon represents nucleotide +1.

Novel variations detected in this study are shown in bold.

HMZ: Homozygote; HTZ: Heterozygote.

Table 2. Intronic and frequent exonic *EIF4G1* gene variants (MAF≥5%) detected in the 251AD PD index cases and 236 healthy controls.

Location	Accession N°.	Nucleotide change[a]	Protein change	Genotypes WT/HET/MUT		Minor allelic frequency (%)		HWE (p-value)	
				Cases	Controls	Cases	Controls	Cases	Controls
Intron3		c.60+20_60+23del		248/3/0	229/7/0	0.60	1.48		
Intron8		c.631-36T>A		251/0/0	235/1/0	0	0.21		
Intron9		c.697+24C>T		250/1/0	236/0/0	0.20	0		
Intron9	rs114840884	c.697+50A>G		250/1/0	235/1/0	0.20	0.21		
Intron9	rs9846954	c.697+93A>T		169/73/9*	134/85/17*	18.1**	25.2**	0.75	0.49
Intron9	rs4912537	c.698-101C>T		163/75/13	147/80/9	20.1	20.8	0.26	0.64
Intron9		c.698-50G>A		251/0/0	235/1/0	0	0.21		
Intron9		c.698-10T>C		251/0/0	235/1/0	0	0.21		
Exon10	rs2178403	c.1294G>A	p.M432V	142/96/13	145/84/7	24.30	20.80	0.53	0.21
Intron10	rs16858641	c.1519+46C>G		250/1/0	236/0/0	0.20	0		
Intron10	rs80195889	c.1520-22T>C		243/7/1	229/7/0	1.79	1.48		
Intron12		c.1796-3C>T		250/1/0	235/1/0	0.20	0.21		
Intron14		c.2089-51T>C		251/0/0	235/1/0	0	0.21		

Intron14	rs114075070	c.2089-28C>T	247/4/0	231/4/0	0.80	0.85		
Intron15		c.2275-71C>T	251/0/0	235/1/0	0	0.21		
Intron15	rs80174971	c.2275-69C>G	249/2/0	236/0/0	0.40	0		
Intron 16		c.2472+57G>T	250/1/0	236/0/0	0.20	0		
Intron 16	rs16858643	c.2473-18A>G	249/2/0	236/0/0	0.40	0		
Intron 18		c.2857-55G>T	251/0/0	235/1/0	0	0.21		
Intron 18	rs17818331	c.2857-38A>G	237/14/0	221/15/0	2.79	3.18		
Intron 19	rs114930389	c. 2962-53T>C	251/0/0	235/1/0	0	0.21		
Intron 21		c.3223-214G>A	251/0/0	235/1/0	0	0.21		
Intron 21	rs35606653	c.3223-120A>G	244/7/0	235/1/0	1.39	0.21		
Intron 21		c.3223-67A>G	251/0/0	235/1/0	0	0.21		
Intron 22	rs2293605	c.3325+16C>T	201/45/5	186/48/2	11.00	11.01	0.20	0.57
Intron 22		c.3325+25G>A	250/1/0	236/0/0	0.20	0		
Intron 26	rs939317	c.3953+9A>G	152/95/4	140/87/9	20.52	22.25	0.01*	0.31
Intron 26		cc.3953+32_3953+33del	250/1/0	236/0/0	0.20	0		
Intron 26	rs1881975	c.3953+252A>G	150/88/13	137/90/9	22.71	22.88	0.98	0.22

Intron 26	rs55772945	c.3953+312C>G		124/111/16	129/85/22	28.49	27.33	0.18	0.15
Intron 26		c.3953+344G>A		250/1/0	235/1/0	0.20	0.21		
Intron 26	rs1879244	c.3954-64T>C		146/99/6	141/86/9	22.11	22.03	0.02*	0.35
Exon 27	rs2230571	c.4005C>T	p.H1335H	126/109/16	129/86/21	28.09	27.12	0.23	0.23
Intron 27	rs115910779	c.4080-85G>A		243/8/0	227/9/0	1.59	1.91		
Intron 30		c.4395+12C>T		250/1/0	236/0/0	0.20	0		
Intron 32	rs62287502	c.4619-95G>T		230/20/1	217/19/0	4.38	4.03	0.44	0.52
Intron 32		c.4619-13C>T		251/0/0	235/1/0	0	0.21		

[a]Human *EIF4G1* cDNA sequence (RefSeq Accession number NM_198241.2) was used as the reference sequence. The A of the ATG translation initiation start codon represents nucleotide +1.

HET: heterozygote; HWE: Hardy Weinberg equilibrium; MAF: Minor Allele Frequency; MUT: mutated; WT: wild-type.

Genotypic and allelic comparisons between patient and control groups: *p=<0.05; **p<0.01.