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SHORT COMMUNICATION

Variants in *eukaryotic translation initiation factor 4G1* in sporadic Parkinson's disease

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Abstract Recently, mutations in *eukaryotic translation initiation factor 4G1* (*EIF4G1*) were reported as a rare cause of familial Parkinson's disease (PD). We screened the 33 exons of *EIF4G1* by high-resolution melting curve analysis for variants in our Central European cohort of 376 PD cases. Variant frequency was assessed in a total of 975 PD cases and 1,014 general population controls. Eight novel nonsynonymous and four synonymous variants were identified. In our cohort, novel and previously identified nonsynonymous variants were very rare. Although it is possible that our general population controls also comprise individuals who have or could develop PD in the future, the presence of the original mutation (*EIF4G1* p.Arg1205 His) in three controls only, raises questions about the causality of this variant with regard to PD.

Keywords Genetics · Rare variants · Parkinson's disease · *EIF4G1*

Introduction

Genome-wide association studies and linkage analyses have identified at least 19 genes associated with idiopathic Parkinson`s disease (PD). Most recently, variants in *eukaryotic translation initiation factor 4G1* (*EIF4G1*) were implicated in familial PD, linking dysfunctional mRNA translation initiation to PD pathogenesis. [1] Here, we assess the role of *EIF4G1* variants in our Central European PD cohort.

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Methods

Using Idaho® melting curve analysis, we screened the 33 exons and exon–intron boundaries of *EIF4G1* in a discovery sample of 376 German PD patients (71.1±9.4 years, 31.6 % female). When altered melting patterns suggested variants, Sanger sequencing ensued. To assess variant frequency, we genotyped the novel as well as four of the five variants previously described in PD (*EIF4G1* c.1505C > T (p.Ala502Val), c.2056G > T (p.Gly686Cys), c.3490A > C (p.Ser1164Arg), and c.3614G > A (p.Arg1205His)) [1] in 975 familial and sporadic PD cases from Austria (n=486, 58.7±11.3 years, 35.4 % female, family history known in n=413, 33.4 % thereof positive for PD in a first or second degree relative), Germany (n=450, 376 of which comprised the discovery sample, 70.2±9.7 years, 32.2 % female, family history known in n=105, 24.7 % thereof positive for PD in a first or second degree relative), and Hungary (n=39, 50.4±10.8 years, 53.9 % female, family history known in n=39, 28.2 % thereof positive for PD in a first- or second-degree relative) and 1,014 general population controls belonging to the KORA-AGE cohort (76.0±6.6 years, 50.1 % female) [2] by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry on the Sequenom platform. The KORA-AGE cohort is a follow-up study of the initial surveys, enriched for older individuals. Individuals known to take dopaminergic medication where excluded from the control sample. All individuals included in this study were Caucasian. German and Austrian PD samples and KORA controls originate from the same geographic region. The small number of Hungarian patients either have an early age of onset or are index patients of larger PD families and were, therefore, genotyped as well. For technical reasons, four novel variants could not be included in the genotyping assay. Haplotype analysis in carriers of the original c. 3614G > A (p.Arg1205His) variant was performed using haplotype-tagging SNPs rs4912537, rs2178403, rs2293605, rs1879244, and rs2230571 and polymorphic markers D3S3609, D3S3578, and D3S3583 by Sanger sequencing. All subjects were diagnosed according to the UK Brain Bank criteria by a senior neurologist specializing in movement disorders. Ethics review board approval and participants' written informed consent were obtained.

Results

In addition to several common and rare synonymous variants, we identified seven nonsynonymous variants, not previously reported in PD, in six individuals. These include c.47C > T (p.Pro16Leu), c.211C > T (p.Pro71Ser, rs113810947), c.953C > T (p.Thr318Ile),c.1622T > G (p.Val541Gly), c.1648G > C (p.Ala550Pro, rs111924994), c.2093G > C (p.Gly698Ala), and c.2149G > C (p.Ala717Pro, rs11396765) as well as c.1456C > T (p.Pro486Ser, rs112545306) previously reported in two individuals suffering from PD [3] (Fig. 1). Similar to the phenotype described [1], all individuals presented with classic PD with an age of onset at 64.5±5.5 years and positive response to dopaminergic therapy. Where available, family history was negative (Table 1).

Overall, the identified variants were very rare in our population. Four—c.47C > T (p.Pro16Leu), c.953C > T (p.Thr318Ile), c.1622T > G (p.Val541Gly), and c.2093G > C (p.Gly698Ala)—were validated in the PD individual in whom they were first identified but were not found in any additional PD subjects. Of these, c.953C > T (p.Thr318Ile), c.1622T > G (p.Val541Gly), and c.2093G > C (p.Gly698Ala) were not present in controls, while c.47C > T (p.Pro16Leu) was identified in three controls. Of the previously reported [1] variants, c.1505C > T (p.Ala502Val) and c.3490A > C (p.Ser1164Arg) were not seen in the 1989 individuals assessed. Five cases and three controls, on the other hand, were heterozygous for c.2056G > T (p.Gly686Cys). Surprisingly, the original mutation, c.3614G > A (p.Arg1205His), which had, so far, only been identified in PD cases [1], was only present in three controls. In the original publication, all eight PD probands heterozygous for c. 3614G > A (p.Arg1205His; out of 4,708 cases and 4,576 controls) shared the same minimal haplotype [1]. Genotyping of five haplotype-tagging SNPs and three

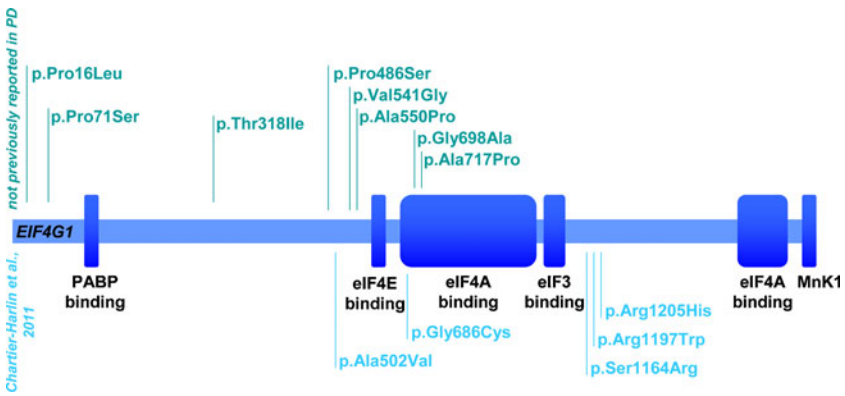

Fig. 1 *EIF4G1* sheme depicting novel and previously described [1] missense variants in individuals with PD and their relative location in relation to known and predicted functional domains. *PABP* polyadenylate binding protein, *eIF* eukaryotic translation initiation factor

Table 1 Rare *EIF4G1* variants identified in individuals with PD, clinical phenotype, and variant frequencies

EIF4G1 variant	Genomic position (hg19)	Exon	Family history	AoO	DD	IS	B	R	RT	PI	L-Dopa/DA	Frequency		Frequency NHLBI-ESP (EA)	Mutation Taster	PolyPhen2
												Cases	Controls			
p.Pro16Leu (c.47C>T)	chr3:184,033,631	1	n/a	n/a	n/a	n/a	+	+	−	++	+	1/975	3/1014	Not found	dc	n/s
p.Pro71Leu (c.211C>T)	chr3:184,035,172	4	n/a	59	4	RT	+	+	+	+	+	n/a	n/a	0.000285	Poly	prob.dam.
p.Ala239Ala (c.717A>G)	chr3:184,039,089	9										1/975	0/1014	0.000142		
p.Thr318lle (c.953C>T)	chr3:184,039,325	9	neg	62	3	B	+	+	−	+	+	1/975	0/1014	Not found	poly	prob.dam.
p.Pro486Ser (c.1456C>T)	chr3:184,039,828	9	n/a	70	1	B	+	+	+	+	+	n/a	n/a	0.00057	poly	benign
p.Val541Gly (c.1622T>G)	chr3:184,040,345	11	n/a	72	3	TR	+	+	+	+	+	1/975	0/1014	Not found	poly	poss. dam.
p.Ala550Pro (c.1648G>C)	chr3:184,040,371	11	n/a	72	3	RT	+	+	+	+	+	n/a	n/a	0.001994	poly	benign
p.Gly698Ala (c.2093G>C)	chr3:184,041,200	14	neg	65	4	B	+	+	−	+	+	1/975	0/1014	Not found	dc	prob. dam.
p.Ala717Pro (c.2149G>C)	chr3:184,041,256	14	neg	59	9	B	+	+	−	+	+	n/a	n/a	Not found	poly	benign
p.Pro992Pro (c.2971A>G)	chr3:184,043,282	20										14/975	10/1014	0.00616		
p.Val141Val (c.4251C>T)	chr3:184,049,143	29										n/a	n/a	0.0245		
p.Ala1517Ala (c.4551C>T)	chr3:184,049,807	32										n/a	n/a	0.00818		
Chartier_A502V		10										0/975	0/1014	0.000285		
Chartier_G686C		14										5/975	3/1014	Not found		
Chartier_S1164R		24										0/975	0/1014	No found		
Chartier_R1197W		24										n/a	n/a	Not found		
Chartier_R1205H		24										0/975	3/1014	Not found		

Four nonsynonymous and two synonymous variants present in our sample in addition to the *EIF4G1* variants previously identified in familial PD [1] were genotyped in 975 cases and 1,014 controls. Additional clinical information and in silico predictions of the damaging potential of the amino acid exchange as assessed by MutationTaster [5] and PolyPhen2 [6] are presented for the newly identified missense variants. Additionally, variant frequencies as found in the approximately 3,500 European American exomes found in the NHLBI exome sequencing project (NHLBI-ESP) are noted for all newly identified and previously reported [1, 3, 4]

n/a Not available, *neg* negative, *AoO* age of onset, *DD* disease duration, *IS* initial symptom, *B* bradykinesia, *R* rigor, *RT* resting tremor, *PI* postural instability, *D* dementia, *DA* dopamine agonist, *dc* disease causing, *poly* polymorphism, *n/s* not scored, *prob. dam.* probably damaging, *poss. dam.* possibly damaging

microsatellite markers indicated that two of our three c.3614G > A (p.Arg1205His) controls could share this minimal haplotype (Table 2). Seven out of the 12 variants identified in our PD cohort were also found in the approximately 3,500 European American exomes pertaining to the NHLBI exome sequencing project [4] (Table 1).

Discussion

Of the newly identified variants, c.47C > T (p.Pro16Leu), c.211C > T (p.Pro71Ser), c.953C > T (p.Thr318Ile), c.1622T > G (p.Val541Gly), and c.2093G > C (p.Gly698Ala) are predicted to damage protein structure, while c.1456C > T (p.Pro486Ser) and c.2149G > C (p.Ala717Pro) are likely functionally neutral [5, 6] (Table 1). Of these, c.2093G > C (p.Gly698Ala) emerges as the best potentially pathogenic candidate. Contrary to most other amino acids affected, the glycine in position 698 is conserved in all vertebrates. The variant, moreover, was ranked most likely to be damaging by two prediction algorithms [5, 6] and is located in the eIF3/eIF4A binding domain necessary for formation of the translation initiation complex (Fig. 1). However, caution is mandated as a nearby variant (c.2056G > T (p.Gly686Cys)), previously only found in two individuals with PD [1], was present in five cases and three controls in our much smaller sample, suggesting that population-specific effects can misconstrue frequency assessment especially with regard to rare genetic variation. Consequently, further assessment of the role of *EIF4G1* variants in PD is warranted.

Haplotype analysis in the three control subjects harboring c. 3614G > A (p.Arg1205His) supports the idea of an ancestral founder mutation. Linkage analysis and segregation in the original family [1] back pathogenicity of this variant and this is not necessarily disparaged by the presence of the variant in our controls. First, we used general population controls and it is not unlikely that some of the controls may have or may develop PD. Second, it is possible that this mutation shows incomplete penetrance or that other protective factors exist. However, the presence of c. 3614G > A (p.Arg1205His) in our control cohort could also indicate that its role in PD pathogenesis is questionable as has just now also been suggested for the *EIF4G1* p.Ala502Val variant initially also reported by Chartier-Harlin et al. [1, 3]. Overall, the *EIF4G1* locus naturally holds a lot of genetic variance [1, 3]. Accordingly, much larger case–control samples than those used in either the original [1], a follow-up [3], or our study will be necessary to answer this question.

Although not common, it still cannot be excluded that rare exonic *EIF4G1* variants of strong effect could play a causative role in PD in rare cases. And their study is important as they can provide significant clues in understanding disease mechanism. This idea is supported by the fact that *LRRK2*, which harbors both rare and common genetic variation contributing to PD development [7, 8], has recently also been implicated in dysfunctional mRNA translation initiation [9].

Table 2 Haplotype of *EIF4G1* p.Arg1205His carriers

Marker ID	KORA_315	KORA_330	KORA_944
D3S3609	163/179	163/167	163/165
rs4912537	***T***	***T*/*C***	***T*/*C***
rs2178403	***G***	***G*/*A***	***G***
rs2293605	***T*/*C***	***T*/*C***	***T*/*C***
p.Arg1205His	***A*/*G***	***A*/*G***	***A*/*G***
rs1879244	***T*/*T***	***T*/*C***	***T***
rs2230571	***C***	***C***	***C*/*T***
D3S3578	*240/240*	***230*/*240***	***230*/*240***
D3S3583	262/**272**	268/270	268/270

Since phase is unknown for all three individuals, where necessary, both alleles are given (with the one pertaining to the described haplotype in bold). Variants comprising the reported minimal haplotype [1] are in italics

Accession numbers

NCBI accessions NM_198241.2 and NP_937884.1 were used to number all variants within the *EIF4G1* gene and eIF4G1 protein. Functional domains were assessed using UniProtKB/Swiss-Prot Q04637 (accessed January 24, 2012).

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