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EIF4G1 gene mutations are not a common cause of Parkinson's disease in the Japanese population

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Abstract

Pathogenic mutations in the *EIF4G1* gene were recently reported as a cause of autosomal dominant parkinsonism. To assess the frequency of *EIF4G1* mutations in the Japanese population we sequenced the entire gene coding region (31 exons) in 95 patients with an apparent autosomal dominant inherited form of Parkinson's disease. We detected three novel point mutations located in a poly-glutamic acid repeat within exon10. These variants were screened through 224 Parkinson's disease cases and 374 normal controls from the Japanese population. We detected the poly-glutamic acid deletion in exon 10 in two additional patients with sporadic Parkinson's disease. Although the *EIF4G1* variants identified in the present study were not observed in control subjects, co-segregation analyses and population-based screening data suggest they are not pathogenic. In conclusion, we did not identify novel or previously reported pathogenic mutations (including the p.A502V and p.R1205H mutants) within *EIF4G1* in the Japanese population, thus future studies are warranted to elucidate the role of this gene in Parkinson's disease.

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Keywords

Parkinson's disease; *EIF4G1*; mutation; genetics

Introduction

Parkinson's disease (PD) is one of the most common movement disorders in the elderly. Pathogenic mutations that result in hereditary forms of PD/parkinsonism are reported in a number of genes and have subsequently directed both functional studies and the generation of disease model systems [1, 2]. The nomination of each new gene for parkinsonism implicates disease pathways and provides a rationale for targeted therapeutic development [3, 4]. Recently, two substitutions in the eukaryotic translation initiation factor 4-gamma 1 protein (*EIF4G1*, MIM#600495); p.R1205H and p.A502V were nominated to cause PD with autosomal dominant inheritance in a number of pedigrees [5]. Furthermore studies in a yeast model revealed the *EIF4G1* ortholog (TIF4632) is a suppressor of α-synuclein toxicity [6].

EIF4G1 is a protein scaffold subunit of the translation initiation complex, EIF4F, which binds the ribosomal 40S. A decrease in the levels of EIF4G1 protein in cells results in a reduction of overall protein synthesis linked to nutrient sensing [7]. Reported pathogenic EIF4G1 substitutions, p.R1205H and p.A502V, were shown to disrupt binding to EIF3E and EIF4E respectively, and result in impaired nutrient sensing and mitochondrial dysfunction [5, 7]. Interestingly, over-expression of EIF4G1 protein has been implicated in cell proliferation as observed in some malignant disorders, especially inflammatory breast cancer [8]. This evidence supports a role for *EIF4G1* mutations in cell survival and potentially the neuronal damage observed in PD. Herein, we set out to examine the occurrence and frequency of mutations in the *EIF4G1* gene among PD patients of Japanese origin.

Subjects and methods

All individuals were collected at Juntendo University, Tokyo and at Mie University, Mie and were of Japanese ethnicity. Patients were diagnosed with PD based on the modified United Kingdom Parkinson's disease society brain bank criteria. DNA was extracted from peripheral blood by standard protocols. A series of 95 patients with autosomal dominant PD had an average of age at onset of 52.7 years ± 11.4 (SD) and a 1:1.32 male to female ratio were selected (Table 1). Family history was defined as one or more affected relatives within 2 degrees of relationship. All variants were then screened through a population-based patient-control series of 224 patients with PD including 43 probands (age at onset 41.7± 14.2 years old and male: female=1:0.95) with possible autosomal dominant PD, 181 sporadic PD cases (age at onset 38.6± 12.3 years old and male: female=1:0.91) and 374 normal controls (age at examination 57.8± 12.6 years old and male: female=1:1.49). The ethical review boards at the Mayo Clinic, Juntendo University and at Mie University approved the study, and all participants provided informed written consent.

Genetic Analysis

The 31 coding exons (exon 3-33, NM_198241.2) of *EIF4G1* were sequenced in 95 patients with apparent autosomal dominant PD. Primer pairs for coding regions of *EIF4G1* (exons 3-33) were used and are available upon request [5]. PCR products were purified from unincorporated nucleotides using Agencourt bead technology (Beverly, MA) with Biomek FX automation (Beckman Coulter, Fullerton, CA). Electropherograms were analyzed with SeqScape v2.1.1 using 3730 DNA Analyzer (ABI, Applied Biosystems, Foster City, CA, USA). In addition, real-time PCR was employed to investigate the role of exon dosage and gene copy number variation was analyzed as previously described [5]. We also screened any novel mutations identified and the previously reported mutants (EIF4G1 p.R1205H and p.A502V) in an additional 43 autosomal dominant PD patients, 181 sporadic patients and 374 controls. We performed allelic cloning using a TOPO® TA Cloning® Kit (Life Technologies, Carlsbad, CA, USA) followed by individual clone PCR and sequencing to assess the phase of three exon 10 variants identified in one PD patient.

Results

We identified novel *EIF4G1* mutations in one patient with autosomal dominant PD (J-19) among the 95 probands (Supplemental Figure 1). Patient J-19 had three point mutations; [p.E463G, c.1388AG>GA] and [p.E465A, c.1394A>C] on the same allele, in exon10 (Figure 1a). However we found the three point mutations in two healthy siblings of patient J-19 (73-year old man and 67-year old woman). In addition, our sequencing analysis identified four novel synonymous variants; p.Q149Q (c.447 A>G), p.K1206K (c.3618G>A), p.T1211T (c.3633G>A), and p.Y1488Y (c.4464C>T) which were observed independently each in a single autosomal dominant PD patient (n=95). No *EIF4G1* gene copy number variations were observed in 95 probands.

Screening an additional 43 patients with autosomal dominant PD and 181 sporadic PD patients, identified two sporadic patient (ID#1558, 1601) with the same three point mutations as patient J-19. Furthermore, one of the two patients (ID#1601) had a known 9bp deletion (rs111659103) in exon 10 (Figure 1b); these exon 10 variants were not observed in our 374 normal control subjects, but rs111659103 is reported on the Exome Variant Server database with a carrier frequency of over 5% .

Discussion

Our comprehensive screening of the coding region of the *EIF4G1*gene in 95 probands from families with apparent autosomal dominant inheritance of PD did not detect any novel pathogenic mutations, nor the p.A502V or p.R1205H variants described in Chartier-Harlin et al.[5]. Several novel variants were identified, including a number of point mutations located in a poly-glutamic acid tract. However the location of the polyglutamic acid tract does not appear to be in a region involved in complex formation or RNA-binding, therefore if these variants do have a functional influence the mechanism is yet to be determined. *In silico* analysis using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) predicts the [p.E463G, c.1388AG>GA] and [p.E465A, c.1394A>C] to be both possibly damaging and

benign based on HumDiv and HumVar measures respectively. In addition, evidence from disease co-segregation analysis within families, *in silico* prediction and population frequency data from the Exome Variant Server database (rs111659103) does not support variants in the polyglutamic acid tract as high penetrant pathogenic factors for parkinsonism.

These findings support the initial report describing a relatively low frequency of *EIF4G1* mutations in 4708 individuals with idiopathic PD (7/4708) [5]. Recently, independent studies examining the frequency of *EIF4G1* variation have identified both the p.A502V and p.R1205H variants in non-diseased individuals, thus demonstrating the importance of replication studies to resolve the role of *EIF4G1* variants in PD pathophysiology (Supplemental Table 1). In addition, findings from other *EIF4G1* gene screening studies suggest that mutations are rare in patients across multiple populations and that the pathogenicity of this gene in PD remains to be resolved (Supplemental Table 1). We conclude from our data that *EIF4G1* mutations are not a common cause of PD in patients of Japanese origin.

Given the technological advances in DNA sequencing approaches an ever increasing number of rare variants will be nominated as pathogenic in PD and related neurodegenerative disorders. As observed for *EIF4G1* variants, even large series of patients and controls may not be sufficient to confer definitive pathogenicity. There will need to be large collaborative consortia efforts as recently reported to determine the true nature of rare variants within the context of disease risk and clinical relevance [9, 10]. In the absence of overwhelming genetic evidence we may have to rely on a functional readout (e.g. PINK1/PARKIN mutation effects on cellular mitophagy), although reliable disease-specific assays are still to be developed for PD-related genes [11, 12]. With the noted caveats in mind further investigations are warranted to confirm the pathogenicity of *EIF4G1* variants in PD and to assess global prevalence and clinical relevance to disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Sundal C, Fujioka S, Uitti RJ, Wszolek ZK. Autosomal dominant Parkinson's disease. Parkinsonism Relat Disord. 2012; 18(Suppl 1):S7–10. [PubMed: 22166459]
2. Bonifati V. Autosomal recessive parkinsonism. Parkinsonism Relat Disord. 2012; 18(Suppl 1):S4–6. [PubMed: 22166450]
3. Valente EM, Arena G, Torosantucci L, Gelmetti V. Molecular pathways in sporadic PD. Parkinsonism Relat Disord. 2012; 18(Suppl 1):S71–3. [PubMed: 22166460]
4. Puschmann A, Bhidayasiri R, Weiner WJ. Synucleinopathies from bench to bedside. Parkinsonism Relat Disord. 2012; 18(Suppl 1):S24–7. [PubMed: 22166445]

5. Chartier-Harlin MC, Dachsel JC, Vilarino-Guell C, Lincoln SJ, Lepretre F, Hulihan MM, et al. Translation initiator EIF4G1 mutations in familial Parkinson disease. Am J Hum Genet. 2011; 89:398–406. [PubMed: 21907011]
6. Yeger-Lotem E, Riva L, Su LJ, Gitler AD, Cashikar AG, King OD, et al. Bridging high-throughput genetic and transcriptional data reveals cellular responses to alpha-synuclein toxicity. Nature genetics. 2009; 41:316–23. [PubMed: 19234470]
7. Ramirez-Valle F, Braunstein S, Zavadil J, Formenti SC, Schneider RJ. eIF4GI links nutrient sensing by mTOR to cell proliferation and inhibition of autophagy. The Journal of cell biology. 2008; 181:293–307. [PubMed: 18426977]
8. Silvera D, Arju R, Darvishian F, Levine PH, Zolfaghari L, Goldberg J, et al. Essential role for eIF4GI overexpression in the pathogenesis of inflammatory breast cancer. Nat Cell Biol. 2009; 11:903–8. [PubMed: 19525934]
9. Lesage S, Brice A. Role of Mendelian genes in “sporadic” Parkinson's disease. Parkinsonism Relat Disord. 2012; 18(Suppl 1):S66–70. [PubMed: 22166458]
10. Ross OA, Soto-Ortolaza AI, Heckman MG, Aasly JO, Abahuni N, Annesi G, et al. Association of LRRK2 exonic variants with susceptibility to Parkinson's disease: a case-control study. Lancet Neurol. 2011; 0:898–908. [PubMed: 21885347]
11. Kawajiri S, Saiki S, Sato S, Sato F, Hatano T, Eguchi H, et al. PINK1 is recruited to mitochondria with parkin and associates with LC3 in mitophagy. FEBS Lett. 2010; 584:1073–9. [PubMed: 20153330]
12. Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, et al. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol. 2010; 12:119–31. [PubMed: 20098416]
13. Tucci A, Charlesworth G, Sheerin UM, Plagnol V, Wood NW, Hardy J. Study of the genetic variability in a Parkinson's Disease gene: EIF4G1. Neuroscience letters. 2012; 518:19–22. [PubMed: 22561553]
14. Lesage S, Condroyer C, Klebe S, Lohmann E, Durif F, Damier P, et al. EIF4G1 in familial Parkinson's disease: pathogenic mutations or rare benign variants? Neurobiology of aging. 2012; 33:2233, e1–e5. [PubMed: 22658323]
15. Schulte EC, Mollenhauer B, Zimprich A, Bereznai B, Lichtner P, Haubenberger D, et al. Variants in eukaryotic translation initiation factor 4G1 in sporadic Parkinson's disease. Neurogenetics. 2012; 13:281–5. [PubMed: 22707335]
16. Nuytemans K, Bademci G, Inchausti V, Dressen A, Kinnamon DD, Mehta A, et al. Whole exome sequencing of rare variants in EIF4G1 and VPS35 in Parkinson disease. Neurology. 2013; 80:982–9. [PubMed: 23408866]
17. Sudhaman S, Behari M, Govindappa ST, Muthane UB, Juyal RC, Thelma BK. VPS35 and EIF4G1 mutations are rare in Parkinson's disease among Indians. Neurobiology of aging. 2013; 34:2442, e1–3. [PubMed: 23726718]
18. Zhao Y, Ho P, Prakash KM, Foo JN, Liu JJ, Au WL, et al. Analysis of EIF4G1 in Parkinson's disease among Asians. Neurobiology of aging. 2013; 34:1311, e5–6. [PubMed: 23092605]
19. Chen Y, Chen K, Song W, Chen X, Cao B, Huang R, et al. VPS35 Asp620Asn and EIF4G1 Arg1205His mutations are rare in Parkinson disease from Southwest China. Neurobiology of aging. 2013; 34:1709, e7–8. [PubMed: 23261770]
20. Yuan L, Song Z, Xu H, Gu S, Zhu A, Gong L, et al. EIF4G1 Ala502Val and Arg1205His variants in Chinese patients with Parkinson disease. Neuroscience letters. 2013; 543:69–71. [PubMed: 23562511]
21. Li K, Tang BS, Guo JF, Lou MX, Lv ZY, Liu ZH, et al. Analysis of EIF4G1 in ethnic Chinese. BMC Neurol. 2013; 13:38. [PubMed: 23617574]
22. Blanckenberg J, Ntsapi C, Carr JA, Bardien S. EIF4G1 R1205H and VPS35 D620N mutations are rare in Parkinson's disease from South Africa. Neurobiology of aging. 2014; 35:445, e1–3. [PubMed: 24080171]

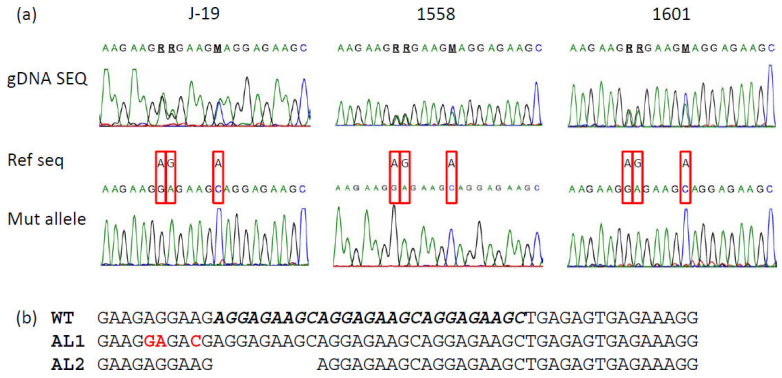

Figure 1.

(a) Genomic sequence of the *EIF4G1*exon 10 from the patients J-19, 1518 and 1601 showing three point mutations; [p.E463G, c.1388AG>GA], and [p.E465A, c.1394A>C], as well as the mutant haplotype from the TOPO TA cloning. The three mutations exist on same allele.

(b) Diagrammatic representation of the wild-type sequence around the poly-glutamic acid repeat and highlighting the three point mutations (AL1; red) and 9bp deletion (AL2; rs111659103) in *EIF4G1* exon 10 from sporadic patient 1601. A three unit perfect repeat (bold in WT allele) precludes determining which unit is rs111659103.

Table 1

Demographic of patients and control subjects

Subjects	Ethnicity	No.	Male to female ratio	Mean age at onset (SD)
Autosomal dominant PD	Japanese	95	1:1.32	52.7 (±11.4)
Replication				
Autosomal dominant PD	Japanese	43	1:0.95	41.7 (±14.2)
Sporadic PD	Japanese	181	1:0.91	38.6 (±12.3)
Control subjects	Japanese	374	1:1.49	

PD; Parkinson's disease

SD; standard deviation