Translation Initiator *EIF4G1* Mutations in Familial Parkinson Disease

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Genome-wide analysis of a multi-incident family with autosomal-dominant parkinsonism has implicated a locus on chromosomal region 3q26-q28. Linkage and disease segregation is explained by a missense mutation c.3614G>A (p.Arg1205His) in eukaryotic translation initiation factor 4-gamma (*EIF4G1*). Subsequent sequence and genotype analysis identified *EIF4G1* c.1505C>T (p.Ala502Val), c.2056G>T (p.Gly686Cys), c.3490A>C (p.Ser1164Arg), c.3589C>T (p.Arg1197Trp) and c.3614G>A (p.Arg1205His) substitutions in affected subjects with familial parkinsonism and idiopathic Lewy body disease but not in control subjects. Despite different countries of origin, persons with *EIF4G1* c.1505C>T (p.Ala502Val) or c.3614G>A (p.Arg1205His) mutations appear to share haplotypes consistent with ancestral founders. eIF4G1 p.Ala502Val and p.Arg1205His disrupt eIF4E or eIF3e binding, although the wild-type protein does not, and render mutant cells more vulnerable to reactive oxidative species. *EIF4G1* mutations implicate mRNA translation initiation in familial parkinsonism and highlight a convergent pathway for monogenic, toxin and perhaps virally-induced Parkinson disease.

Introduction

Parkinson disease (PD [MIM 168600]) is characterized clinically by asymmetric resting tremor, bradykinesia, muscle rigidity, and postural instability.[1] Dopaminergic loss and Lewy bodies in surviving neurons of the *substantia nigra* support a pathologic diagnosis.[2] Although considered a sporadic illness, 10%–30% of individuals with PD report a first-degree relative with parkinsonism.[3] Linkage and sequence analyses performed in multi-incident families with parkinsonism have discovered deleterious mutations in α-synuclein (*SNCA* [MIM 163890]), leucine-rich repeat kinase 2 (*LRRK2* [MIM 609007]), vesicular protein sorting 35 (*VPS35* (MIM 601501]), Parkin (*PARK2* [MIM 602544]), PTEN induced putative kinase 1 (*PINK1* [MIM 608309]), DJ-1 (*PARK7* [MIM 602533]) and ATP13A2 (*PARK9* [MIM 610515]).[4,5] Although familial parkinsonism attributed to mutant genes is uncommon, the molecular etiology discovered might be generalizable to idiopathic PD. For instance, antibodies to α-synuclein robustly stain

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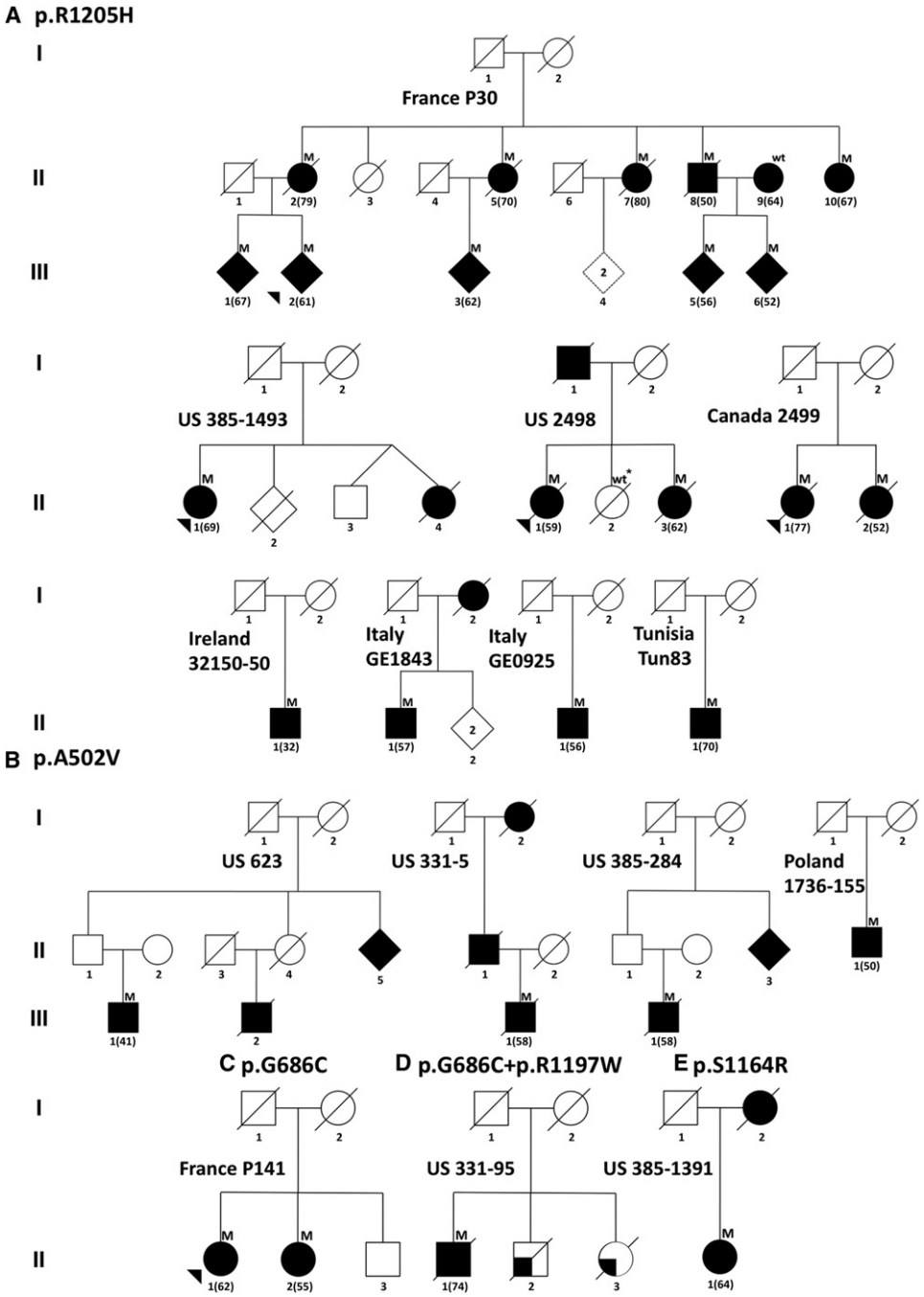

Figure 1. Pedigrees with *EIF4G1* Mutations and Parkinsonism
Individual pedigrees are numbered and their country of origin is indicated. Only pedigrees with an (A) *EIF4G1* c.3614G>A (p.Arg1205His), (B) c.1505C>T (p.Ala502Val), (C) c.2056G>T (p.Gly686Cys), (D) c.2056G>T (p.Gly686Cys) and c.3589C>T (p.Arg1197Trp) and (E) c.3490A>C (p.Ser1164Arg) mutations are illustrated. Filled symbols indicate affected individuals with parkinsonism; the age of symptom onset is shown in parentheses. Quarter-filled symbols indicate individuals with dementia. To protect confidentiality, the pedigrees do not show some individuals and siblings and/or gender is sometimes disguised with a diamond. All samples genotyped are indicted as heterozygous mutations (M) or as wild-type (wt). The asterisk (*) indicate that this individual did not fulfill the criteria for PD, but presented resting tremor and akinesia.

Lewy bodies and neuritic pathology,[6] whereas mutations in Parkin and *PINK1* have emphasized the role of stress-induced mitochondrial dysfunction in PD.[7]

We have performed genome-wide linkage analysis of a multi-incident northern French family (P30; Figure 1A) with autosomal-dominant late-onset parkinsonism for which known genetic causes of parkinsonism were excluded (unpublished data).[8] Linkage analysis identified two regions with suggestive two-point LOD scores > 2 ($\theta = 0$), a 31 cM interval between D3S1763 and D3S1580,

and a 15 cM interval between D5S2055 and D5S393. After saturation of both loci with short tandem repeat (STR) markers, significant linkage was obtained only for chromosomal region 3q26-q28. Genomic analysis subsequently led to the identification of eukaryotic translation initiation factor 4-gamma 1 (*EIF4G1*) mutations (protein eIF4G1 [MIM 600495]) in affected subjects with familial parkinsonism and idiopathic Lewy body disease. eIF4G1 is a component of the translation initiation complex, eIF4F.[9,10] We demonstrate that the two most frequent mutations impair complex formation, consistent with a dominant-negative loss of function, and are associated with mitochondrial dysfunction when cells are stressed.

Subjects and Methods

Subjects Ascertainment

The institutional review boards of all participating institutions approved the protocol (institutional review boards France CPP 94/07), and informed consent was obtained from all affected and control subjects. Participating individuals were examined by neurologists specializing in movement disorders. A full history, including a family history and a neurological examination, was completed for each patient. A clinical diagnosis of PD was determined by the presence of at least two of three cardinal signs (resting tremor, bradykinesia, and rigidity), improvement through adequate dopaminergic therapy (when tried), and the absence of atypical features or other causes of parkinsonism. Clinical criteria for probable and possible PD were consistent with former classifications.[1,2] Familial history is defined herein as one or more affected relatives within two degrees of relationship.

Linkage Analysis

Peripheral blood samples were collected and genomic DNA was extracted with standard techniques. Tri- and tetra-nucleotide repeat genome-wide genotyping was completed by the mammalian genotyping service of Marshfield in family P30, for 403 markers at approximately 10 cM resolution. Linkage analysis employed MLINK with a dominant model for two-point LOD scores and SIMWALK2 for nonparametric statistics and haplotype analysis.[11,12] The frequency of the deleterious allele was set at 0.0001; marker-allele frequencies were from CEPH or determined empirically. The map positions for each marker were taken from Marshfield and Rutgers combined linkage physical map.[13] For tightly linked loci with no observed recombinants the intermarker genetic distances were assigned as 0.01 cM.

Sequencing Analysis and Mutation Screening

Gene sequencing of all coding exons in the 3q26-q28 interval was performed for the three affected members of family P30 (individuals with symptom onset of 61, 62, and 56 years [Figure 1A, III-2, III-3, III-5]) with an ABI 3730 sequencer with SeqScape v2.5 analysis software (Applied Biosystems). Electrophoregrams were compared with CEPH 1331-01 and -02 control subjects obtained from Genethon and the human reference sequence from the UCSC database. Primers designed for amplification of all exons including exon-intron junctions of *EIF4G1* are provided in Table S1, available online. Mutations segregating with the disease were further assessed in the other P30 members (n = 23), in control subjects of the northern French (n = 146), and in other subjects of European descent (n = 370). RefSeq accessions NM_198241.2 and NP_937884.1 were used to number all variants within the *EIF4G1* gene and protein.

Further sequencing of all *EIF4G1* coding exons was then performed to identify other mutations in additional subjects affected with autosomal-dominant parkinsonism (n = 95) or neuropathologically confirmed Lewy body disease (n = 130) and ethnically matched controls (n = 185).

In order to test whether *EIF4G1* missense mutations might be found in idiopathic PD, we genotyped a large case-control series consisting of 4430 affected and 3671 control subjects of European descent (North America: 2092 PD [130 Lewy body disease], 1666 controls [provided by Z.K.W., R.J.U., D.W.D., R.F., D.M.M., PROGENI]; Norway: 775 PD, 614 controls [J.O.A.]; France: 574 PD, 469 controls [M.-C.C.-H., A.D.]; Ireland: 400 PD, 460 controls [D.G., T.L., M.H.]; Poland: 397 PD, 366 controls [A.K.-W., G.O.]; Italy: 192 PD, 96 controls [A.R.B., E.M.V.]) and 278 affected and 379 control subjects of Arab-Berber ethnicity from Tunisia [F.H., R.A.G.]. Genotyping was performed with the Sequenom MassArray iPLEX platform (San Diego, CA). All primer sequences are available on Table S1. All mutations discovered were confirmed by direct sequencing.

Chromosome 3 Haplotype Analysis

STR and SNP markers were chosen to span the *EIF4G1* locus, including D3S3037, Chr3_179.425, D3S3730, D3S3699, Chr3_182.108, D3S2312, D3S2314, Chr3_184.176, Chr3_184.543, Chr3_184.806, D3S1571, Chr3_185.212, Chr3_185.320, Chr3_185.398, Chr3_185.425, Chr3_185.464, D3S3609, Chr3_185.526, D3S3578, Chr3_185.619, Chr3_185.633, Chr3_185.653, Chr3_185.692, D3S3583, Chr3_185.822, Chr3_185.860, Chr3_185.870, D3S3592, Chr3_186.199, D3S1530, D3S1262, D3S2436, D3S3686, D3S3651, and D3S1580 (Table S2). Eighteen of these STR markers are novel (prefixed with Chr3_ as "D segment" numbers are no longer available) and were designed by searching for repeat polymorphisms in 6.77 Mb of genomic sequence spanning the locus. One primer of each pair was labeled with a fluorescent tag; amplified sequences are available on Table S1. PCR reactions were performed under standard conditions and products were run on an ABI 3730 genetic analyzer. Results were analyzed with GeneMapper 4.0 software (Applied Biosystems). Five SNPs located in *EIF4G1*, rs4912537, rs2178403, rs2293605, rs1879244 and rs2230571, were included. Marker-allele frequencies for the European population were obtained from the CEPH genotype database, the Human HapMap database, or estimated by genotyping 100 unrelated healthy subjects from North America of European descent. NCBI build 36.1 is referenced throughout.

Testing for *EIF4G1* Copy-Number Variation

To determine whole-gene copy number, primer pairs, and probes were designed against exon 10, 18, and 24 of *EIF4G1*. An endogenous control assay was designed against exon 5 of the presenilin 2 gene. All primers and probes were purchased from Applied Biosystems and sequences are available on Table S1. Quantitative PCR was carried out with TaqMan expression chemistry protocol; 25 ng genomic DNA was amplified with 0.25 μl primer probe and 2.5 μl TaqMan 2X Universal PCR Master Mix (Applied Biosystems). The thermal cycle conditions were performed at 50°C for 2 min and at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s for denaturation and at 60°C for 1 min for annealing and extension. All assays were performed in triplicate on the ABI 7900HT

Fast Real-Time PCR System and analyzed with ABI SDS 2.2.2 Software (Applied Biosystems).

EIF4G1 Cloning and Coimmunoprecipitation Studies of Protein-Complex Binding Partners

Full-length *EIF4G1* wild-type (WT) and a dominant negative mutant were amplified from plasmids as previously described[14] and subcloned into pcDNA6.2-V5 and pcDNA6.2/C-EmGFP-Dest expression vectors. The eIF4G1 p.Ala502Val and p.Arg1205His mutations were subsequently introduced with site-specific mutagenesis (primers available in Table S1). HEK293T cells, maintained in Opti-mem 1+GlutaMAX1 with 10% of fetal calf serum and penicillin/streptomycin (all GIBCO) at 37°C and 5% CO2, were transfected with cDNA encoding the respective WT or mutant eIF4G1-V5 proteins with Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). Cells were harvested 48 hr after transfection and lysed in 50 mM Tris/HCL, 150 mM NaCl, and 0.1% Triton X-100. To avoid unspecific binding during immunoprecipitation, we precleared crude lysates by rotating them at 4°C for 1 hr with Protein A/G ultralink resin (Thermo Scientific) followed by centrifugation at 13,000 × *g* for 10 min. After performing a BCA Protein assay (Pierce), equal amounts of supernatant were combined with protein G Dyna beads (Invitrogen) preconjugated with monoclonal mouse anti-V5 antibodies (Invitrogen) and rotated on a spinning wheel for 4 hr at 4°C. The resulting immunocomplex was stringently washed with intraperitoneal (IP) buffer/PBS and eluted in SDS-Sample buffer (Invitrogen). Immunoprecipitated and coprecipitated proteins were analyzed with SDS PAGE/immunoblot technologies. For protein detection, monoclonal anti-V5 (Invitrogen) and polyclonal rabbit eIF4E, eIF3e, and eIF4A (Abcam) were applied. ImageJ was used to quantify the relative amounts of copurified interactors eIF4A1, eIF4E, and eIF3e, taking into account the amounts in the full lysates and the amount of immunoprecipitated eIF4G1 proteins. One-way ANOVA was applied for statistical analysis.

Assessment of Mitochondrial Function by Flow Cytometry

HEK293T cells were transfected with plasmids encoding GFP-tagged eIF4G1 proteins (WT, p.Ala502Val, and p.Arg1205His) and subsequently subjected to viability studies with fluorescence activated cell sorting (FACS) analysis. Incorporation of the mitochondrial-permeable dye tetramethyl rhodamine ethyl ester (TMRE; Invitrogen) has been shown to reflect mitochondrial activity and was used as an indicator for cellular viability. Cells, maintained at 37°C in Opti-mem 1+GlutaMAX1 with 10% fetal calf serum medium (both GIBCO), were seeded at a density of 150,000 cells/6-well and transfected 24 hr prior to FACS analysis. Duplicates of each condition were treated with either 0 or 0.5 mM H_2O_2 for 6 hr. Labeling with 100 nM TMRE was performed by directly adding TMRE from a DMSO stock solution (0.2 mM) to the growth medium and incubating for 30 min at 37°C. After two washing steps with warm PBS containing 1% FBS, cells were trypsinized, resuspended, and immediately subjected to FACS analysis. We established specific settings for the separation into transfected (GFP positive) and untransfected (GFP negative) as well as viable (TMRE positive) and TMRE negative cells by using appropriate controls including (1) untransfected, no TMRE; (2) transfected, no TMRE; (3) untransfected, TMRE stained; and (4) transfected, TMRE stained samples. For each sample, 100,000 events were acquired and subsequently analyzed. After removal of background counts and the exclusion of untransfected (GFP negative) cells, average TMRE loading values were based on at least 25,000 cells. The number of TMRE positive cells were calculated as the percentage of the total number of GFP positive cells in the respective sample.

Results

Genetic and Physical Mapping

Genome-wide analysis of the P30 pedigree led us to identify two regions with suggestive two-point LOD scores > 2 ($\theta = 0$), a 31 cM interval between D3S1763 and D3S1580, and a 15 cM interval between D5S2055 and D5S393. The two loci on chromosomes 3 and 5 were saturated with STRs at <2.0 cM resolution to maximize genetic information, and genotype segregation with disease was reanalyzed with additional DNA samples from family P30. Significant linkage was obtained only for chromosomal region 3q26-q28 (mLOD = 3.01) with a haplotype shared by all affected relatives.

Identification of an *EIF4G1* Mutation in Family P30

In silico analysis revealed that the critical interval contained 159 genes (1432 exons). We sequenced each of these along with their donor and acceptor splice sites by using genomic DNA samples from three affected first cousins of P30 (Figure 1A; III-2, III-3, III-5). We found 236 known SNPs and 33 novel variants with only five that were present in all three affected family members sequenced; three intronic variants were located more than 20 bases from exon-intron boundaries. Of those novel variants, only *EIF4G1* mutation c.3614G>A (p.Arg1205His) was found to segregate with disease in all ten blood-related, affected family members (family P30 two-point LOD = 3.55, $\theta = 0$). The mutation was absent in 146 unrelated control subjects from the same ethnical and geographical origin and in 370 unrelated North American control subjects of European descent. Because chromosome 3q26-q28 is a fragile site in cancer and genomic multiplications are associated with malignancy,[10] probes in *EIF4G1* were used to grossly assess copy number variants but none were found (n = 225 affected subjects).

eIF4G1 c.3614G>A is located in exon 24 of a 33 exon gene. It predicts the substitution of a conserved arginine to histidine at residue 1205 (p.Arg1205His) of the protein. A cross-species alignment of the eIF4G1 demonstrated the p.Arg1205His is highly conserved across species (Figure S1). In silico analysis predicts the mutation might be damaging to protein function (Polyphen-2 PSIC score = 0.99).

Screening of *EIF4G1* c.3614G>A (p.Arg1205His) in Individuals with PD

To further investigate the pathogenicity of the *EIF4G1* c.3614G>A (p.Arg1205His), we genotyped 4050 control subjects and 4708 individuals with idiopathic PD. We identified nine affected heterozygotes from seven families originating from the US (385-1493, 2498), Canada (2499),

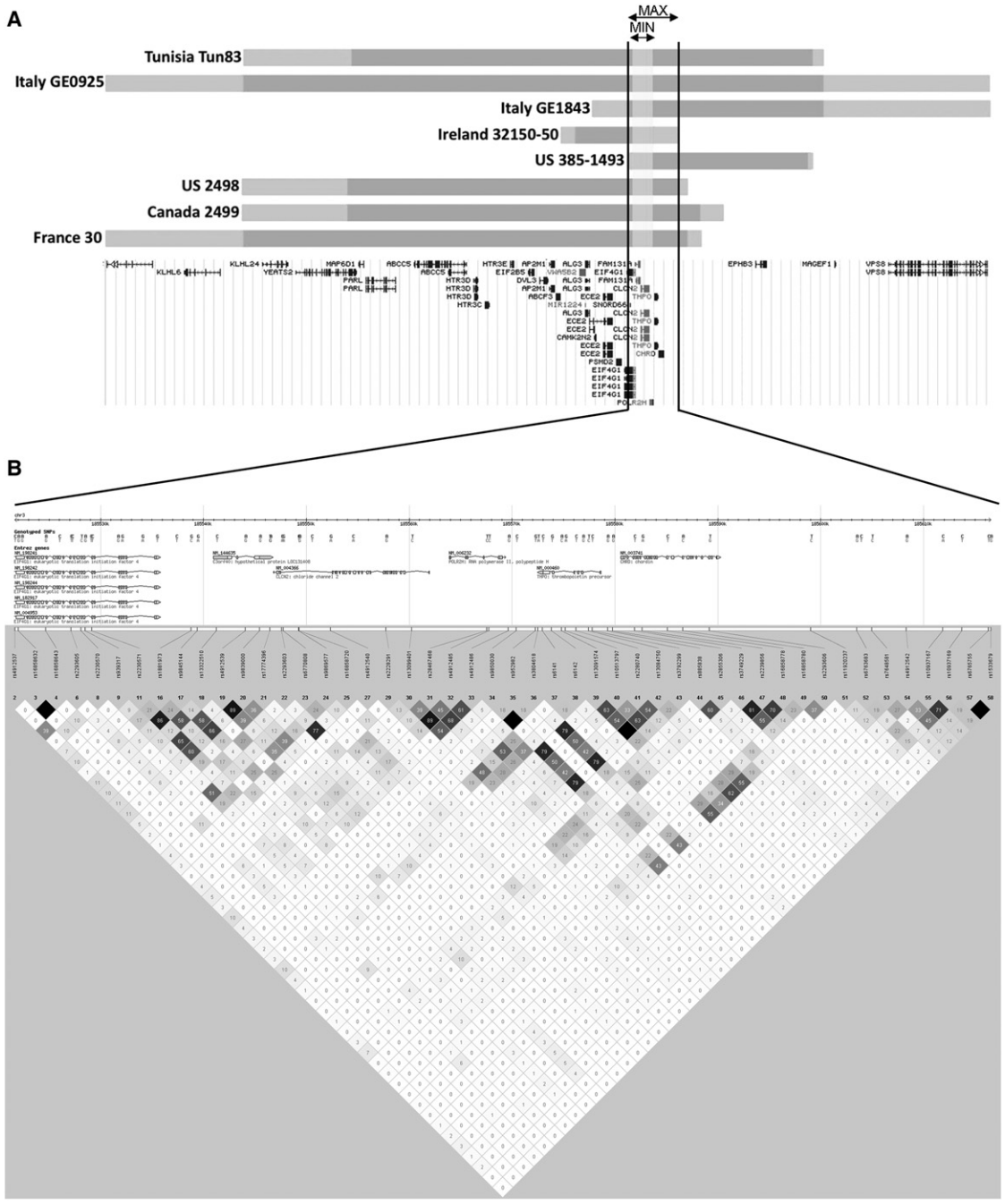

Figure 2. Disease-Segregating *EIF4G1* c.3614G>A Haplotypes among Families
(A) Comparison of haplotype data among all families with *EIF4G1* mutations shows alleles between rs4912537 and D3S3578 are shared (Table S2). The maximum shared interval is 99,330 bp (MAX; chr3:185,519,706–185,619,035; gray and blue bars) and contains six gene transcripts, *EIF4G1*, *FAM131A*, *CLCN2*, *POLR2H*, *THPO*, and *CHRD*. The minimal shared interval is 45,676 bp (MIN; chr3:185,521,663-185,567,338; gray) and harbors four genes (*EIF4G1*, *FAM131A*, *CLCN2*, and *POLR2H*). *EIF4G1* c.3614G>A (p.Arg1205His) is the only novel coding variant that segregates with disease.
(B) The CEU haplotype map suggests negligible linkage disequilibrium across the rs4912537–D3S3578 region.

Ireland (32150- 50), Italy (GE0925 and GE1843), and Tunisia (Tun83) (Figure 1A). A family history of parkinsonism was not evident in the Irish (32150-50), one Italian (GE0925), or the Tunisian (Tun83) subjects. In contrast, eIF4G1 p.Arg1205His was absent in control subjects.

To determine whether the *EIF4G1* c.3614G>A (p.Arg1205His) mutation segregates with disease in these seven cases, we genotyped 42 STRs distributed across the 3q26-q28 locus flanking *EIF4G1* c.3614G>A (Figure 2A; Table S2A). Haplotype analysis shows that each affected member of all seven families shares a minimal interval of 45,676bp (chr3:185,521,663-185,567,338) in common with family P30. With the exception of *EIF4G1* c.3614G>A (p.Arg1205His) no other novel coding variant

segregates with the disease in this shared haplotype, in which linkage disequilibrium between neighboring variants is marginal (Figure 2B). The results suggest the deleterious *EIF4G1* c.3614G>A (p.Arg1205His) mutation originates from an ancestral founder and segregates with disease in seemingly unrelated families.

Screening for Novel *EIF4G1* Variants in Other Families

To further evaluate the *EIF4G1* mutations in PD, the 31 coding exons were sequenced in 95 randomly selected affected probands with autosomal-dominant parkinsonism, 130 pathologically-defined cases with Lewy body disease, and 185 ethnically matched controls. Sequence analysis identified eight novel coding variants in affected subjects (4 missense, 4 silent), three novel changes in controls (two missense, one silent), and three novel coding variants in affected and control subjects (Table S3). The four missense mutations not observed in controls were c.1505C>T (p.Ala502Val), c.2056G>T (p.Gly686Cys), c.3490A>C (p.Ser1164Arg), and c.3589C>T (p.Arg1197Trp) (Table S3). They were detected in two affected probands with parkinsonism (US 623, US 385-1391) and two autopsied cases with Lewy body disease (US 331-5, US 331-95) (Figure 1B).

These four variants were subsequently genotyped in a case-control series consisting of 4483 individuals with idiopathic PD and 3865 age, gender, and ethnically matched control subjects. Screening identified only affected heterozygotes p.Ala502Val (US 385-284 and Poland 1736-155) and p.Gly686Cys (2 with PD in the family French P141), whereas the p.Ser1164Arg and p.Arg1197Trp mutations were not observed again in affected or control subjects.

Consistent with eIF4G1 p.Arg1205His, the p.Gly686Cys, p.Ser1164Arg, and p.Arg1197Trp mutations are also evolutionarily conserved in mammals (Figure S1). Similarly, eIF4G1 p.Ala502Val is conserved in most mammals with the exception of the rabbit that has a valine at this position. Limited cross-species conservation typically argues against pathogenicity, but the first alpha-synuclein p.Ala53Thr mutation linked to PD is a notable precedent.[15] Assessment of segregation with disease was not possible for p.Ala502Val, p.Gly686Cys, p.Ser1164Arg, and p.Arg1197Trp mutations, and as a consequence genetic evidence for pathogenicity is limited. STR marker analysis of chromosomal region 3q26-q28 in all heterozygotes with p.Ala502Val suggested an ancestral founder, although the phase was only available for one pedigree (Table S2A).

Clinical Findings and Neuropathological Analyses

Within family P30, the clinical phenotype among *EIF4G1* c.3614G>A (p.Arg1205His) heterozygotes is consistent with late-onset PD, with a mean onset of 64 ± 10.3 standard deviation (SD) years (n = 10), but spans a broad range (50–80 years) (Figure 1A). Symptoms start insidiously with asymmetric resting tremor or akinetic rigidity and become progressively mixed. The parkinsonism has a relatively long, mild course, and cognition seems preserved. Patients' symptoms respond well to L-DOPA, but many remain untreated or use only dopamine agonists. In clinically symptomatic individuals' dopaminergic imaging with I-*123 Ioflupan*, DaTSCAN is abnormal and asymmetric.

The clinical phenotype among unrelated subjects with a heterozygous *EIF4G1* c.3614G>A (p.Arg1205His) mutation was comparable to family P30 with a mean symptom onset of 59 ± 13.0 SD years (n = 9), range 32–77 years (Figure 1A). Overall, affected subjects with an *EIF4G1* c.1505C>T (p.Ala502Val) mutation have a mean age of symptom onset of 52 ± 8.1 SD years (range 41–58 years, n = 4) (Figure 1B).

The ages at onset of subjects who have PD with other *EIF4G1* substitutions (c.2056G>T [p.Gly686Cys] and c.3490A>C [p.Ser1164Arg]) were 55, 62, and 64, respectively, in the France P141 and US 385-1391 families. *EIF4G1* mutations were also observed in two autopsy-confirmed cases with Lewy body disease: an *EIF4G1* c.1505C>T (p.Ala502Val) mutation in family US 331-5 and the double *EIF4G1* mutant c.2056G>T (p.Gly686Cys) and c.3589C>T (p.Arg1197Trp) in family US 331-95 (Figures 1B and 1D). The Lewy body pathology in these cases is presented in Figure S2.

Functional Analyses of eIF4G1 p.Arg1205His and p.Ala502Val Substitutions

eIF4G1 p.Arg1205His is just distal to the putative eIF3e binding domain (amino acids 1015–1118; Figure S3), a key partner of the translation initiation complex that recruits the 40 S ribosomal subunit.[16–18] Thus, we evaluated WT and mutant eIF4G1 binding to eIF3e. Plasmids containing eIF4G1 WT or eIF4G1 p.Arg1205His or eIF4G1 LacZ were transfected into HEK293 cells. Coimmunoprecipitation studies of protein lysates from mutant eIF4G1-transfected cells show that p.Arg1205His perturbs eIF3e binding, but the WT protein does not (Figure 3).

eIF4G1 p.Ala502Val is the most frequent substitution encountered in other familial cases, although genetic evidence for segregation with disease and thus pathogenicity is limited. Its physical location predicts that the substitution might perturb eIF4E binding (amino acids 612-618). Coimmunoprecipitation studies of protein lysates from mutant eIF4G1 transfected cells show p.Ala502Val perturbs eIF4E binding compared to WT protein (Figure 3). Both p.Ala502Val and p.Arg1205His are located within or proximal to the eIF4A-interacting domains (amino acids 761-988 and 1240-1450) but do not appear to affect its binding, although a nonsignificant trend toward increased binding was observed for p.Ala502Val.

When TMRE, a marker for mitochondrial membrane polarization, was applied to either WT or mutant (p.Ala502Val; p.Arg1205His) eIF4G1 transfected cells, no differences could be detected under basal conditions.[10] However, with hydroperoxide treatment, a profound loss in mitochondrial membrane potential was observed for both mutations but not for cells overexpressing WT protein (Figure 4). Complementary results were obtained

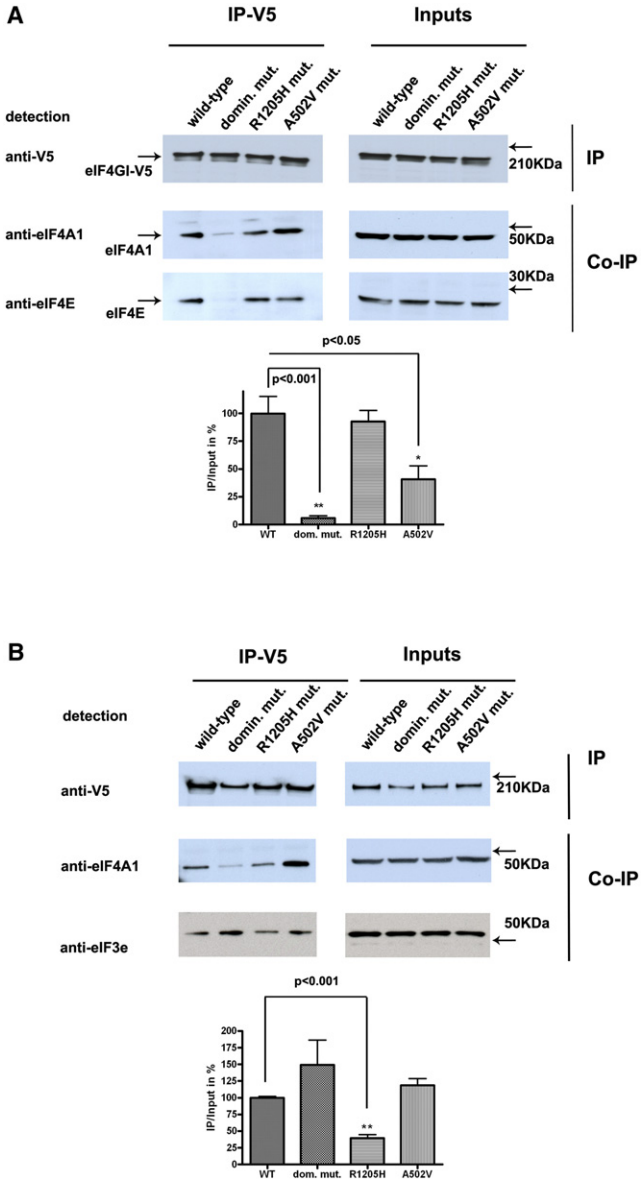

Figure 3. Coimmunoprecipitation Studies of eIF4G1 p.Ala502Val and p.Arg1205His with eIF4A and eIF3e

Coimmunoprecipitation (CO-IP) of endogenous eIF4A, eIF4E, and eIF3e with transfected full-length eIF4G1-V5 (WT, a dominant negative mutant,[13] or the p.Ala502Val and p.Arg1205His mutants) in HEK293T cells.

(A) The p.Ala502Val mutant perturbs the interaction with eIF4E but not eIF4A (IP-V5). The inputs on the upper right panel represent the amounts of transfected eIF4G1-V5 (WT and mutants) and the endogenous proteins (eIF4A1 or eIF4E) introduced in the immunoprecipitation assay.

(B) Coimmunoprecipitation of eIF3e with eIF4G1-V5 (IP left; inputs right). In the presence of the p.Arg1205His mutation significantly reduced amounts of eIF3e protein are coprecipitated with eIF4G1-V5. Experiments were performed on three separate occasions as depicted in the graphs. Representative immunoblots are shown.

with patient-derived lymphoblastoid cell lines (data not shown). When starvation was used as an alternate stressor, the induction of autophagy was comparable in WT and mutant (p.Ala502Val; p.Arg1205His) overexpressing cells. The lack of response might reflect the presence of endogenous eIF4G1 (Figure S4).[9]

Discussion

eIF4G1 is the core scaffold of a multisubunit translation initiation complex that regulates the translation initiation of mRNAs encoding mitochondrial, cell survival and growth genes in response to different stresses.[9,10] The pathogenicity of *EIF4G1* mutations is supported by (1) the segregation of eIF4G1 p.Arg1205His with disease in a family with multi-incident autosomal-dominant, parkinsonism; (2) the absence of eIF4G1 p.Ala502Val and p.Arg1205His in control subjects (n = 4050), although observed in several multi-incident families with autosomal-dominant late-onset PD; (3) an eIF4G1 p.Arg1205His haplotype suggestive of one ancestral founder, and delimiting the candidate gene interval; (4) the sequence conservation of eIF4G1p.Ala502Val and p.Arg1205His substitutions suggesting these mutations might be functionally deleterious; and (5) impaired binding of eIF4G1 p.Ala502Val and p.Arg1205His to eIF4E or eIF3e, interactions that are normally required for translation initiation.[17,18] Both p.Ala502Val and p.Arg1205His directly impair formation of the larger eIF4 complex and support the genetic argument for a dominant-negative loss-of-function compatible with an age-dependent neurodegenerative disorder; (6) the loss of mitochondrial membrane potential and viability in transfected cells subjected to oxidative stress. The functional data presented are cursory and further analyses of mitochondrial biogenesis, autophagy, and transcription and translation, in additional models is warranted. A caveat of linkage within a multi-incident family is that there remains a remote possibility of a missing segregating variant, most likely within the disease-segregating haplotype. Although rare, additional *EIF4G1* mutations in other families will be important to discover.

EIF4G1 mutations directly implicate mRNA translation initiation in parkinsonism and might help unify other monogenic forms, toxin, and perhaps virally-induced disease within a convergent pathway.[19,20] Availability of eIF4E is generally the rate-limiting step of translation initiation and is largely determined by phosphorylation of eIF4E-binding proteins (4E-BP) through the mammalian target of the rapamycin (mTOR) pathway.[9,21] Constitutive activity of mTOR signaling normally leads to phosphorylation of 4E-BPs; this dislodges eIF4E and enables assembly of the eIF4F complex to promote cap-dependent translation (Figure S3).[18] Notably, 4E-BP is a substrate of human Lrrk2 and the *Drosophila* ortholog (Lrrk).[22] Lrrk2 pathogenic mutations cause hyperphosphorylation of 4E-BP leading to reduced oxidative stress resistance and dopaminergic neurodegeneration. Conversely, inhibition of mTOR signaling during development or overexpression of 4E-BP in *Drosophila* mutants with *PINK1* or *Parkin* loss-of-function suppresses the flies' pathologic phenotypes.[23]

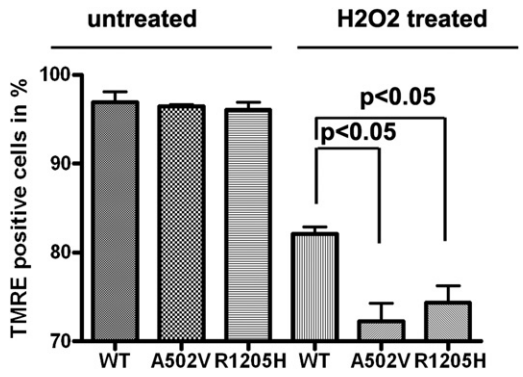

Figure 4. Fluorescence-Activated Cell-Sorting Analysis of Mutant and Control Cell Lines HEK293T Cells Transiently Overexpressing WT or Mutant EIF4G1 (p.Ala502Val, p.Arg1205His)
eIF4G1-GFP proteins were subjected to FACS analysis, with TMRE labeling applied as a marker of mitochondrial polarization. Untreated eIF4G1-GFP-positive cells are comparable in terms of TMRE loading. In contrast, with hydrogen peroxide treatment p.Ala502Val- and p.Arg1205His eIF4G1-GFP-positive cells show a 10% decrease in cell survival compared to WT cells. For each sample, 100,000 events were acquired and subsequently analyzed. After the removal of background counts and the exclusion of untransfected (GFP negative) cells, average TMRE loading values were based on at least 25,000 cells. The number of TMRE-positive cells was calculated as a percentage of the total number of GFP-positive cells in the respective sample. Experiments with two duplicates per condition were performed on three separate occasions.

The variability in clinical and pathologic presentation of affected members of *EIF4G1* families is similar to genetically-defined parkinsonism but most consistent with idiopathic, late-onset Lewy body PD. Although *EIF4G1* mutations represent a molecular mechanism for PD, genetic defects in translation have been implicated in many neurological diseases.[24] Most are private mutations in orphan families. Nevertheless, the three-fold increase in rare *EIF4G1* coding variants in PD compared to control subjects warrants further study. Common polymorphic variability in the *EIF4G1* locus was not associated with PD in case-control samples.

These data suggest that eIF4G1 p.Ala502Val and p.Arg1205His substitutions affect scaffold function and consequently impair the ability of cells to rapidly and dynamically respond to stress, presumably through changes in the translation of existing mRNAs essential to cell survival.[9,10] Perturbations in mTOR/4E-BP signaling and eIF4G1-mediated changes in mRNA translation will be important to elucidate in neurons in vivo. The pathway highlighted might represent a central theme in PD for which targeted interventions already have therapeutic potential.[25]

Supplemental Data

Supplemental Data include four figures and three tables and can be found with this article online at http://www.cell.com/AJHG/.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

Human Polymorphism Study Center (CEPH) database, http://www.cephb.fr
dbSNP homepage, http://www.ncbi.nlm.nih.gov/SNP/
Genethon, http://www.genethon.fr

Haploview, http://www.broad.mit.edu/mpg/haploview/
International HapMap Project, http://www.hapmap.org
MAP-O-MAT genotype database, http://compgen.rutgers.edu/mapomat/
National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/
PolyPhen, http://genetics.bwh.harvard.edu/pph2/
RefSeq, http://www.ncbi.nlm.nih.gov/RefSeq/
UCSC human genome browser, http://genome.cse.ucsc.edu/cgi-bin/hgGateway

Accession Numbers

NCBI accessions NM_198241.2 and NP_937884.1 were used to number all variants within the *EIF4G1* gene and eIF4G1protein.

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Supplemental Data

Translation Initiator *EIF4G1* Mutations in Familial Parkinson Disease

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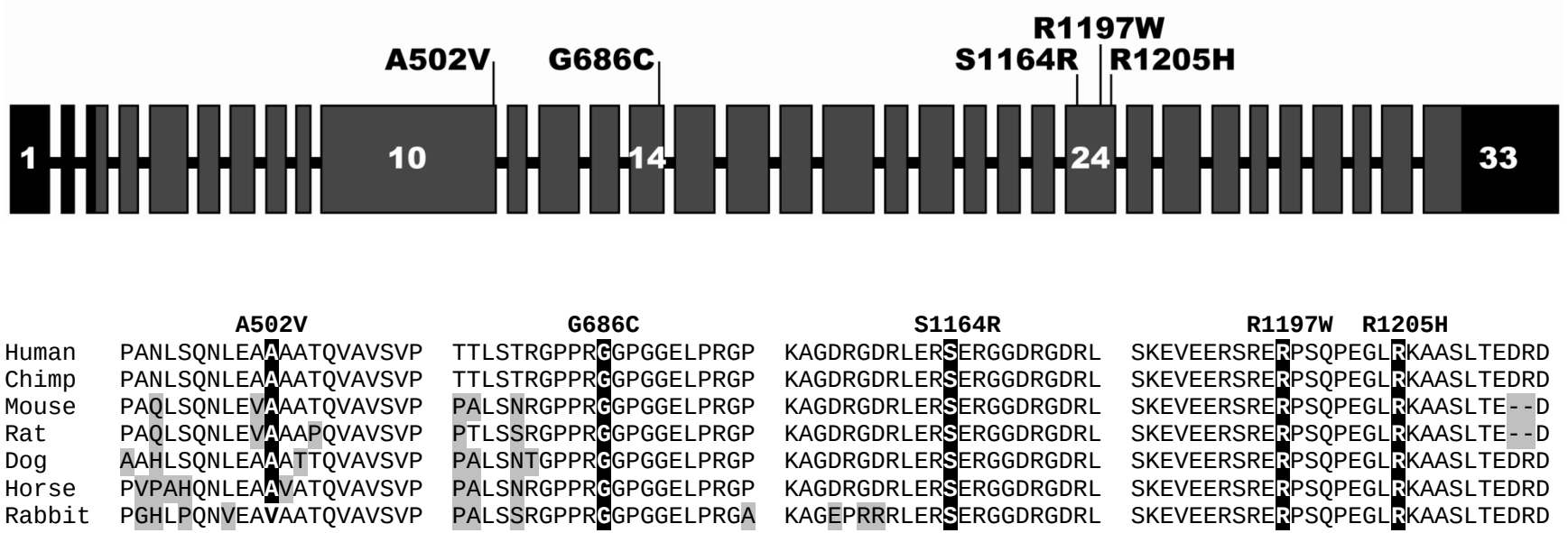

Figure S1. Novel eIF4G1 Mutations Observed Only in Cases with Cross-Species Conservation

The *EIF4G1* gene structure spans 185,515,050-185,535,840 bp of chromosome 3q27.1. The upper panel is a schematic of *EIF4G1*, the mutation nomenclature given with respect to RefSeq identifier NM_198241. Exons are drawn to scale and represented with grey boxes with untranslated regions in black. The lower panel highlights the conservation of mutations identified in patients with PD. Protein homologs were aligned using ClustalW. Genebank accession numbers: NP_937884, *Homo sapiens*; XP_001145407, *Pan troglodytes*; NP_666053, *Mus musculus*; XP_213569, *Rattus norvegicus*; ENSCAFP00000018694, *Canis vulgaris*; NP_001157343, *Equus caballus;* NP_001076136, *Oryctolagus cuniculus*.

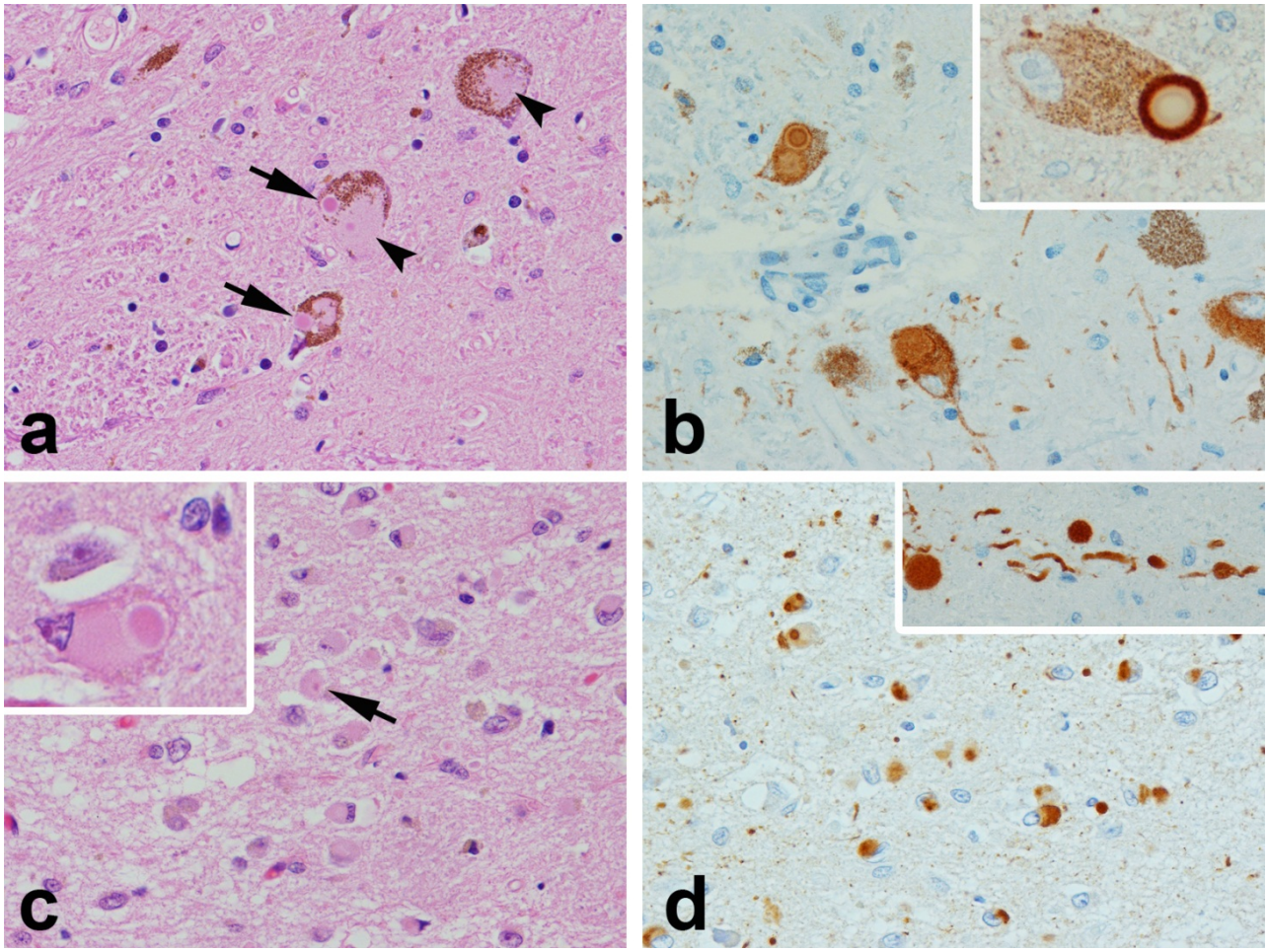

Figure S2. Lewy Pathology in *EIF4G1* Mutation Carriers

Panels a) and c) are from a carrier with *EIF4G1* c.2056G>T (p.Gly686Cys) and c.3589C>T (p.Arg1197Trp) mutations (331-95), whereas panels b) and d) are from a carrier with *EIF4G1* c.1505C>T (p.Ala502Val). a) *substantia nigra* Lewy bodies (arrows) and pale bodies (arrow heads); b) *substantia nigra* Lewy bodies (α-synuclein immunohistochemistry); inset locus ceruleus); c) amygdala Lewy bodies, some with hyaline appearance (arrow); inset: cortical Lewy body, and d) amygdala Lewy bodies, some with hyaline appearance; inset: Lewy neurites, medulla. Of note, antibodies to eIF4G1 did not stain Lewy body pathology.

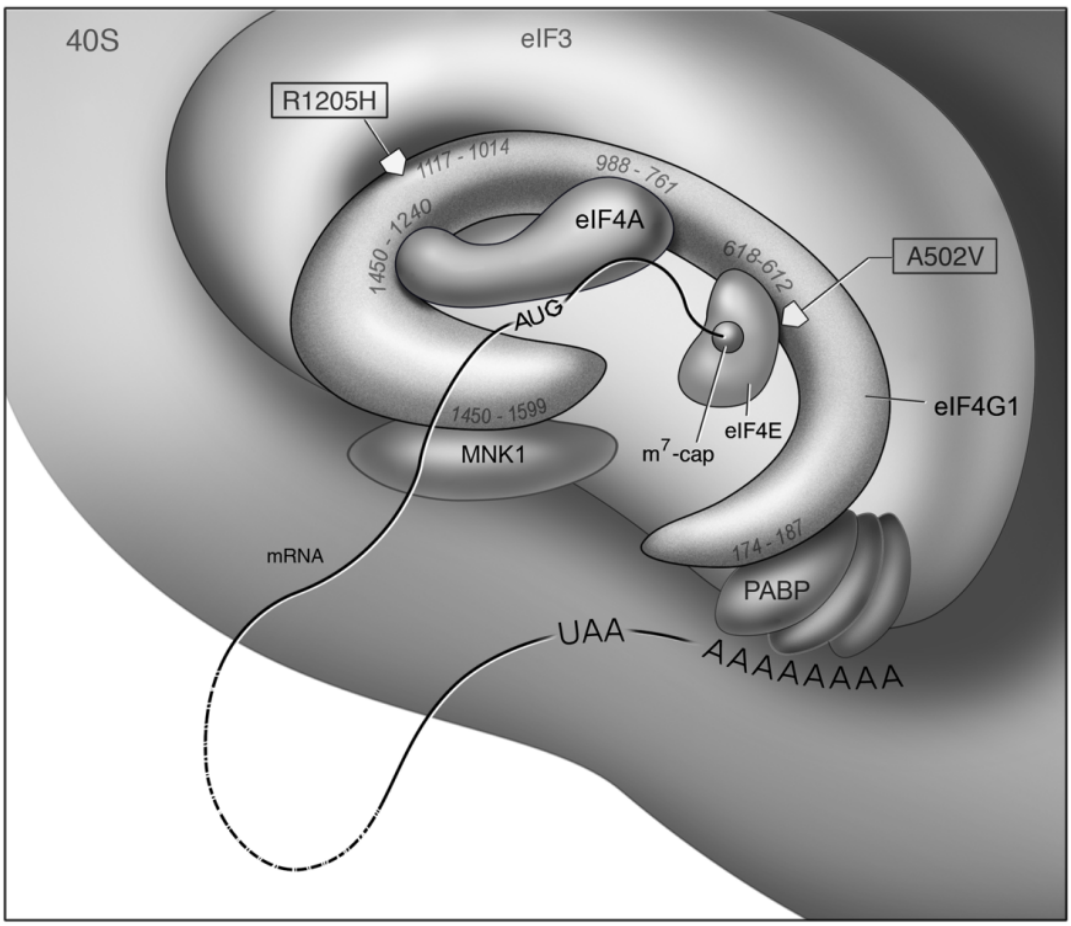

Figure S3. Ideogram of eIF4G1 Protein Interactions

The eIF4G1 N-terminus binds eIF4E (the mRNA m7GTP cap binding protein) and PAPB (poly-adenine binding protein which attaches to the mRNA tail) proteins thereby circularizing the mRNA message. The central eIF4G1 domain recruits eIF4A (an ATP dependent helicase that unwinds double stranded RNA to expose the 5' AUG start codon) and eIF3.[16] The C-terminal of eIF4G1 contains additional binding sites for eIF4A and regulatory factors including Mnk1 (MAP kinase signal-integrating serine/threonine kinase 1). Together eIF3 and eIF4F bind the ribosomal 40S subunit and methionyl tRNA (Met-tRNAiMet, recruited by GTP-bound eIF2). Met-tRNAiMet then scans the mRNA to find the AUG start codon to initiate translation. eIF4G1 interactions with binding proteins are based on a previous review.[16] eIF4G1 amino acid numbering is based on UniProtKB/Swiss-Prot entry Q04637, without glutamine at position 697. Several isoforms have been reported but the codon for Q697 was not observed in *EIF4G1* sequencing nor in genomic databases. The approximate positions of *EIF4G1* c.1505C>T (p.Ala502Val) and c.3614G>A (p.Arg1205His) mutation are highlighted.

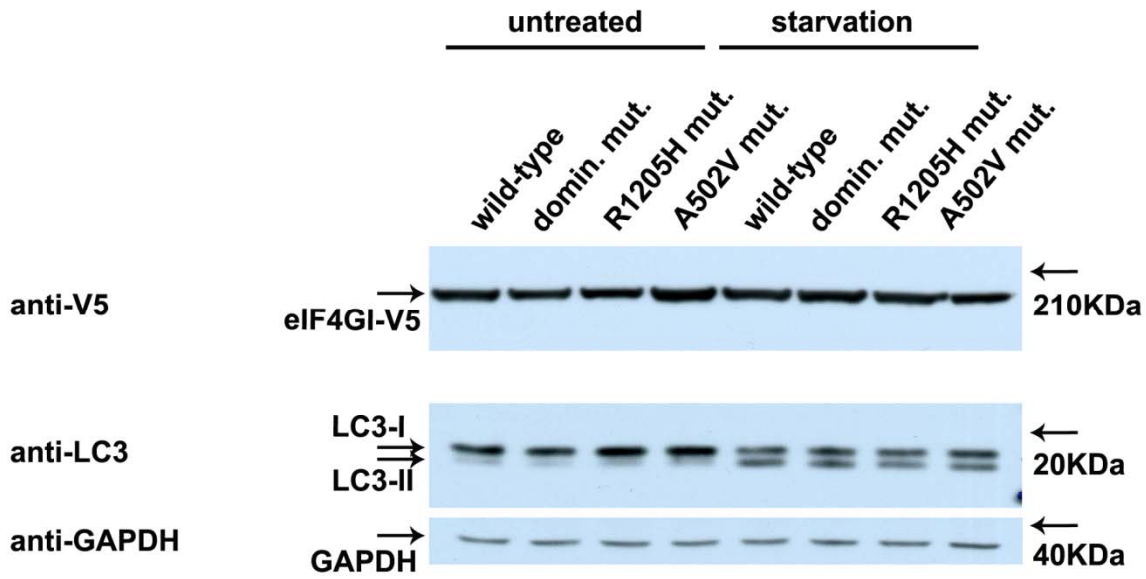

Figure S4. Starvation Induced Autophagy

HEK293T cells were seeded and transfected with WT or mutant eIF4G1-V5. 20 hours post-transfection full growth medium (Opti-mem 1+GlutaMAX1 with 10% of fetal calf serum (both Gibco) was replaced with starvation medium (EBSS, HyClone). Cells were harvested, lysed and subjected to Western blot analysis using standard techniques. For protein detection, antibodies against V5 (to detect the respective eIF4G1-V5 proteins) (Invitrogen), LC3 (Cell Signaling) and GAPDH (to account for potential errors in the assay; Meridian Life Science) were applied.

Starvation induced autophagy HEK293T cells transiently over-expressing WT or mutant eIF4G1-V5 proteins were subjected to starvation conditions for 3 hours. Induction of autophagy as indicated by lipidation of LC3-I (conversion to LC3-II) can be observed in a comparable manner in both WT and mutant eIF4G1 transfected cells. To account for potential errors in the assay the housekeeping protein GAPDH was used. However, depletion of eIF4G1 in cells only causes modest alterations in very specific pathways.[9;18] Overexpressing mutant proteins in the presence of endogenous eIF4G1 might not be sufficiently sensitive to detect changes.

Table S1. Primer and Probe Sequences

a) *EIF4G1* sequencing primers

Primers ID	Forward	Reverse
EIF4G1_ex1-2	GCATCAACCCTTTCACCG	CAGAGGATTAAGGCCAGGTATG
EIF4G1_ex3-4	CCCACAGTACTTCTTTCCAGG	GATGAGTTTAGATGCTCCCTTG
EIF4G1_ex5	TTGACAGACACCTAATTCCCTG	TTCCTCCTCAAGTCACTGCTC
EIF4G1_ex6-7	GTTTCTGGCACCTACCTACCTG	GGTACCCAGTTCAAGTCACTCC
EIF4G1_ex8.1	TGGAGTGACTTGAACTGGGTAC	GCTCATGAATTTCCACTGTGTG
EIF4G1_ex8.2	CTCTCTCCAGAACCCACTC	CAGGGACCCAGAAACATGTC
EIF4G1_ex9-10	ATACTGTTCTTTGGCTGCTTTC	AGAGGCTTCCACTGATCTGC
EIF4G1_ex11-12	GTATGCTGAAGAAAGGGTTGAG	ACCACTACTACACGCCTCCAG
EIF4G1_ex13	TGATTCCGTGTCTCAGTGCC	TGTTGGGAGGGACAGGAGAG
EIF4G1_ex14	CCCTCTTTGCTTCTTTTTG	GGAAGAGGCACAAGTTGGAG
EIF4G1_ex15	CTCCAACTTGTGCCTCTTCC	CAAGACACCACTACCTCCATTC
EIF4G1_ex16-17	GAGTGCTTATTGCTAGGTTTGG	ATGATGTCAGGGAAGGCAAG
EIF4G1_ex18-19	CCTTGTCTTGCCTTCCCTG	AGAGGAAACATTCAGCTGTCAC
EIF4G1_ex20-21	GGACTGGGCCATTCACTACC	TCTAACCCAAGGGCTCCAAC
EIF4G1_ex22	TGCTAAGAACAAGGCCCAACAG	CTAGTCCCAAGGCAGCCAATG
EIF4G1_ex23-24	TTGGCTGCCTTGGGACTAG	CTTATGCAAGGTTCCAAGGGTC
EIF4G1_ex25-26	GGTTCCATAGTTGATGCCCTAG	CATCACTCCACTCCACCAATG
EIF4G1_ex27-28	TAGGAATGGAAGGGCTTTGC	GCCTGTATGGGAGAAACAAATG
EIF4G1_ex29-30	GGGCAGCAAGAATGAAGACTG	GGCTGTGTCTAACTCCTTCCAC
EIF4G1_ex31	GATTAGCATCCTGTCAGAG	ACATCACACTACAGGCACCCAC

b) Mutation specific sequenom primers

SNP_ID	1st-PCRP	2nd-PCRP	UEP_SEQ
p.A502V	ACGTTGGATGTCCAACCACACCTTACCTTG	ACGTTGGATGAGAGAGTACCCCTATTCCAG	CTCAGAATTTGGAGGCAG
p.G686C	ACGTTGGATGTTTACCCAGCCCCACTCAC	ACGTTGGATGTTGGCCGGACAACCCTTAG	GCGTGGGCCCCCAAGG

p.S1164R	ACGTTGGATGAAAGCTGGAGACCGAGGAGA	ACGTTGGATGTCCGCTTGGTAGCAGGTGTC	GTCCCCTCCCCGTTCAC
p.R1197W	ACGTTGGATGATCCTCCGTGAGGCTAGCTG	ACGTTGGATGTCAGCAAGGAAGTGGAGGAG	GGAGCGGAGTAGAGAA
p.R1205H	ACGTTGGATGTTCCTCTCCCAGACTCTCAC	ACGTTGGATGTAGAGAACGGCCCTCCCAG	CCTCAGTGAGGCTAGCTGCCTTG

c) Haplotype Markers

Marker ID	Position	Forward	Reverse	CEPH 1331-01	CEPH 1331-02
D3S3037	178,924,190	GGATTACATTTCTAATCTGGAACG	TTGAGACATGTAACTTTTAATACGC		
Chr3_179.425	179,425,620	GTATGAGAAAGCTAAGGCAGGAAG	CTGGCTGGTTGTAAGATGAATG	288/290	292/294
D3S3730	180,029,294	GACTGGAAAATTCAGCCTCTA	AAGATGAGTCCTGAGCATGT		
D3S3699	180,779,738	GACCTTTGTTAGATACATAGTTTGC	CCAGCCAACCCTGTCT		
Chr3_182.108	182,108,831	AAGAAGGCAGGGCATGGTAG	TAGGGTAGTGGCATCATGAATG	228/228	228/232
D3S2312	182,873,855	AGGGAGCCCATATCATGCC	TACAGGTGTGAGCCACCGT		
D3S2314	183,613,071	AGGGTTGTTGTGGGATTTGAA	GCTGTGCTCTATTGTTAAGAC		
Chr3_184.176	184,176,772	CGTACCACTCAGAATCCTTCAATG	GGGATGAGGGAATAAGCCATG	259/259	259/265
Chr3_184.543	184,543,103	CCCCTGTAGTTTCTCAGACTC	CGTTGTCCTGAGCATTTTAC	160/162	160/174
Chr3_184.806	184,806,816	CTTCAAGAATCCCAACAAAC	AGCACTTTAAAGATGGCTTG	213/221	221/223
D3S1571	185,022,606	ACAGTGGCTGATGCCTT	CACAGGTGGGCACTACAT		
Chr3_185.212	185,212,491	AAAAGGGTAACGAGAGATCC	CAGTGGTAACATCTTGCATAAC	247/247	227/235
Chr3_185.320	185,320,825	CACACCATTGCAATCTACC	GACCTTCAAATATTGTCTCC	292/292	272/288
Chr3_185.398	185,398,321	TTGTGCATGAGGAAATACAG	AGGAGAATCCCTTGAACC	267/269	269/271
Chr3_185.425	185,425,698	AGTCAAGCTAAGACCCAGAG	GGCGATTTTAACTTTGATCC	331/335	331/331
Chr3_185.464	185,464,120	TCAACATGGCAAAACCCTGT	GATGTTATGTGATTTTCTTAATTTTCC	304/312	296/298
D3S3609	185,519,706	AGCTGGGGACCAGTCT	CGAGAGTAACTTGTACGGTG		
Chr3_185.526	185,526,620	AGACTAACTGGTTGGATTGCTG	TGAATGGGCTGGATCTGG	138/140	138/138
D3S3578	185,567,338	GATTGGGTTACAGAGTAGCCACAC	TCTGAAATGTAGACTGCCCTTTG		
Chr3_185.619	185,619,035	TTTCAAAGAATAACCCTCAGCG	CAAATGATTTGCCCACCTTG	197/197	201/201
Chr3_185.633	185,633,367	GTTGTAGAAAGCCGAGATTG	ACCCTTGAGCAGTATTTCAG	307/307	303/305
Chr3_185.653	185,653,111	CAGAGTCAGTTCGCCAGGTG	GGGCTCAAGTGACAATCCTG	307/307	303/305

Chr3_185.692	185,692,505	AACAAAAGCGAAACTCTGTC	GAGGGAGACTTCATTTTCAC	165/171	156/168
D3S3583	185,767,793	TGCAAAGTCACAGATGTCCA	CGAGAGGCACCAGAGTGTT		
Chr3_185.822	185,822,045	TAGTTGAGATGGGGTTTCAC	CTGAAGAATCCCTCTTTTCC	350/354	350/356
Chr3_185.860	185,859,628	AACTCTGCCCTTTACCTTGGAG	CGCTTGTCTTTCGACTGGG	321/325	321/325
Chr3_185.870	185,870,551	CTTTGGGCTATCTATGACACGG	TGATGTGGACATCCCAAACC	352/354	352/352
D3S3592	185,893,115	GCAGTTCTGAGTGATTTACCA	TCATCTGAGGTGTCTGATTG		
Chr3_186.199	186,198,962	TGTAGTAGCCCATGTGAGAG	AATTCTCAGCCATCTACACTG	319/319	319/319
D3S1530	186,866,252	GAGGCATGAGAATCGCTTG	TCCTTTTTCCTTCACACGTG		
D3S1262	187,706,172	CGGCCCTAGGATATTTTCAA	CCAGTTTTTATGGACGGGGT		
D3S2436	188,317,806	TCCAATCTCATCCAGGTTTC	ATGCCCATCAATCAATGAGT		
D3S3686	188,900,150	AGGGTATTTCATTCCCATTG	CCAGGTTACGCCAAGTG		
D3S3651	189,123,836	AGTGTGCTCTGGTTTTCTC	TTCGATATGAACTTGCTTATTG		
D3S1580	190,025,486	CCACCATACTCCAGTGATCC	TGACCATCTGTCCTGCCTT		

d) CNV primer and probe sequences

CNV probes	Forward	Reverse	Probe
PSEN2_ex5	CATCAGCCCTTTGCCTTCTC	GGTGTTCAGCACGGAGTTGA	TTCACTGAGGACACACCC
EIF4G1_ex10	CACCATCCCCAGTCTTGGAA	TTGTCATAGTGTCCCCAGGAATAGA	CCTAATCTCGCAGTCCTC
EIF4G1_ex18	CTGTGTGGTCAAACTGCTTAAGAAC	TGGTGAGCAGACGACAAAGG	GAAGAGTCCCTTGAGTG
EIF4G1_ex24	GCGCGGACACCTGCTA	CCGCTCCTCCACTTCCTTG	CCAAGCGGAGCTTCAG

e) Site-specific mutagenesis primers

	Forward	Reverse
EIF4G1_A502V	CAGAATTTGGAGGCAGTAGCAGCCACTCAAGTG	CACTTGAGTGGCTGCTACTGCCTCCAAATTCTG
EIF4G1_R1205H	CCTGAGGGGCTGCACAAGGCAGCTAGC	GCTAGCTGCCTTGTGCAGCCCCTCAGG

Table S2. Chromosome 3q26-27 Haplotypes of Families with *EIF4G1* c.3614G>A (p.Arg1205His) or c.1505C>T (p.Ala502Val) Mutations

a)

Marker ID	Position	France P30	Canada 2499	US 2498	US 385-1493	Ireland 32150-50	Italy GE1843	Italy GE0925	Tunisia Tun83	Shared Allele CEPH Freq.
D3S3037	178,924,190	213	NS	205	193/217	205/209	209/217	197/221	213	
Chr3_179.425	179,425,620	294	NS	290/298	290/292	290/294	288/294	288/290	286/292	
D3S3730	180,029,294	134	NS	142/152	140/142	140/150	138/146	138/156	140/158	
D3S3699	180,779,738	244	NS	248	244/246	244/246	250	250	250	
Chr3_182.108	182,108,831	228	NS	134	228	228/232	224/232	228/230	132/134	
D3S2312	182,873,855	242	NS	232	246/266	242/246	226/250	234/250	232	
D3S2314	183,613,071	222	NS	234/242	214/218	218/222	218/222	210	234/246	
Chr3_184.176	184,176,772	259	NS	214	265/269	259/261	259/261	259/263	214/218	
Chr3_184.543	184,543,103	160	NS	160	170	170/172	160/168	170	166/176	
Chr3_184.806	184,806,816	**229**	NS	235	221/229	213/225	223/229	223/**229**	225	229=3.1%
D3S1571	185,022,606	**172**	**172**	**172**	172	160/174	172/174	**172**	**172**/182	172=46.0%
Chr3_185.212	185,212,491	**247**	**247**	**247**	247	235/247	227/247	227/**247**	**247**	247=18.8%
Chr3_185.320	185,320,825	**300**	**300**	**300**	300	288/292	288/300	272/**300**	292/**300**	300=20.8%
Chr3_185.398	185,398,321	**265**	**265**	**265**	265/269	269	265	**265**/269	**265**/267	265=13.5%
Chr3_185.425	185,425,698	**331**	**331**	**331**	331	**331**/333	331	329/**331**	**331**	331=70.5%
Chr3_185.464	185,464,120	**298**	**298**	**298**	298	**298**/300	302/308	296/**298**	296/**298**	298=19.6%
D3S3609	185,519,706	**165**	**165**	**165**	173	**165**/179	**165**/173	**165**/169	**165**/173	165=3.6%
rs4912537	185,521,663	**T**	**T**	**T**	**T**	**T**/C	**T**/C	**T**	**T**	T=22.5%
rs2178403	185,522,368	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	G=75.0%
Chr3_185.526	185,526,620	**138**	**138**	**138**	**138**	136/**138**	136/**138**	**138**	**138**	138=72.8%
rs2293605	185,527,127	**T**	**T**	T	**T**	**T**/C	**T**/C	**T**	**T**	T=10.8%
p.Arg1205His	185,527,883	**A**	**A**	**A**	**A**/G	**A**/G	**A**/G	**A**/G	**A**/G	-
rs1879244	185,529,049	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	T=77.1%
rs2230571	185,529,164	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	C=74.2%
D3S3578	185,567,338	**240**	**240**	230/**240**	**240**	230/**240**	232/**240**	**240**	**240**	240=32.0%
Chr3_185.619	185,619,035	**201**	**201**	199/**201**	**201**/207	199	**201**/203	**201**/207	**201**/207	201=45.3%
Chr3_185.633	185,633,367	**239**	**239**	237	**239**/259	237	**239**/241	**239**	**239**/241	239=54.3%
Chr3_185.653	185,653,111	307	**303**	305	**303**	303/307	**303**	**303**/307	**303**	303=53.3%
Chr3_185.692	185,692,505	168	168	168	**165**/168	168/171	150/**165**	150/**165**	**165**/168	165=16.7%
D3S3583	185,767,793	272	272	276	272/**274**	272	**274**	**274**	272/**274**	274=14.3%

Chr3_185.822	185,822,045	356	356	356/360	350/**356**	350	348/**356**	**356**	350/**356**	356=32.3%
Chr3_185.860	185,859,628	321/325	321	321	**321**	321/323	307/**321**	**321**	**321**/325	321=41.5%
Chr3_185.870	185,870,551	356	352	352	356	352/354	**352**	348/**352**	**352**	352=42.4%
D3S3592	185,893,115	167	171	169	167	159/167	**167**/169	165/**167**	159/169	167=23.1%
Chr3_186.199	186,198,962	319	319	319	319	319/321	323	319/327	319	
D3S1530	186,866,252	246	254	242	250/254	238/254	254/258	242/250	242/246	
D3S1262	187,706,172	120	-	-	126	100/122	112/116	116/118	112/116	
D3S2436	188,317,806	172	172	172	168/172	168/172	168/172	164/172	168/172	
D3S3686	188,900,150	108	124	122/130	122/126	108/124	122/128	126/130	128/130	
D3S3651	189,123,836	254	252	254	248/254	252/254	252/254	248/252	250/252	
D3S1580	190,025,486	NS	-	-	145/149	145/153	145/157	149/153	139/141	

b)

Marker ID	Position	US 623	US 385-284	US 331-5	Poland 1736-155	Shared Allele CEPH Freq.
D3S3037	178,924,190	217	209/213	189/217	213/217	
Chr3_179.425	179,425,620	296	288/290	290/296	290	
D3S3730	180,029,294	152	152/154	140/148	140/150	
D3S3699	180,779,738	250	248/250	244/252	248/250	
Chr3_182.108	182,108,831	228	228	224/232	224/228	
D3S2312	182,873,855	242	230/246	234/246	238/242	
D3S2314	183,613,071	214/218	206/214	218	218/222	
Chr3_184.176	184,176,772	261	257/**259**	**259**	259/261	259=52.2%
Chr3_184.543	184,543,103	174	**166**/172	**166**/174	170/172	166=8.3%
Chr3_184.806	184,806,816	221	**221**/225	**221**	221/225	221=18.8%
D3S1571	185,022,606	172	**172**	160/**172**	172	172=46.0%
Chr3_185.212	185,212,491	247	235/**247**	241/**247**	237/247	247=18.8%
Chr3_185.320	185,320,825	292	**292**/296	**292**/292	288/292	292=19.8%
Chr3_185.398	185,398,321	269	**267**/275	**267**/271	267	267=20.8%
Chr3_185.425	185,425,698	333/335	331/**335**	331/**335**	331/335	335=8.0%
Chr3_185.464	185,464,120	308	**308**/310	304/**308**	300/308	308=3.3%
D3S3609	185,519,706	169	**171**/183	**171**	169/175	171=10.7%
rs4912537	185,521,663	**C**	**C**	**C**	**C**	C=77.5%
rs2178403	185,522,368	**A**	**A**	**A**/G	**A**/G	A=25.0%
p.Ala502Val	185,522,571	**T**	**T**/C	**T**/C	**T**/C	-
Chr3_185.526	185,526,620	**138**	**138**	**138**	136/**138**	138=72.8%

rs2293605	185,527,127	**C**	**C**	**C**	**C**	C=89.2%
rs1879244	185,529,049	**C**	**C**	**C**/T	**C**/T	C=22.9%
rs2230571	185,529,164	**C**	**C**	**C**/T	**C**	C=74.2%
D3S3578	185,567,338	**230**	**230**/240	**230**	**230**/232	230=33.9%
Chr3_185.619	185,619,035	**199**	**199**/207	197/**199**	**199**	199=31.4%
Chr3_185.633	185,633,367	**239**	**239**/241	**239**	**239**	239=54.3%
Chr3_185.653	185,653,111	**311**	303/**311**	307/**311**	303/**311**	311=1.1%
Chr3_185.692	185,692,505	**165**	159/**165**	156/**165**	159/**165**	165=16.7%
D3S3583	185,767,793	**272**	**272**	**272**/276	**272**	272=41.1%
Chr3_185.822	185,822,045	**356**	350/**356**	**356**	**356**	356=32.3%
Chr3_185.860	185,859,628	**321**	**321**/325	**321**	**321**	321=41.5%
Chr3_185.870	185,870,551	**356**	**356**	**356**	352/360	356=3.3%
D3S3592	185,893,115	**167**	161/**167**	**167**	167	167=23.0%
Chr3_186.199	186,198,962	**326**	**326**	319/**326**	319/326	326=7.3%
D3S1530	186,866,252	**246**	**246**/250	250	246/254	246=20.0%
D3S1262	187,706,172	100	112/122	100/116	112/116	
D3S2436	188,317,806	168	168/172	164/172	168	
D3S3686	188,900,150	128	108/122	122/126	122/124	
D3S3651	189,123,836	252	250/252	252/254	248/250	
D3S1580	190,025,486	139	143/153	143/155	145/153	

Markers are shown with their physical locations (NCBI Build 36.1); STR allele sizes are given in base-pairs consistent with CEPH standards (available on request). SNPs (denoted rs-) and *EIF4G1* mutations are given as nucleotides. Panel on the right shows genetic variability shared by *EIF4G1* c.3614G>A (p.Arg1205His) carriers, panel on the left shows genetic variability shared by c.1505C>T (p.Ala502Val) carriers. Mutant haplotypes containing alleles shared between two or more families are highlighted in grey (for phase-unknown markers both alleles are given). Allele frequencies for European populations are shown. NS, Not shared.

Table S3. Variants Identified by Sequencing *EIF4G1* in Familial Probands and Healthy Controls

rs#/ss#	Exon	AA	Genotypes	Cases			Controls		
Cases only									
ss229051897	10	A502V	CC/CT/TT	210	2	0	181	0	0
ss229051277	14	G686C	GG/GT/TT	211	1	0	181	0	0
ss229051284	17	E831E	AA/AG/GG	211	1	0	182	0	0
ss229051286	17	L844L	TT/CT/CC	211	1	0	182	0	0
ss229051288	18	T941T	CC/CT/TT	211	1	0	182	0	0
ss229051913	20	P992P	AA/AG/GG	208	3	0	183	0	0
ss229051293	24	S1164R	AA/AC/CC	211	1	0	185	0	0
ss229051296	24	R1197W	CC/CT/TT	211	1	0	185	0	0
ss229051297	24	R1205H	GG/AG/AA	211	1	0	185	0	0
rs73053766	25	N1257S	AA/AG/GG	210	1	0	185	0	0
Controls only									
ss229051246	4	T35T	GG/GA/AA	212	0	0	180	1	0
ss229051255	5	P71S	CC/CT/TT	212	0	0	181	1	0
rs13319149	7	T161A	AA/AG/GG	212	0	0	181	1	0
rs16858632	8	Y311C	AA/AG/GG	212	0	0	181	1	0
rs62287499	16	I806V	AA/GA/GG	212	0	0	179	1	0
ss229051282	17	T829S	AA/TA/TT	212	0	0	181	1	0
Cases and controls									
rs2178403	10	M432V	GG/AG/AA	121	78	13	90	78	13
ss229051265	10	G466_A468del	WT/HET/HOM	209	3	0	179	2	0
rs35629949	25	P1229A	CC/CG/GG	209	3	0	183	2	0
rs2230570	25	L1233P	TT/CT/CC	209	3	0	181	4	0
rs2230571	27	H1335H	CC/CT/TT	115	85	12	114	68	5
rs76779558	29	V1417V	CC/CT/TT	201	11	0	176	6	0
ss229051311	30	F1461F	CC/CT/TT	210	2	0	181	2	0
ss229051315	30	D1462D	CC/CT/TT	203	9	0	178	5	0
rs11559218	32	D1517D	CC/CT/TT	205	4	0	180	2	0

Exons and amino acid positions are given to RefSeq identifier NM_198241. Known variants are indicated by their rs numbers, novel variants have been given ss numbers. Genotype counts for cases and controls are provided. Although not statistically significant there are three-fold more novel coding variants (denoted by ss notation) identified in cases only compared with controls only.