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Sequence variants in eukaryotic translation initiation factor 4-gamma (eIF4G1) are associated with Lewy body dementia

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Abstract

We recently reported a missense mutation and four variants in eukaryotic translation initiation factor 4-gamma (*EIF4G1*) associated with parkinsonism, dementia or both. In those with a positive family history, the mode of inheritance was autosomal dominant. Detailed neuropathologic descriptions of individuals with *EIF4G1* genetic variants have not been reported. Herein, we report neuropathologic findings of three individuals from two American families with *EIF4G1* variants. The patients had initial clinical presentations of dementia or parkinsonism and all had dementia at the time of autopsy. One family carried an *EIF4G1* double variant, c.2056G>T (p.G686C) and c.3589C>T (p.R1197W), and one family carried variant c.1505C>T (p.A502V). All three patients also carried at least one ε4 allele of apolipoprotein E. One individual presented with cognitive impairment without significant parkinsonism; one presented with memory problems followed by bradykinesia; and the third presented with cardinal signs of Parkinson's disease, followed more than a year later by cognitive dysfunction. Pathological examination showed diffuse cortical Lewy bodies and Lewy neurites in all patients. A small subset of Lewy bodies and Lewy neurites were immunopositive for eIF4G1. All patients had moderate to frequent non-neuritic, cortical amyloid plaques, mostly medial temporal neurofibrillary pathology (Braak neurofibrillary tangle stages of II to IV), and minimal or no TDP-43 pathology. The results suggest that in some patients variants in *EIF4G1* can be associated with pathology that has a high likelihood of association with clinical features of dementia with Lewy bodies.

Keywords

APOE; dementia with Lewy bodies; diffuse Lewy body disease; *EIF4G1*; parkinsonism; α-synuclein; tau

Introduction

Lewy body dementia is an umbrella term that includes dementia with Lewy bodies (DLB) and Parkinson's disease with dementia (PDD) [32]. These two entities share many clinical features, including parkinsonism, cognitive impairment, psychiatric symptoms, autonomic

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Conflict of Interest
The authors declare that they have no conflict of interest.

dysfunction,, neuroleptic sensitivity, and rapid eye movement sleep behavior disorder [35]. There is currently no way to distinguish DLB from PDD other than the timing of dementia, with dementia occurring early in DLB and after at least one year for PDD [36].

DLB and PDD are associated with a range of α-synuclein immunoreactive lesions, including Lewy bodies, Lewy neurites, and dot-like structures [49], in aggregate referred to as Lewy-related pathology [9]. The distribution of Lewy-related pathology can be classified as diffuse, transitional, or brainstem predominant [28]. Both DLB and PDD are associated with diffuse neocortical Lew-related pathology [20]. Alzheimer type pathology, including senile plaques (SP) and neurofibrillary tangles (NFT) [15,21,33], as well as cerebral amyloid angiopathy and vascular pathology may coexist with DLB and PDD [21,64].

Most cases of DLB and PDD are sporadic; however, several mutant genes or loci have been reported in familial parkinsonism [1,4,37,43,48,65]. Recently, we discovered a potentially pathogenic mutation and four variants in eukaryotic translation initiation factor 4-gamma (*EIF4G1*) in patients with familial and sporadic parkinsonism [7]. The mode of inheritance is autosomal dominant in those with a positive family history and Lewy bodies were present in two patients with *EIF4G1* variants, but neuropathologic descriptions of these patients was not reported. In this communication, we describe clinical and genealogical findings as well as detailed neuropathologic description of three patients with *EIF4G1* variants.

Materials and methods

Case material

We reviewed medical records submitted to the brain bank at Mayo Clinic Jacksonville, Florida, to obtain clinical information on the patients with *EIF4G1* variants. We also performed genealogical studies of the families (Families A and B) by interviewing other family members (Fig. 1). The clinical diagnosis of DLB was made according to third report of the DLB Consortium [36], and the diagnosis of PDD was made based on Movement Disorder Society diagnostic criteria for dementia associated with Parkinson's disease [13]. This study was approved by the institutional review board of Mayo Clinic. Written informed consent was obtained from all participating family members. Autopsies were performed after written informed consent from the legal next-of-kin.

Genetic evaluation

DNA from frozen brain tissue of autopsy patients and blood specimens from living family members was obtained with standard protocols and screened for mutations in *EIF4G1.* We also performed genotype analysis of apolipoprotein E (*APOE*), and we assessed a genetic variant (rs356165) in the α-synuclein gene (*SNCA)* that has been reported to be a risk factor for PD [47] as well as the haplotype of the tau gene (*MAPT*) by assessing (rs1052553) that has also been shown to be a risk factor for PD [62]. All genotypes were determined using Applied Biosystems TaqMan chemistry and analyzed with SDS 2.2.2 software on an Applied Biosystems 7900HT Fast Real-Time PCR System (primer sequences are available upon request).

Neuropathological methods

Macroscopic and microscopic evaluations of the three brains were performed at the brain bank for neurodegenerative disorders at Mayo Clinic in Jacksonville, Florida. The left hemibrain was fixed in formalin, photographed, dissected and sampled for histology according to a standardized protocol. Tissue sections were embedded in paraffin, and 5 μm thick sections were mounted on glass slides for histological studies and immunohistochemistry. The areas sampled were middle frontal gyrus, superior temporal

gyrus, inferior parietal lobule, motor cortex, visual cortex, cingulate cortex, superior frontal gyrus, posterior hippocampus at level of lateral geniculate nucleus, anterior hippocampus at level of the entorhinal cortex, amygdala, basal nucleus of Meynert, caudate nucleus, putamen, nucleus accumbens, thalamus including subthalamic nucleus, midbrain, pons, medulla, cerebellar vermis, cerebellar hemisphere with deep nuclei, olfactory bulb, and spinal cord (if available). Paraffin-embedded sections were stained with hematoxylin and eosin (H&E) and thioflavin S. Select sections were processed for Bielschowsky or Gallyas silver stains.

Immunohistochemistry

Immunohistochemistry was done on 5-μm thick sections of formalin-fixed paraffin-embedded tissue, after removing paraffin in three 5-minute washes in xylene, rehydration with three 2-minute washes in a graded series of ethanol (100%, 100%, 95%) and finally dH_2O. Immunohistochemistry was performed on a DAKO AutostainerPlus (DAKO, Carpinteria, CA, USA) with the DAKO EnVision™ + System-HRP (diaminobenzidine) and 5% normal goat serum (in Tris-buffered saline) used to block nonspecific antibody binding. The sections were dehydrated and cover slipped after light hematoxylin counterstaining.

Sections for frontal, temporal, parietal, cingulate, hippocampus, amygdala, basal nucleus of Meynert, lentiform nucleus, midbrain, pons, medulla and olfactory bulb were processed for immunohistochemistry for α-synuclein (NACP [19], rabbit polyclonal; 1:3,000) using a protocol that has comparable sensitivity and specificity to commercial α-synuclein antibodies [2]. All patients had tau immunohistochemistry on at least frontal cortex, hippocampus, amygdala, basal nucleus of Meynert, lentiform nucleus, and midbrain with a monoclonal antibody to phospho-tau (CP13; pSer202 [59], mouse monoclonal IgG_1, 1:1000, a gift from Dr. Peter Davies, Albert Einstein College of Medicine, Bronx, NY, USA). The amygdala was screened for argyrophilic grains with immunohistochemistry with ET3 [14] (mouse monoclonal to 4R tau; IgG_1, 1:1000, a gift from Dr. Peter Davies, Albert Einstein College of Medicine, Bronx, NY, USA). Sections of hippocampus and amygdala were screened for TDP-43 pathology (rabbit polyclonal Ig to C-terminal epitope; MC2085 [66], 1:1500, from Dr. Leonard Petrucelli, Mayo Clinic Jacksonville). Sections of frontal and occipital lobe were immunostained for Aβ40 and Aβ42 (clone# 13.1.1 for Aβ40 and clone# 21.3.1 for Aβ42 [30], both mouse monoclonal IgG_1; 1:1,000; from Dr. Pritam Das, Mayo Clinic Jacksonville). Sections processed for α-synuclein, ET3, and Aβ were pretreated with steam heat and 95% formic acid; all others with just steam heat.

Immunohistochemistry was performed for eIF4G1 (rabbit polyclonal #ab2609; 1:100, Abcam, Cambridge, MA) on cingulate gyrus, hippocampus, amygdala, basal nucleus of Meynert, lentiform nucleus, midbrain, pons, and medulla on patients and controls (neurologically normal and sporadic DLB).

Double staining for EIF4G1 and α-synuclein

To determine the relationship to eIF4G1 immunoreactivity to Lewy-related pathology, double immunohistochemistry was performed with rabbit polyclonal antibody to eIF4G1 and mouse monoclonal antibody to α-synuclein (LB509; 1:100; Invitrogen, Camarillo, CA). To maximize detection of Lewy-related pathology, sections were pretreated with protease (Sigma protease XXIV; 0.25 mg/ml) for 6 minutes at room temperature. The sections were incubated with anti-eIF4G1 at room temperature for 45 minutes. After incubation with the primary antibody, the sections were treated with Anti-Rabbit Envision-Plus System-HRP (DAKO, Santa Barbara, CA) for 30 min. Peroxidase labeling was visualized with a solution containing 0.02 mg/mL 3, 3′diaminobenzidine (DAB). After washing in dH_20, the sections were treated with Doublestain Block Reagent (DAKO, Santa Barbara, CA) for 3 minutes.

The second primary antibody, LB509, was applied at room temperature for 45 minutes. The sections were then treated with Rabbit/mouse link (Dako, Santa Barbara, CA) for 10 minutes followed by alkaline phosphatase labeled polymer (DAKO, Santa Barbara, CA) for 30 min. Alkaline phosphatase labeling was detected with Vector Blue Alkaline Phosphatase Substrate kit III (Vector Laboratories, Burlingame, CA). The sections were dehydrated and cover slipped without counterstaining.

Quantitative neuropathologic assessments

Thioflavin-S fluorescent microscopy was used to assess Alzheimer type pathology. Counts of SP (at x100 magnification), NFT (at x400 magnification), and scores of cerebral amyloid angiopathy (CAA) severity were ascertained in cortical and limbic regions. The density and distribution of NFT were used to assign a Braak NFT stage [5] as in previous studies [40]. Presence and density of neuritic plaques was assessed on thioflavin S fluorescent microscopy according to CERAD [38]. In addition to Braak NFT stage, a Thal amyloid phase [52] was also assigned based upon counts of SP with thioflavin S fluorescent microscopy in six cortical regions, four sectors of the hippocampus and two subregions of the amygdala, as well as semiquantitative SP scores (none, mild, moderate and severe) in the basal ganglia and cerebellum. Because midbrain is not routinely evaluated for SP with thioflavin S fluorescent microscopy, we used counts in hippocampal CA4 subfield as a surrogate, since CA4 plaques are highly correlated with brainstem plaques [52]. A composite score for Alzheimer type neuropathologic change was assigned according to National Institute on Aging-Alzheimer Association (NIA-AA) recommendations [39].

Lewy bodies were counted in 5 cortical regions and in the amygdala with x200 magnification, and the Braak PD stage was determined according to distribution of Lewy-related pathology [6]. Neuritic pathology in CA2/3 sector of hippocampus [11] was graded on a 4-point scale (none, mild, moderate, severe). Cases were assigned a neuropathologic likelihood that they would be associated with clinical features of DLB based upon the relative distribution of Lewy-related pathology with respect to severity of Alzheimer type pathology as recommended in the third report of the Consortium for Dementia with Lewy bodies [36], the validity of which we have independently confirmed [15]. The degree of neuronal loss in key subcortical nuclei (basal nucleus of Meynert, substantia nigra, locus ceruleus and dorsal motor nucleus of the vagus) was graded on H&E stained sections as none, sparse, moderate or severe. The degree of tau pathology and TDP-43 pathology in cortical and subcortical areas (frontal cortex, parahippocampal gyrus, amygdala, dentate fascia, hippocampal endplate, CA2/3, CA1, subiculum, basal nucleus of Meynert and locus ceruleus) was assessed on immunostained sections as none, sparse, moderate, or frequent.

Results

Genealogical and genetic findings

The subjects examined were from two American families, designated Families A and B.

Family A—Genealogic information for Family A (Fig. 1a) includes 24 individuals, spanning four generations. There are five affected members, and the pattern of inheritance is consistent with autosomal dominant. Case 1 (II-2) harbors the eIF4G1 double variant p.G686C and p.R1197W as previously described [7]. Case 2 (II-3) and one of the children of II-2 have the same mutations as the proband. The other four family members in the third generation for whom DNA was available for testing had wild type eIF4G1. Genotype analysis revealed that both Case 1 and Case 2 were homozygous for *APOE* ε4. They were also both homozygous for the minor allele (A) in rs356165 in *SNCA*, and homozygous for H1 with respect to the *MAPT* haplotype.

Family B—Genealogic information for Family B (Fig. 1b) includes nine individuals, spanning three generations, with two affected individuals. Case 3 (III-1) harbors the eIF4G1 p.A502V variant as previously described [7] as well as being heterozygous for *APOE* ε4 (ε3/ε4), homozygous for the minor (A) allele at rs356165 in *SNCA*, and heterozygous for H1 (H1H2) *MAPT* haplotype.

Clinical findings

Clinical information was obtained on five affected patients and one asymptomatic family member from Family A as well as two affected patients from Family B. The mean age of symptomatic disease onset of affected patients was approximately 70 years, and mean disease duration was almost 8 years. All five affected patients in Family A had dementia, and the proband had both parkinsonism and dementia. Detailed clinical information and blood samples were not available for the proband's mother (I-2), older brother (II-1) or sister (II-4). Both affected patients in Family B presented with dementia. Subject II-1 had dementia and died at age 85; however, detailed clinical information was unavailable. The clinical phenotypes of the affected individuals are summarized in Table 1.

Clinical vignettes of three autopsy patients

Family A/Case 1 (II-2)—This right-handed woman had hypothyroidism and depression due to a partial thyroidectomy, but she had been on adequate thyroid hormone replacement therapy. She did not have any other major medical problems. At age 74, she displayed signs of memory impairment. At age 75, physical examination noted bradykinesia. At age 76, she was diagnosed with Alzheimer's disease and was prescribed donepezil. By age 79, she had gradually developed agitation and anxiety followed by insomnia and combative behavior. At age 80, she had severe cognitive impairment and was almost completely bedridden. She died at age 81. According to available medical records, no other neurological abnormalities, such as language disorders or psychiatric abnormalities were evident during her illness. Based on the available medical information, her diagnosis is consistent with "clinically possible" DLB [36].

Family A/Case 2 (II-3)—This right-handed man, the younger brother of Case 1, developed resting tremor in his right hand at the age of 80. At age 85, he developed delusions and hallucinations. He reported that a family member was in the house, and he spoke to him when he was alone. When he stood up, he experienced mild orthostatic changes. His bladder and bowel function were normal. On neurological examination at age 86, his language was coherent and fluent without paraphasic or phonemic errors. He had slight reduction of facial expression, mild bradykinesia, resting tremor, and stooped posture. He had mild cogwheel rigidity with asymmetry (right more than left). He also had subtle intermittent dystonic posturing of his right foot. His gait was wide based, and he showed a positive Romberg sign. There were no signs of cerebellar dysfunction. The stride height and length were slightly reduced. He was able to turn with three to four steps with some instability. Especially on the right side, he had reduced arm swing during walking. He did not have retropulsion. He scored 14 points on the Unified Parkinson's Disease Rating Scale, and he was completely independent. MRI showed generalized mild cerebral atrophy appropriate for age, and there was moderate chronic small vessel disease involving the cerebral white matter. He was treated with levodopa and had clinical response. At age 88, he developed memory problems followed by confusion. He also became occasionally agitated and sometimes threw things or struck caregivers. He was diagnosed with Parkinson's disease and severe cognitive impairment. His gait became unsteady, and he sometimes fell. He had a trouble with urinary incontinence, and he wandered at night. He died at age 89. Based upon available medical information, his diagnosis is consistent with "clinically probable" PDD [13].

Family B/Case 3 (III-1)—This right-handed man had been well until age 57. His past medical history included hypertension and hypertriglyceridemia. At age 58, he began to have difficulties with tasks that had previously been easy for him to accomplish, such as installing a garage door, building a fence or performing electrical work. He sought neurologic evaluation for declining functional abilities. An MRI at the time revealed isolated punctate hyperintensities in the anterior limb of the internal capsule. He gradually developed depression. He retired early at age 60; and thereafter had progressive cognitive impairment, especially declining short-term memory. He often misplaced things, such as his car keys. He developed visual hallucinations. He misinterpreted objects, such as a tree for a person. He also described vivid dreams and briefly had difficulties distinguishing reality from his dreams. Neurological examination was notable for increased ankle jerks on deep tendon reflex testing. At age 62, he had problems remembering information, such as names of people and their telephone numbers. On clinical examination, he was alert, but had slurred speech. He scored 28 out of 30 points on the Mini-Mental State Examination (MMSE). He had problems copying intersecting pentagons and correctly drawing a clock. He was thought to have Alzheimer's disease. Donepezil was prescribed, and he had some improvement. He was also treated for depression with fluoxetine, with some improvement of depression. At age 63, he often became disoriented in the middle of the night and was unable to recall the location of his bedroom. On examination, he was alert and oriented. He knew the names of the presidents; however, he was unable to remember the year he was married, and he had difficulties with serial "sevens." On the MMSE, he scored 27 out of 30. He recalled 1 of 3 three words in one minute, but was not able to draw intercepting pentagons. There were no other neurological abnormalities, such as parkinsonism, behavioral or psychiatric problems during his illness. He died at age 66. Based on available medical information, his diagnosis is consistent with "clinically possible" DLB [36].

Neuropathological findings

Summary of microscopic findings, including Lewy-related pathology, Alzheimer type pathology, as well as tau and TDP-43 pathology are summarized in Tables 2, 3, and 4, respectively.

Family A/Case 1—The fixed left hemibrain weighed 570 grams; the calculated whole brain weight, 1140 grams. Macroscopic examination revealed no significant focal atrophy; however, the frontal horn of the lateral ventricle was mildly dilated. Neuromelanin pigmentation of the substantia nigra and locus ceruleus was moderately decreased.

Microscopic examination revealed neuronal loss and gliosis in the basal nucleus of Meynert, substantia nigra (Fig. 2a), locus ceruleus, and dorsal motor nucleus of the vagus. Lewy-related pathology was frequent in the parahippocampal cortex (Fig. 3d), amygdala (Fig. 3g), and basal nucleus of Meynert (Fig. 3j); but also detected in the hippocampal CA2 subfield (Fig. 3a). With thioflavin-S fluorescent microscopy, non-neuritic SP were detected in frontal, temporal, and parietal cortices, with NFT relatively limited to entorhinal cortex, subiculum, and amygdala (Table 3). Tau immunocytochemistry revealed tau positive pathology in the parahippocampal cortex, hippocampus, amygdala, basal nucleus of Meynert, and locus ceruleus. Thioflavin S fluorescent microscopy and Aβ immunohistochemistry revealed severe amyloid angiopathy in the frontal (Fig. 4a) and visual cortices. Sparse TDP-43 positive neuronal cytoplasmic inclusions were detected only in the amygdala (Fig. 4g). The pathologic diagnosis was diffuse Lewy body disease (Braak PD stage: 6) with concomitant Alzheimer's pathology (Braak neurofibrillary tangle stage III), as well as amyloid angiopathy. The likelihood that pathologic findings would be associated with a DLB clinical syndrome is "high" [36].

Family A/Case 2—The fixed left hemibrain weighed 650 grams; calculated whole brain weight, 1300 grams. Macroscopic examination revealed mild atrophy of the frontal lobe and enlargement of the frontal and temporal horns of the lateral ventricle. Neuromelanin pigmentation of the substantia nigra and the locus ceruleus was moderately decreased.

Microscopic examination revealed moderate neuronal loss in the basal nucleus of Meynert and minimal in the substantia nigra (Fig. 2b). Immunohistochemistry for α-synuclein confirmed Lewy bodies in the parahippocampal cortex (Fig. 3e), basal nucleus of Meynert (Fig. 3k), and the amygdala (Fig. 3h), although they were sparse in the latter. Lewy neurites were abundant in hippocampal CA2 sector (Fig. 3b) and the basal nucleus of Meynert (Fig. 3k). NFT were sparse in subiculum and amygdala. With thioflavin-S fluorescent microscopy, non-neuritic SP were detected in the frontal (Fig. 4b), temporal, parietal cortexes, and entorhinal cortexes. Tau positive pathology was mainly located in the parahippocampal cortex, hippocampus (CA1-3, subiculum). Aβ immunohistochemistry revealed severe amyloid angiopathy in the frontal and visual cortices (Fig. 4b). There was no TDP-43 positive pathology in the basal forebrain or amygdala (Fig. 4h). The pathologic diagnosis was diffuse Lewy body disease (Braak PD stage: 6) with concomitant Alzheimer's pathology (Braak neurofibrillary tangle stage III-IV), as well as amyloid angiopathy. The likelihood that pathologic findings would be associated with a DLB clinical syndrome is "high" [36].

Family B/Case 3—The fixed left hemibrain weighed 760 grams; calculated whole brain weight, 1520 grams. The cerebral hemisphere had a normal configuration; however, neuromelanin pigmentation of substantia nigra and locus ceruleus was slightly decreased.

Microscopic examination revealed moderate neuronal loss in the basal nucleus of Meynert, substantia nigra (Fig. 2c) and locus ceruleus. Immunohistochemistry for α-synuclein revealed Lewy bodies in the amygdala (Fig. 3i) and the basal nucleus of Meynert (Fig. 3l). Many Lewy neurites were also present in hippocampal CA2 sector (Fig. 3c) and the basal nucleus of Meynert (Fig. 3l). With thioflavin-S fluorescent microscopy, non-neuritic SP were detected in frontal (Fig. 4c), temporal, parietal, visual, and entorhinal cortexes. NFT were sparse in the cortex, subcortical areas, and brainstem. Tau positive pathology was mainly found in the parahippocampal cortex, amygdala, and hippocampus. Aβ immunohistochemistry revealed SP in the frontal and visual cortices (Fig. 4c). There was no TDP-43 positive pathology in the basal forebrain or amygdala (Fig. 4i). The pathologic diagnosis was diffuse Lewy body disease (Braak PD stage: 6) with concomitant Alzheimer's pathology (Braak neurofibrillary tangle stage II-III). The likelihood that pathologic findings would be associated with a DLB clinical syndrome is "high" [36].

eIF4G1 immunohistochemistry

Immunohistochemistry for eIF4G1 was performed on paraffin sections with a commercially available rabbit polyclonal antibody. Antigen retrieval methods included steam heat and pH 9 target retrieval solutions. Positive controls for immunohistochemistry were sections of human intestine (not shown), which had strong cytoplasmic immunoreactivity in submucosal and myenteric plexus ganglion cells, as well as finely granular cytoplasmic staining of enteric epithelial cells, but not goblet cells. There was also immunoreactivity of vascular smooth muscle cells. In the brain, immunoreactivity was most prominent in neurons and vascular smooth muscle cells. Neurons had diffuse cytoplasmic staining as well as coarse granules in some neurons, including cortical pyramidal neurons (Fig. 5a). There was no significant difference in staining between normal controls and sporadic DLB compared with patients with genetic variants in *EIF4G1*. In neurons with Lewy bodies, there was cytoplasmic eIF4G1 immunoreactivity and minimal granular staining in a small

proportion of cortical type Lewy bodies (Fig. 5b). Brainstem type Lewy bodies in substantia nigra were mostly completely negative (Fig. 5c), but isolated Lewy bodies had weak immunoreactivity (Fig. 5d). Double immunohistochemistry for eIF4G1 and α-synuclein confirmed findings with single labeling. Specifically, most Lewy bodies and Lewy neurites had no co-localization with eIF4G1 (Fig. 5e), and only a few intra-neuritic hyaline type Lewy bodies had weak granular immunoreactivity in the central zone with α-synuclein immunoreactivity located at the periphery of the inclusion (Fig. 5f).

Discussion

We present clinical and pathological details of three individuals from two unrelated families whose affected members presented with dementia or parkinsonism or both, and who had genetic variants in *EIF4G1*. In Family A, harboring the eIF4G1 double variant, p.G686C and p.R1197W, all affected individuals had dementia; one patient had parkinsonism followed by dementia more than one year later. In Family B, harboring the eIF4G1 p.A502V variant, there were two affected patients and both had dementia with psychiatric features, but without parkinsonism.

The degree of neuronal loss in the substantia nigra correlated with clinical severity of extrapyramidal motor signs. Moderate-to-severe neuronal loss and Parkinsonism were noted in Cases 1 and 2. In fact, Case 2 was diagnosed with PD and treated with levodopa with good response. Case 3 had minimal neuronal loss in the substantia nigra, and correspondingly no Parkinsonism. It is increasingly recognized that a subset of DLB patients may have minimal extrapyramidal signs and also minimal neuronal loss in the substantia nigra [54,55]. Kosaka has referred to such cases as “cerebral type” Lewy body disease [27].

Neuronal loss was moderate-to-severe in basal nucleus of Meynert in all patients consistent with findings in Lewy body dementia presenting with DLB or PDD [45]. In all patients immunohistochemistry for α-synuclein revealed Lewy-related pathology in neocortical regions as well as limbic and brainstem regions consistent with diffuse Lewy body disease [28]. Mild-to-moderate Alzheimer type pathology was observed in all patients, but none had advanced pathology (A3, B3, C3) using the NIA-AA classification and none had greater than “intermediate” Alzheimer neuropathologic change [39]. The combination of diffuse Lewy body disease with mild-to-moderate Alzheimer’s pathology would predict with “high likelihood” that the pathologic findings would be associated with a DLB clinical syndrome that includes dementia and at least two of the following cardinal clinical signs: parkinsonism, visual hallucinations, and fluctuation in level of consciousness [36]. Two patients met criteria for clinically possible DLB [36] and the third, clinically probable PDD [13], given the timing of cognitive deficits more than one year after clinically established PD.

Severe cerebral amyloid angiopathy was detected in Cases 1 and 2 from Family A, both of whom were homozygous for *APOE* ε4, but not in Case 3 from Family B, who was heterozygous for *APOE* ε4. It is known that amyloid angiopathy is influenced by *APOE* genotype [18]. The greater severity of CAA in homozygous patients fits with this observation. It is unclear if *APOE* ε4 is associated with risk of PD or age of onset of PD [26,31,44,57,60]. On the other hand, there is more consistent evidence of an association between *APOE* ε4 and Alzheimer type pathology in PD [1,3,34,51]. While not conclusive, several studies have indicated an increased frequency of *APOE* ε4 in diffuse Lewy body disease [1,23,42], especially in association with concomitant Alzheimer type pathology [10,22]. It is of interest that familial DLB has also been shown to be associated with *APOE* ε4 [42,53,58]. The possibility of gene-gene interaction in families with *APOE* ε4 and *EIF4G1* variants needs to be explored in future studies.

All patients had mild tau pathology; however, this is a common in Lewy body dementia (DLB and PDD) when accompanied by Alzheimer type pathology or age-related medial temporal tauopathies such as argyrophilic grain disease. None of the patients in small series had argyrophilic grain disease. Both patients from Family A were homozygous for *MAPT* H1, and the patient from Family B was heterozygous for *MAPT* H1. The significance of *MAPT* variants on Alzheimer type pathology [41] has recently been called into question, with evidence that H1 may actually be associated with decreased Alzheimer type neurofibrillary pathology [61]. Homozygous AA alleles for rs356165 in the *SNCA* gene are the genotype most commonly seen in controls; therefore, the effect of this genotype on α-synuclein pathology in patients harboring *EIF4G1* variants is uncertain.

TDP-43 positive inclusions were detected in the amygdala in Case 1, although they were sparse and not detected in other brain regions. This is the region of the brain most vulnerable to TDP-43 pathology in neurologically normal elderly individuals [16,63], which suggests that in this patient, TDP-43 pathology may be a coincidental finding.

Immunohistochemistry for eIF4G1 was performed on paraffin embedded tissue from cases and controls revealing cytoplasmic staining in neurons as well as non-neuronal cells, most notably vascular smooth muscle cells. Some neurons had coarse cytoplasmic granules. Further studies are needed to determine the nature of these granules. It is worth noting, however, the eIF4G1 is a recognized component of a subset of cytoplasmic RNA granules referred to as stress granules [24]. For the most part, neither Lewy bodies nor Lewy neurites were immunoreactive for eIF4G1. A few Lewy bodies had weak immunoreactivity, which may represent nonspecific sequestration of cytoplasmic constituents in brainstem type Lewy bodies as noted in other studies for neurotransmitter synthetic enzymes [12]. Sporadic DLB cases were no different from patients harboring *EIF4G1* genetic variants.

eIF4G1 is a subunit of the protein complex eIF4F that plays a central role in the recruitment of ribosomes to mRNA for translational control [46,50]; therefore, eIF4G1 is critical for the regulation of protein synthesis [17]. eIF4G1 depletion has been shown to play a role in cell proliferation, bioenergetics, impaired mitochondrial function, and changes in nutrient sensing [46]. Mammalian target of rapamycin complex 1 (mTORC1) is composed of mammalian target of rapamycin (mTOR), raptor, and lst8 [25]. mTOR functions as a nutrient, energy, and redox sensor that controls protein synthesis via eIF4G1 [46]. It has been shown in Lewy body disease and mouse α-synuclein models that mTOR is elevated in the brain compared with controls and that mTOR immunoreactivity is increased in neurons with α-synuclein pathology [8]. At the present, there is no evidence of a direct relationship between eIF4G1 and Lewy-related pathology; the mechanism by which genetic variants in *EIF4G1* may be associated with pathology consistent with high likelihood of DLB is unclear.

The functional significance of the eIF4G1 p.A502V variant has been linked to its effects on scaffold function of eIF4F and the ability of cells to rapidly and dynamically respond to stress [7]. On the other hand, the other eIF4G1 sequence variants — p.G686C, p.R1197W, and p.A502V — have not been proven to be pathogenic, although it should be noted that the p.G686C variant has not been found in clinically normal controls. Both p.R1197W and p.A502V have recently been identified in clinically normal controls [29,56]; however, *in vitro* functional studies have not been reported with these variants.

This present study has certain limitations. Most notably, it is a retrospective study, and the quality of medical and family history information was variable for both patients and other family members. In addition, the families are small, making it impossible to demonstrate segregation of the genetic variants with a clinical disease phenotype. Finally, *in vitro* studies

demonstrating pathogenicity of eIF4G1 variants, which was beyond the scope of this clinicopathologic study, have not been performed; however, they are clearly needed in the future for these and other eIF4G1 variants.

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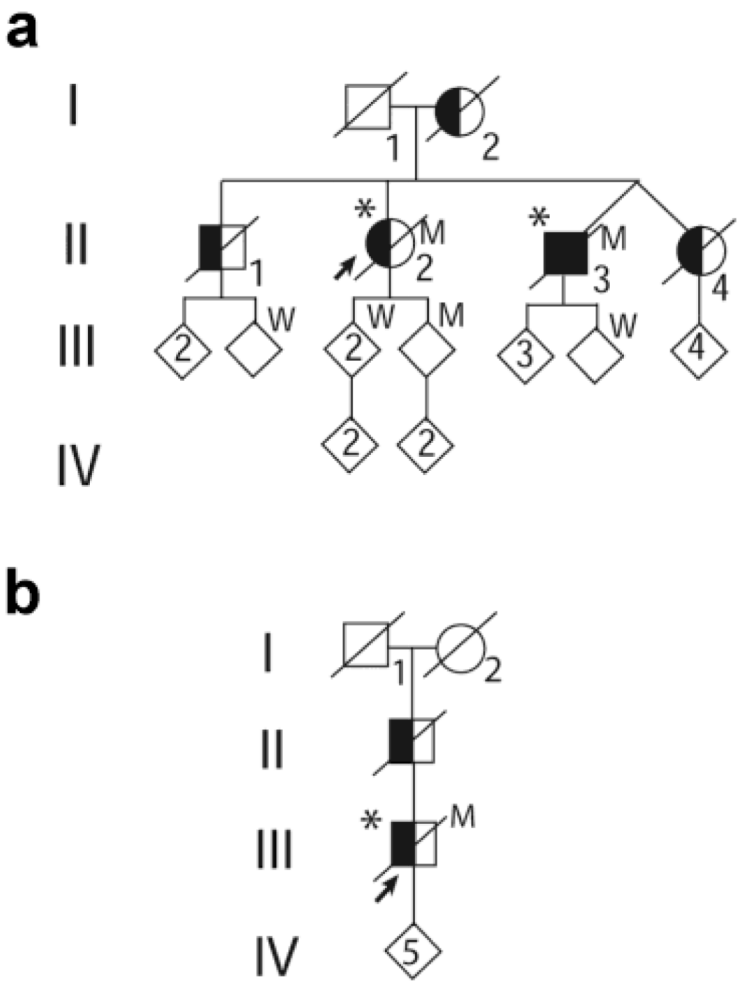

Fig. 1. Pedigrees of families

(a) Family A with the eIF4G1 double variant p.G686C and p.R1197W, (b) Family B with eIF4G1 p.A502V variant. Standard symbols were used. Round symbols indicate females, squares males, diagonal lines indicate the individual is deceased. Diamonds were used to disguise gender. The solid arrowhead indicates the proband. Black full-filled symbols indicate individuals with cognitive impairment and Parkinsonism, left half-filled symbols indicate cognitive impairment. * indicates autopsy patients. Mutation carriers (M) and wild-type (W) are indicated. *EIF4G1*: eukaryotic translation initiation factor 4-gamma 1 isoform

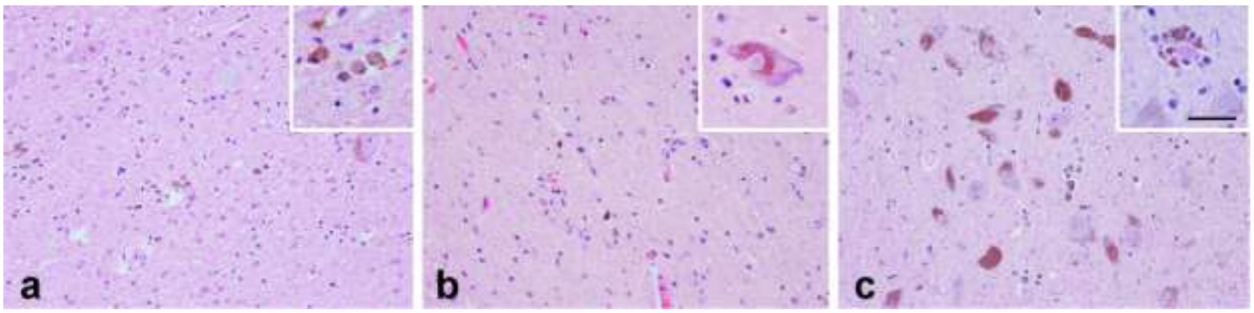

Fig. 2. Neuronal loss in substantia nigra

Substantia nigra ventrolateral pars compacta in Case 1 (a), Case 2 (b) and Case 3 (c). Note severe neuronal loss in Case 1, but less in Cases 2 and 3. Inset I (a) shows cluster is pigment laden macrophages. Inset in (b) shows Lewy body; Inset in (c) shows neuronophagia. (bar = 50 μm for a, b, c and 30 μm for insets)

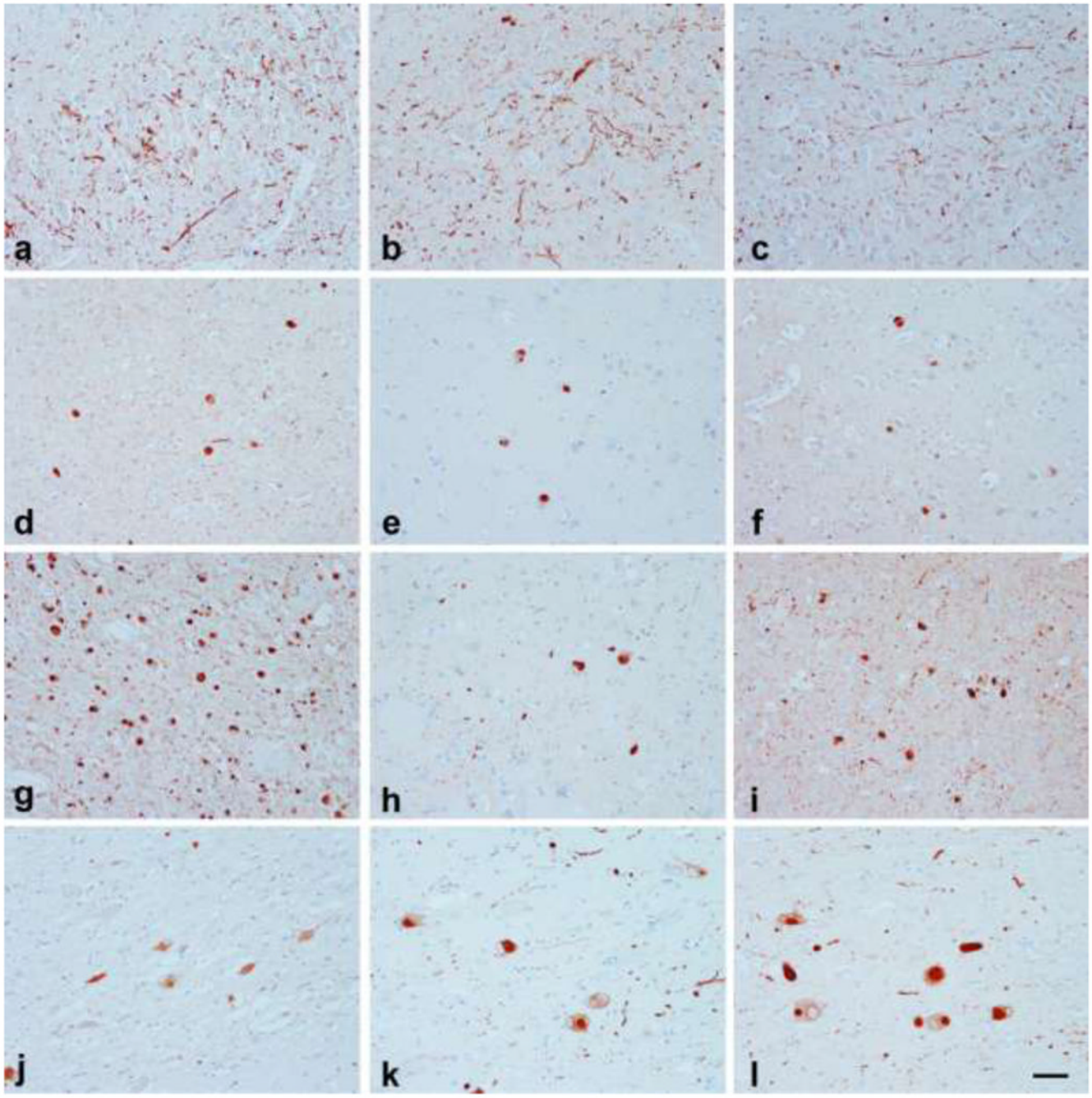

Fig. 3. Synuclein pathology

Hippocampal CA2/3 sector in Case 1 (a), Case 2 (b) and Case 3 (c). All cases have many Lewy neurites. Parahippocampal cortex in Case 1 (d), Case 2 (e) and Case 3 (f). Moderate-to-frequent Lewy bodies, with many Lewy neurites in Case 1. Amygdala in Case 1 (g), Case 2 (h) and Case 3 (i). Many Lewy bodies and Lewy neurites in Cases 1 and 3, but sparse Lewy bodies and Lewy neurites in Case 2. Basal nucleus of Meynert in Case 1 (j), Case 2 (k) and Case 3 (l). Many Lewy bodies and Lewy neurites in all three patients. (bar in i = 50 μm for a, b, c, d, e, f, g, h & i)

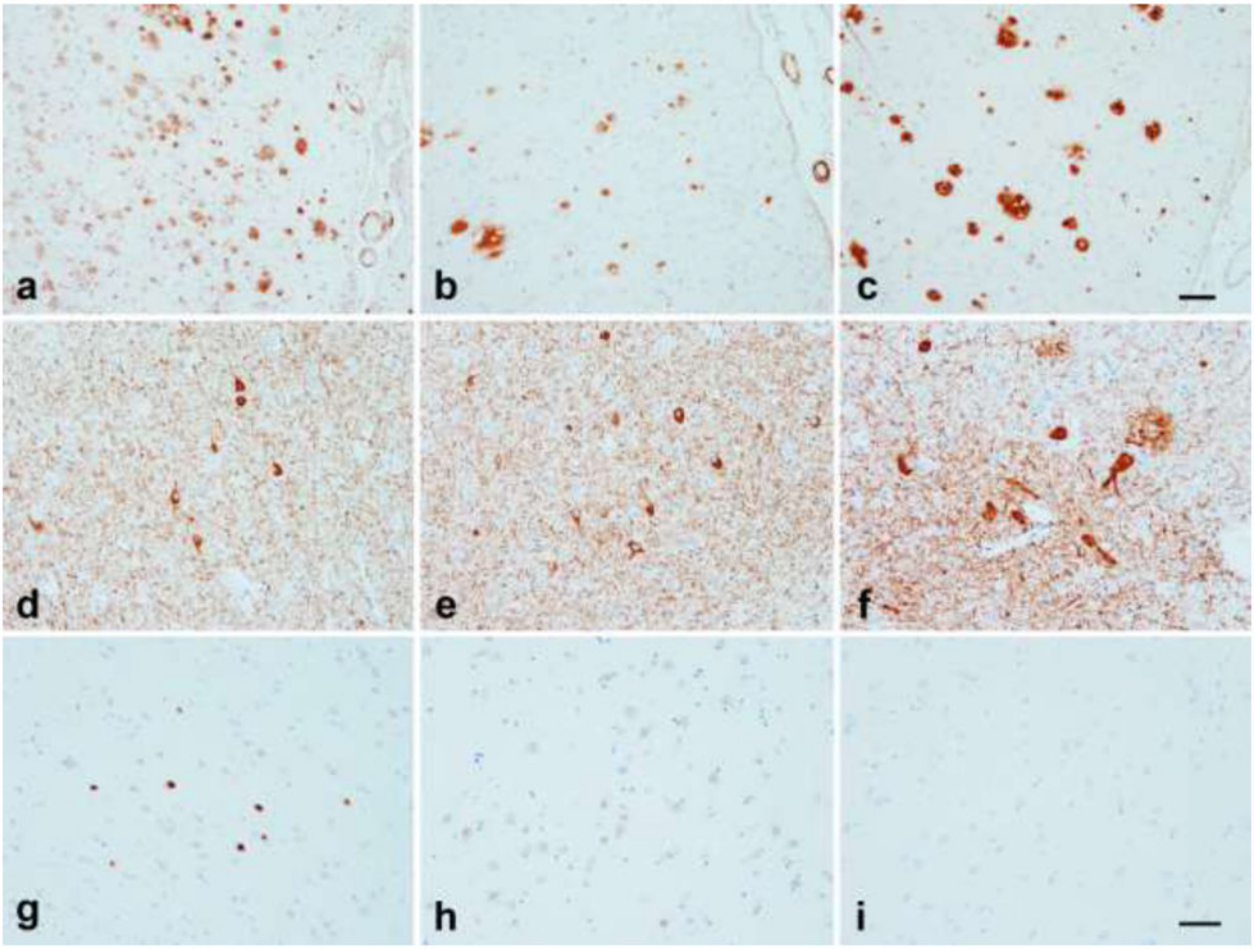

Fig. 4. Alzheimer type pathology

Frontal cortex Aβ42 immunohistochemistry in Case 1 (a), Case 2 (b) and Case 3 (c). Many diffuse plaques and CAA in Case 1, moderate plaques and CAA in Case 2, and moderate plaques without CAA in Case 3 Entorhinal phospho-tau in Case 1 (d), Case 2 (e) and Case 3 (f). Many neuropil threads and sparse NFT in all; sparse neuritic plaques in Case 3. Amygdala TDP-43 in Case 1 (g), Case 2 (h) and Case 3 (i). Sparse TDP-43-positive neuronal cytoplasmic inclusions in Case 1. (bar in c = 90 μm for a, b & c =; bar in i = 50 μm for d, e, f, g, h & i).

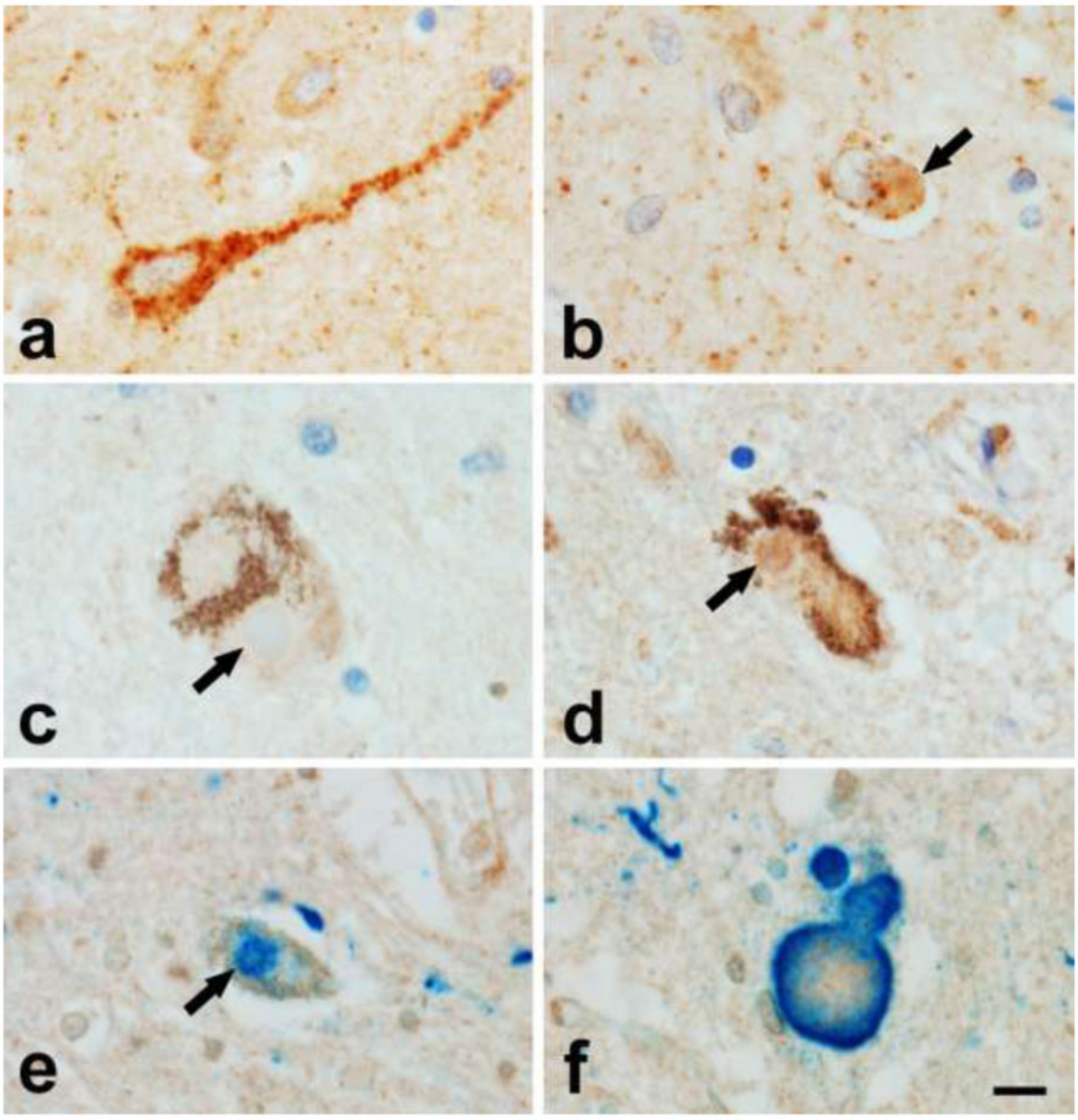

Fig. 5. eIF4G1 immunohistochemistry

Single labeling eIF4G1 immunohistochemistry (a, b, c and d) and double labeling immunohistochemistry for eIF4G1 (brown) and α-synuclein (blue) (e and f) in Case 1 (a, b, e) and Case 2 (c, d and f). (a) A pyramidal neuron from cingulate cortex of Case 1 has diffuse somatodendritic cytoplasmic immunoreactivity as well as cytoplasmic granules. (b) A cortical type Lewy body (arrow) in a small nonpyramidal neuron in lower cortical layer of cingulate gyrus has weak eIF4G1 immunoreactivity. (c) A brainstem type Lewy body (arrow) in the substantia nigra has no eIF4G1 immunoreactivity. (d) A brainstem type Lewy body (arrow) in the substantia nigra has weak eIF4G1 immunoreactivity. (e) A brainstem type Lewy body (arrow) in the basal forebrain has α-synuclein (blue), but no eIF4G1 (brown) immunoreactivity. (d) An intraneuritic hyaline Lewy body in the basal forebrain has weak eIF4G1 (brown) immunoreactivity in the center and α-synuclein (blue) immunoreactivity at the periphery of the inclusion. (bar = 6 μm for all panels).

Table 1

Clinical phenotype and mutation status of affected individuals

Autopsy Case		AAO	AAD	Initial symptom	Clinical phenotype	*Age at last evaluation*	*Final clinical diagnosis*	*APOE*	*SNCA*	*MAPT*
Family A (EIF4G1 p.G686C and p.R1197W variants)										
	I-1	na	82	na	Dementia	na	AD			
	II-1	80†	82	na	Dementia	na	AD			
1	**II-2**	74	81	Memory impairment	Memory impairment, bradykinesia, agitation, anxiety, insomnia, combative behavior	80	AD	ε4/ε4	AA	H1H1
2	**II-3**	80	89	Resting tremor	Parkinsonism*, hallucinations, orthostatic hypotension, dystonia, gait disturbance, memory impairment, agitation	89	PDD	ε4/ε4	AA	H1H1
	II-4	75†	na	na	Dementia	na	AD			
	III-5				Asymptomatic					
Family B (EIF4G1 p.A502V variant)										
	II-1	na	na	na	Dementia	na	na			
3	**III-1**	57	66	Difficulty of performing the simple tasks	Cognitive impairment, depression, hallucinations, dysarthria	64	AD	ε3/ε4	AA	H1H2

AAD: age at death, AAO: age at symptomatic disease onset, APOE: apolipoprotein E, DD: disease duration, eIF4G1: eukaryotic translation initiation factor 4-gamma, MAPT: microtubule associated protein tau, na: not available, SNCA: α-synuclein,

† age when contacted with the individual,

* resting tremor, bradykinesia, stooped posture, cogwheel rigidity, facial masking, and reduced arm swing

Table 2

Summary of microscopic Lewy body pathology

	Family A				Family B	
	Case 1 II-2		Case 2 II-3		Case 3 III-1	
	NL	LB	NL	LB	NL	LB
Frontal cortex		1–2		1–4		1–2
Temporal cortex		3–6		2–3		1–2
Parietal cortex		1–3		1–2		1–4
Cingulate cortex		3–5		12–14		5–8
Parahippocampal cortex		14–20		8–10		2–11
Amygdala (corticomedial)		20–30		4–6		5–12
Basal nucleus of Meynert	+++	10–15	++	15–20	++	25–35
Substantia nigra	+++	0–4	+	0–3	+	0–3
Locus ceruleus	+	8–10	na	na	+	6–9
Dorsal motor nucleus of the vagus	+	10	+	0–3	+	0
Hippocampus CA2/3 neurites		+++		+++		++
Braak PD stage		6		6		6
Lewy body type		DLBD		DLBD		DLBD

CDLB: consortium on dementia with Lewy bodies, DLBD: diffuse Lewy body disease, LB: Lewy body, NL: neuronal loss, PD: Parkinson’s disease, − = none, + = sparse, ++ = moderate, +++ = severe

Table 3

Summary of microscopic Alzheimer's pathology (thioflavin S fluorescent microscopy)

	Family A						Family B		
	Case 1 II-2			Case 2 II-3			Case 3 III-1		
	SP	NFT	CAA	SP	NFT	CAA	SP	NFT	CAA
Frontal cortex	>50	0	++	30–45	0	+	>50	0–1	−
Temporal cortex	>50	0	+	>50	0–1	+	>50	0	−
Parietal cortex	>50	0	++	15–25	0–1	+	>50	0	−
Visual cortex	0–1	0	+++	3–10	0	++	16–26	0	−
Motor cortex	0–1	0	+++	0	0	+	9–12	0	−
Entorhinal cortex	>50	0–1		>50	10–20		>50	4–5	
Hippocampus									
Endplate	5–6	0		0	0		0	0	
CA2-3	0–1	0		0	0–1		0	0	
CA1	2–7	0		0	1–2		0–1	0–1	
Subiculum	16–22	2–5		1–3	1–3		5–13	0–1	
Amygdala (corticomedial)	>50	1–3		5–10	0		13–16	13–16	
Basal nucleus of Meynert		0–1			0–1			0–1	
Cerebellar vermis	+		+	−		+	−		−
Brain weight (grams)		1140			1300			1520	
Braak stage		III			III–IV			II–III	
Thal phase		5			3			3	
CERAD NP score		none			sparse			sparse	
NIA-AA score		A3, B2, C0			A2, B2, C1			A2, B1, C1	
AD neuropathologic score		intermediate			intermediate			low	

CAA: cerebral amyloid angiopathy, NFT: neurofibrillary tangle, SP: senile plaque, − = none, + = sparse, ++ = moderate, +++ = severe

Table 4

Summary of microscopic tau and TDP-43 pathology

	Family A				Family B	
	Case 1 II-2		Case 2 II-3		Case 3 III-1	
	Tau	TDP-43	Tau	TDP-43	Tau	TDP-43
Frontal cortex	−		+		−	
Parahippocampal cortex	++	−	+++	−	++	−
Amygdala	+++	+	++	−	++	−
Hippocampus						
Dentate fascia	+	−	+	−	+	−
Endplate	+	−		−	+	−
CA2-3	++	−	++	−	+++	−
CA1	++	−	++	−	++	−
Subiculum	+++	−	++	−	++	−
Basal nucleus of Meynert	+	−		−	+	−
Locus ceruleus	++		+		−	

− = none, + = sparse, ++ = moderate, +++ = severe