

Progranulin Mutations in Primary Progressive Aphasia

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The PPA1 and PPA3 Families

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Background: Primary progressive aphasia (PPA) is a language-based dementia characterized by fluent or non-fluent language disorder as its principal feature.

Objective: To describe progranulin gene mutations in 2 families with PPA.

Design: Report of affected families.

Setting: Academic research.

Patients: Two families, PPA1 and PPA3, were studied. Genomic DNA was isolated from 3 of 4 siblings in PPA1, from all 3 siblings in PPA3, and from more than 200 control subjects.

Main Outcome Measures: All 12 coding exons of the progranulin gene and the 5' and 3' untranslated regions were amplified by polymerase chain reaction and were sequenced in both directions using relevant primers.

Results: Both affected members of PPA1 for whom DNA was available and both affected sisters of PPA3 had a progranulin gene mutation not found in the unaffected siblings or in the controls. The mutations likely cause a null allele and a reduction in the level of functional progranulin protein. Both affected members of PPA1 with autopsies had frontotemporal lobar degeneration with tau-negative ubiquitinated inclusions.

Conclusions: To our knowledge, these are the only known families in which affected members display phenotypical homogeneity for PPA in the initial stages of the disease. In both families, the disease segregated with progranulin gene mutations. Whether progranulin dysfunction also extends to sporadic PPA and how it affects the initial anatomical specificity of neurodegeneration remain to be determined.

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PRIMARY PROGRESSIVE APHASIA (PPA) is a neurodegenerative syndrome with abnormalities of syntax, spelling, word use, or comprehension as initial symptoms. The resulting aphasia can be fluent or nonfluent and terms such as *progressive nonfluent aphasia* and *semantic dementia* have been used to denote PPA subtypes.¹ As PPA progresses, it can blend into the symptoms of corticobasal degeneration or the behavioral variant of frontotemporal lobar degeneration (FTLD).² The unique features of PPA are the initial predominance of the language deficit and the corresponding initial selectivity of the neurodegeneration for the language network.

Approximately 30% of patients with PPA show the neuropathologic features of Alzheimer disease, occasionally with an unusual distribution of lesions.^{3,4} The re-

maining 70% have the neuropathologic features of FTLD, with various combinations of gliosis, spongiosis, tauopathy, neuronal loss, and ubiquitinated inclusions.⁴⁻⁶

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Familial forms of progressive aphasia have also been described. However, the aphasia in most cases lacks the specificity that is characteristic of PPA or emerges as 1 of several dementia phenotypes within the same family. The hereditary inclusion body myopathy, Paget disease of bone, and frontotemporal dementia syndrome (linked to chromosome 9p13.3-p12) can initially manifest as aphasia, but the language disorder is usually accompanied by other cognitive and muscle disorders.⁷ The most common linkage of familial progressive aphasia is to chromosome 17.⁸⁻¹¹ Autopsy

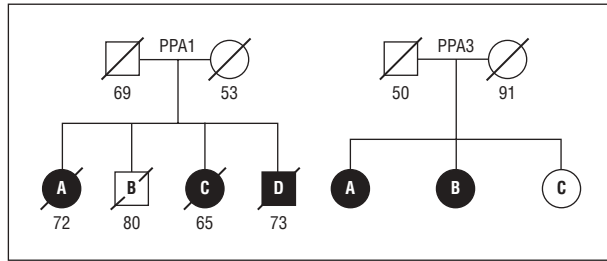


Figure 1. The PPA1 and PPA3 families. PPA1:B and PPA3:C were unaffected, with neither dementia nor progranulin gene mutations. Squares represent men; circles, women; solid symbols, affected members; open symbols, unaffected members; diagonal lines, deceased; and numbers, age at death in years.

findings have revealed FTLD pathologic features with tau or ubiquitin inclusions. In most of these families, as in the case of the family with hereditary dysphasic disinhibition dementia described by Lendon et al,⁸ affected members may have early and prominent impairments in memory, behavior, or executive function, lacking the distinctive clinical specificity of PPA. In other families, some family members have had the behavioral variant of FTLD and others the nonfluent variant of PPA.¹¹ An exception is the family described by Krefft et al,¹² in which all 3 affected siblings in a kindred of 4 manifested typical PPA.

Recently, a genetic cause of FTLD with tau-negative ubiquitinated inclusions was traced to progranulin (*PGRN*) gene mutations on chromosome 17q21.31.^{13,14} Herein, we report the presence of progranulin gene mutations in the family described by Krefft et al¹² and in a previously unreported family in which 2 of 3 siblings have PPA. To our knowledge, these are the only 2 known families in which the disease displays the PPA phenotype in all affected individuals.

REPORT OF FAMILIES

CLINICAL INFORMATION

Primary progressive aphasia is diagnosed when a progressive language impairment (manifested by deficits of naming, word finding, or word comprehension) emerges in relative isolation, without major disturbances of memory, personality, or face and object recognition.¹⁵ Impairments in other areas of cognition and behavior may eventually arise, but the language disorder remains the major problem for approximately the first 2 years. All 5 affected individuals in the PPA1 and PPA3 families fulfilled these inclusion and exclusion criteria for PPA.

No left-handedness or learning disabilities were reported in either kindred. The mother of the PPA1 probands died at age 53 years, and the father of the PPA3 probands died at age 50 years. The mother of the PPA3 probands is deceased and has a clinical history of late-onset dementia of the Alzheimer type.

PPA1 Family

The clinical features of the PPA1 family were described previously in greater detail by Krefft et al.¹² There were 4 siblings, 3 of whom had typical PPA (**Figure 1**).

PPA1:A. Word-finding and object-naming difficulties manifested in PPA1:A at the age of 60 years, followed by right-hand clumsiness 4 years later. No memory or personality changes were reported during the first 4 years. Neuropsychological examination 5 years after onset revealed intermittently fluent speech, frequently interrupted by word-finding pauses and semantic paraphasias. Naming and language comprehension were impaired. Nonverbal memory was much better than verbal memory, and her behavior was appropriate. No face or object recognition deficits were noted. Seven years after onset, she was socially appropriate and living independently. Eventually, progressive deterioration led to mutism and inability to use the right side. Neuroimaging showed left frontal atrophy. She died 12 years after onset, at the age of 72 years. No autopsy was performed.

PPA1:B. PPA1:B, the unaffected brother of PPA1:A, died at the age of 80 years. There was no dementia or cognitive impairments other than those associated with age. The brain weighed 1280 g and had Braak stage III neurofibrillary changes and several cortical microinfarcts in the watershed regions. These pathologic findings are consistent with normal aging and are compatible with age-appropriate mental function.¹⁶

PPA1:C. In PPA1:C, aphasic word finding and object-naming difficulties manifested at the age of 61 years. One year after onset, she could no longer speak in complete sentences. At 3 years after onset, she could only produce yes or no answers, her word comprehension was impaired, and she had stopped reading. However, no impairment of face or object recognition was noted. On examination, she answered no to all questions and followed no verbal commands. She became complacent, would get lost on walks, and developed right-hand tremor. Axial rigidity with bilateral cogwheeling and a pill-rolling tremor of the right hand were noted. Neuroimaging showed progressive left perisylvian atrophy. She died mute at the age of 65 years. The autopsy findings led to a principal diagnosis of FTLD with tau-negative ubiquitinated inclusions.¹²

PPA1:D. Word-finding difficulties manifested at age 65 years in PPA1:D. He substituted words for names of people and objects. Eighteen months after onset, he had no dysarthria, but his category fluency (ability to generate words belonging to a specified type of object) was decreased. By 3 years after onset, he was severely paraphasic and dysfluent, and his comprehension was decreased. Memory and daily living activities, including active involvement in carpentry, were unchanged. No face-recognition abnormalities were observed. Isolated incidents of socially inappropriate behaviors were noted 2 to 3 years after onset. Neuroimaging showed progressive left perisylvian atrophy. He died at age 73 years. The brain weighed 940 g and showed severe perisylvian atrophy and superficial cortical spongiosis (**Figure 2**). There were ubiquitin-positive neurites and neuronal inclusions (cytoplasmic and intranuclear) consistent with a principal diagnosis of FTLD with tau-

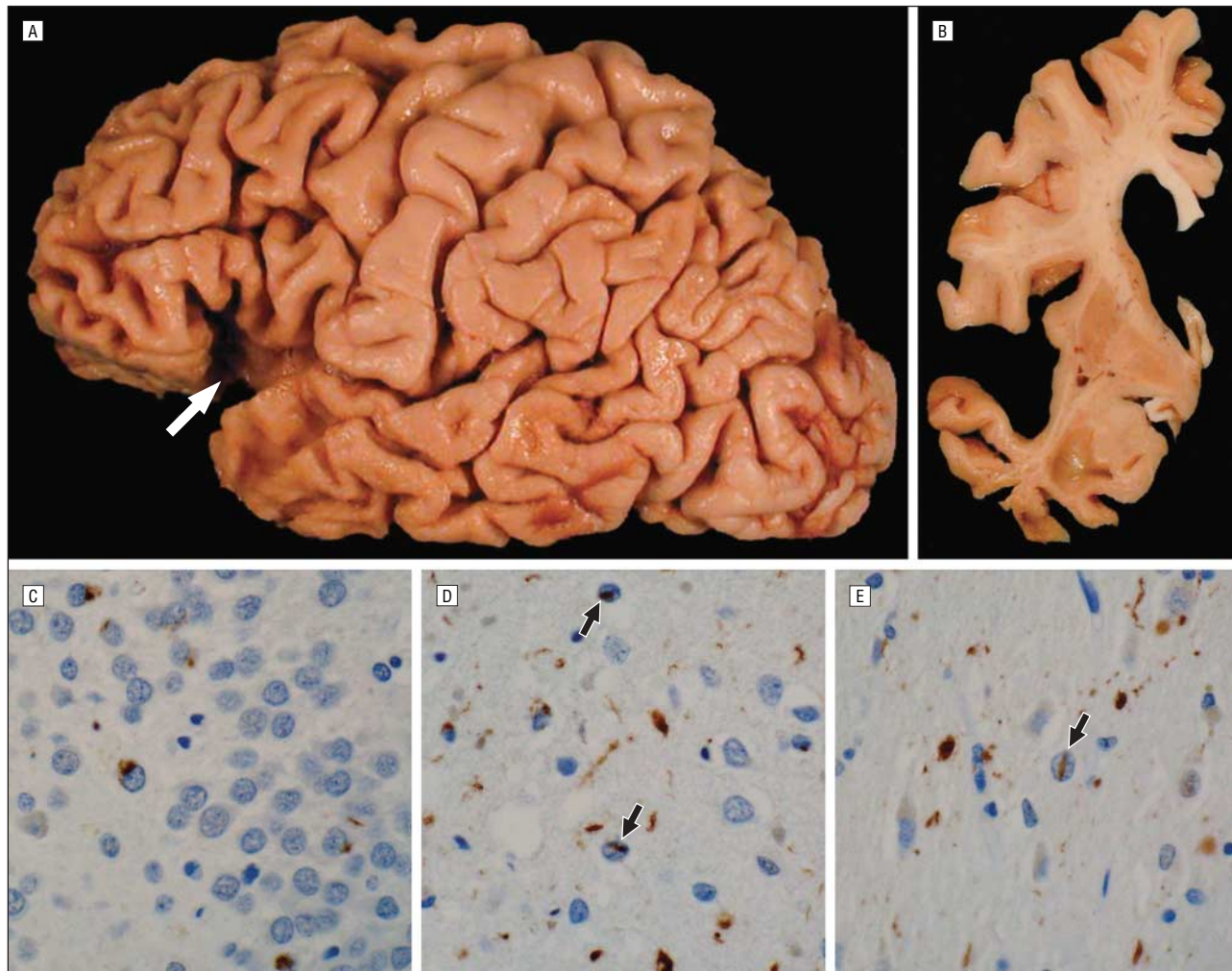


Figure 2. Neuropathologic findings in affected patient PPA1:D. A, The left hemisphere shows diffuse cortical atrophy that is accentuated in the perisylvian region. Note the marked widening of the sylvian fissure (arrow). B, A coronal section at the level of the columns of the fornix reveals enlargement of the frontal horn of the lateral ventricle, widening of the transverse fissure, and thinning of the cortical ribbon of the opercular frontal and temporal cortices, as well as of the inferior insular cortex. C-E, Ubiquitin immunohistochemistry shows sparse cytoplasmic inclusions of the hippocampal dentate fascia (C), while the temporal cortex (D) has many short neuritic processes and neuronal intranuclear inclusions (arrows) in superficial cortical layers, and the striatal pathologic condition (E) is characterized by dystrophic neurites and by neuronal intranuclear inclusions (arrow).

negative ubiquitinated inclusions and no neurofibrillary tangles (Braak stage, 0).

PPA3 Family

The PPA3 family has 3 members. Two have typical PPA (Figure 1).

PPA3:A. Seventy-year-old PPA3:A had manifested symptom onset at age 65 years, starting with word-finding difficulties and decreased speech output. She reported that she was “losing words” and could not finish sentences. One year after symptom onset, she had reduced speech output but no dysarthria. She had nonfluent aphasia with effortful, agrammatical, and paraphasic speech but with preserved comprehension at the single-word and single-phrase levels. Naming was impaired. She could repeat “Today is a very nice day” but not “No ifs, ands, or buts.” Reading comprehension was impaired only for sentences with complex grammatical structure. There was no apraxia. She was able to copy a complex figure from

memory and was fully oriented, with no evidence of visuospatial impairments. Her performance on Trail-Making Test B, a test of executive function, was slow but in the average range. A diagnosis of PPA was made. Three years after onset, the aphasia had progressed further, but the affect was noted as normal, with the motor examination results within normal limits. There was no evidence of object or face recognition impairments, and her husband reported that her memory for recent events was intact. The word-finding difficulties progressed relentlessly. She is mute and does not seem to understand what is being said to her. She is also incontinent and was placed in a nursing home. Results of electroencephalography, magnetic resonance imaging, and carotid ultrasonography were unremarkable within the first year after onset.

PPA3:B. Sixty-five-year-old PPA3:B experienced symptom onset at the age of 62 years, consisting of progressive word-finding impairments. Her speech was described as slow and markedly anomnic but not dysarthric. She was unable to write a full sentence. Runs of fluent

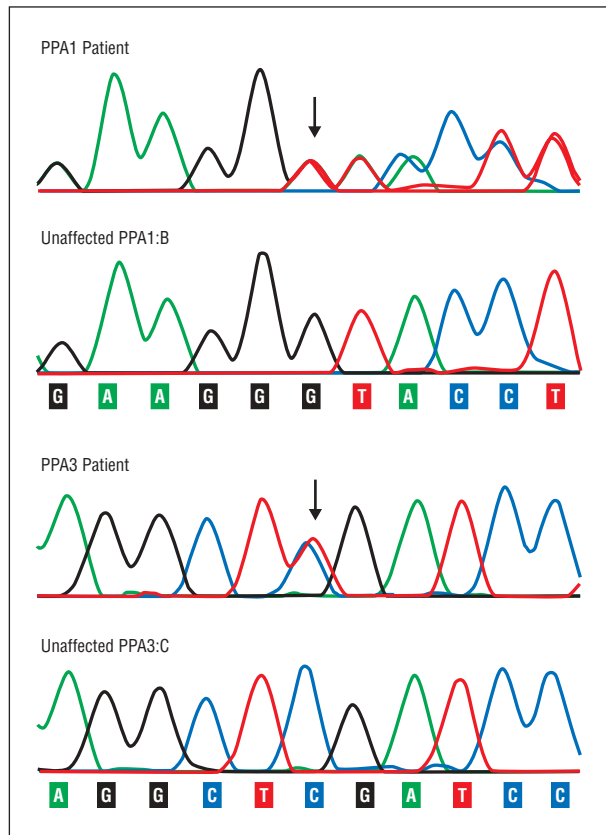


Figure 3. Genomic sequence chromatograms showing the mutations found in the PPA1 and PPA3 families. A and B, Analysis of exon 9 (arrow) in an affected member of the PPA1 family shows the single nucleotide deletion, in contrast to the unaffected member PPA1:B. C and D, Analysis of exon 11 (arrow) in an affected member of the PPA3 family shows the C>T transition that creates a premature stop codon, which is not seen in the unaffected member PPA3:C.

speech could alternate with dysfluent runs, interrupted by numerous word-finding pauses. Category fluency (ability to generate animal names) was reduced, but confrontation naming was intact. Intermittent language comprehension deficits were noted 1 year after onset. The initial diagnosis was PPA because aphasia was the most prominent feature of the clinical picture. Personality, memory for events, and daily living activities were described as intact during the first 18 to 24 months, except for some intermittent difficulties with driving. No face or object recognition deficits were noted. A mild right-hand tremor was present at the onset and progressed to a state of clumsiness and rigidity on the right side. She has minimal speech output and has difficulty with language comprehension. She is unable to use the right upper and lower extremities. She recently started to show uncharacteristic irritability but continues to live at home with her husband. Electroencephalography showed left-sided temporal rhythmic delta waves, and magnetic resonance imaging revealed diffuse biparietal but not hippocampal atrophy.

PPA3:C. At age 58 years, PPA3:C is in good health. She is asymptomatic with respect to cognition, personality, and motor function.

Genomic DNA was isolated from whole blood. The 12 coding exons and the 5' and 3' untranslated regions of *PGRN* were amplified by standard 25- μ L polymerase chain reaction using Qiagen products (Qiagen, Valencia, Calif) and primers as previously described.¹³ The resulting polymerase chain reaction products were purified using MultiScreen plates (Millipore, Billerica, Mass) and were sequenced in both directions on an ABI 3730 real-time system that detects and quantitates nucleic acid using the relevant polymerase chain reaction primers and Big Dye chemistry (Applied Biosystems, Foster City, Calif).

In PPA1, a progranulin gene mutation (c.998delG, p.Gly333ValfsX28) was found in 2 affected siblings (PPA1:C and PPA1:D) but not in the unaffected sibling (**Figure 3**). A DNA sample was not available from the third affected sibling (PPA1:A). The frameshift mutation caused by this single-base deletion mutation results in the introduction of a premature termination codon.

In the 2 affected sisters of PPA3, a nonsense mutation created by a C>T transition was observed on 1 of the *PGRN* alleles in exon 11 at position c.1477 (Figure 3). This change converts an arginine codon into a premature termination codon (p.Arg493X). This mutation was not observed in the unaffected sister. Based on previous findings,¹³ the mutant RNA in both families is likely subject to nonsense-mediated decay, which is the same as for other *PGRN* mutations that introduce premature termination codons. This will in turn cause a reduction in the level of functional *PGRN* messenger RNA and protein, consistent with a haploinsufficiency mechanism. These 2 mutations were not observed in more than 200 North American control samples.

COMMENT

In 2 unrelated families, each with multiple affected siblings, PPA was associated with *PGRN* mutations. In the PPA1 family, in which 3 autopsies were performed, FTLD with tau-negative ubiquitinated inclusions was found in the 2 affected members but not in the unaffected sibling. In contrast to other familial progressive aphasia that lack the typical PPA phenotype (as in many affected members of families with hereditary dysphasia disinhibition dementia⁸) or that display phenotypical heterogeneity of behavioral presentation in some family members and aphasic presentation in others (as in the recently described Belgian kindred¹¹), these are the only known families (to our knowledge) in which each of the multiple affected individuals displayed the PPA phenotype as defined by the emergence of a relatively isolated aphasia unaccompanied by prominent amnesia, personality change of associative agnosia in the initial stages.

Language fluency and comprehension varied among patients and changed as the disease progressed. There was no consistent fit with the progressive nonfluent aphasia or semantic dementia designations, with the common denominator being aphasia (as defined by errors of word finding, object naming, or word comprehension) that initially manifested in relative isolation, without major

impairments of memory, personality, or object recognition. The common feature was the preferential involvement of the language network, although there was considerable heterogeneity in the nature of the language impairment. Eventually, all affected members had a severe loss of fluency. In each family, some of the affected members developed right-sided motor impairments suggestive of corticobasal degeneration. The PPA-PGRN phenotype may overlap with the corticobasal degeneration syndrome. The early emergence of the right-sided tremor in PPA3:B suggests that asymmetrical extrapyramidal findings should be included among the contributory criteria for a PPA diagnosis.

Three additional patients with PPA and PGRN mutations were recently described; all 3 had a family history of dementia, but none had other family members with PPA.¹⁷ These 3 patients,¹⁷ the Belgian kindred with nonfluent PPA,^{11,14} and the 2 families in this report demonstrate that PGRN mutations may emerge as important genetic causes of familial PPA. Whether PGRN gene expression also plays a role in sporadic PPA remains to be seen.

Progranulin mutations can also give rise to behavioral variants of FTL and can display considerable heterogeneity within the same family.¹³ The apparent initial phenotypical homogeneity in the PPA1 and PPA3 families may reflect an interaction between the PGRN mutation and a specific genetic background that makes the language network particularly susceptible to neurodegeneration. Such interactions are known in dominantly inherited prion diseases in which the nature of the polymorphism in codon 129 determines whether the predominant neurodegeneration will be in the thalamus or the cerebral cortex.¹⁸ A parallel possibility in patients with null mutations of PGRN is that variants in the normal allele may affect expression levels and distribution of the wild-type functional protein. Alternatively, the language network may harbor a developmental or acquired vulnerability that can become a locus of least resistance for the anatomical distribution of a subsequent neurodegeneration caused by the PGRN mutation. The higher incidence of learning disabilities and dyslexia in patients with PPA and their first-degree relatives provides indirect support for this hypothesis.³ Even in the PPA1 and PPA3 families, however, the neurodegeneration became widespread, reflecting a fundamental metabolic abnormality that eventually spreads beyond the initial area of preferential vulnerability.

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