HDDD2 Is a Familial Frontotemporal Lobar Degeneration with Ubiquitin-Positive, Tau-Negative Inclusions Caused by a Missense Mutation in the Signal Peptide of Progranulin

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Objective: Familial autosomal dominant frontotemporal dementia with ubiquitin-positive, tau-negative inclusions in the brain linked to 17q21-22 recently has been reported to carry null mutations in the progranulin gene (PGRN). Hereditary dysphasic disinhibition dementia (HDDD) is a frontotemporal dementia with prominent changes in behavior and language deficits. A previous study found significant linkage to chromosome 17 in a HDDD family (HDDD2), but no mutation in the MAPT gene. Longitudinal follow-up has enabled us to identify new cases and to further characterize the dementia in this family. The goals of this study were to develop research criteria to classify the different clinical expressions of dementia observed in this large kindred, to identify the causal mutation in affected individuals and correlate this with phenotypic characteristics in this pedigree, and to assess the neuropathological characteristics using immunohistochemical techniques.

Methods: In this study we describe a detailed clinical, pathological and mutation analysis of the HDDD2 kindred.

Results: Neuropathologically, HDDD2 represents a familial frontotemporal lobar degeneration with ubiquitin-positive, tau-negative inclusions (FTLD-U). We developed research classification criteria and identified three distinct diagnostic thresholds, which helped localize the disease locus. The chromosomal region with the strongest evidence of linkage lies within the minimum critical region for FTLD-U. Sequencing of each exon of the PGRN gene led to the identification of a novel missense mutation, Ala-9 Asp, within the signal peptide.

Interpretation: HDDD2 is an FTLD-U caused by a missense mutation in the PGRN gene that cosegregates with the disease and with the disease haplotype in at-risk individuals. This mutation is the first reported pathogenic missense mutation in the signal peptide of the PGRN gene causing FTLD-U. In light of the previous reports of null mutations and its position in the gene, two possible pathological mechanisms are proposed: (1) the protein may accumulate within the endoplasmic reticulum due to inefficient secretion; and (2) mutant RNA may have a lower expression because of degradation via nonsense-mediated decay.

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Frontotemporal dementia (FTD) is characterized by early behavioral and personality changes, language deterioration, and later in the course of the disease, dementia and parkinsonism.1 FTD shows familial aggregation with a family history of dementia present in 41% of FTD cases.2 Late pyramidal and extrapyrami-
dral signs are present in some cases. Neuropathologically, cases of sporadic and familial frontotemporal lobar degeneration (FTLD) show stereotypical features: atrophy of the frontal and temporal lobes with variable involvement of the basal ganglia that is characterized by neuronal loss, status spongiosus, and reactive astrogliosis. FTLD may be caused by a wide spectrum of disorders including those characterized by abnormal glial and neuronal inclusions of aggregated microtubule-associated protein tau, diseases with ubiquitin inclusions, and a minority that do not show any detectable abnormal cellular aggregates. Some of the FTLD disorders, including Pick’s disease, progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain diseases, and FTD with parkinsonism linked to chromosome 17, collectively are referred to as tauopathies. The majority of FTLD, however, is not characterized by tauopathies, but instead contain other abnormal protein aggregates. These disorders include FTLD with ubiquitin-positive, tau-negative inclusions (FTLD-U), also called FTLD-motor neuron disease type, neuronal intermediate filament inclusion disease, and inclusion body myopathy with Paget’s disease and frontotemporal dementia. Some FTLD cases have no discernible inclusions and are called dementia lacking distinctive histopathology.

Initial genetic studies of familial FTD with evidence of linkage at 17q21 and tau-immunoreactive inclusions identified mutations in the gene encoding microtubule-associated protein tau (MAPT). However, several FTD families with significant linkage (logarithm of odds [LOD] score > 3) to the same region did not show any mutations in the coding and flanking intronic sequences of the tau gene. Subsequent neuropathological studies using ubiquitin immunohistochemistry identified these cases as FTLD-U. Recently, null mutations in the progranulin gene (PGRN) have been described in several FTLD-U kindreds showing linkage to chromosome 17.

PGRN was first characterized as a putative family of growth factors constitutively expressed in several tissues. PGRN is implicated in cell proliferation, wound repair, and anchorage independent growth. Interestingly, when overexpressed, it causes tumorigenesis. In brain, PGRN is expressed in Purkinje cells, pyramidal cells of the hippocampus, and some cerebral cortical neurons.

We have previously described HDDD2 as an autosomal dominant kindred with prominent dysphasia and behavioral disturbances and characterized neuropathologically, before ubiquitin immunohistochemistry, as FTLD. Previously, no mutation in any of the known dementia-causing genes was identified in this kindred. Since 1993, we have performed longitudinal assessments in consenting family members to further characterize the clinical and neuropathological characteristics of HDDD2. In addition to the new neuropathological findings reported here, we have also reevaluated linkage data in a larger series of family members and undertaken mutation analysis of the recently identified FTLD-U gene, progranulin.

Subjects and Methods

Subjects

All procedures reported were approved by the Washington University School of Medicine Human Studies Committee. Family members older than 18 years were included after informed consent was obtained. All individuals were interviewed by an experienced research clinician or nurse, using a modified version of the Family History Interview developed by the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD). For individuals with neurological symptoms, the visit included a clinical and neurological examination and a consensus history derived from the individual or nearest relatives, or both. When possible, available medical records also were analyzed. HDDD2 is a large, multiply affected, multigeneration kindred with FTLD-U segregating in one branch of the pedigree. Several individuals both within the FTLD-U branch and also in the extended kindred exhibit different clinical presentations including dementia of the Alzheimer’s type (DAT), rather than classic dysphasia. This variability in clinical phenotype raises the possibility that the disease in this family exhibits variable expressivity, or that the dementias observed in this kindred have more than one cause.

Genetic Analysis

High molecular weight DNA was extracted from blood or brain tissue according to standard procedures. DNA was available for 82 (18 affected) family members with complete clinical information. Because the primary goal of this study was to determine the genetic cause of HDDD, we established clinical and pathological criteria to classify individuals with definite, probable, or possible HDDD (Table 1). These criteria were based on the presence of frontal lobe signs and prominent early memory loss in family members plus confirmation of FTLD-U (or FTLD-motor neuron disease [MND] type) using established neuropathological diagnostic criteria.

For linkage analysis, we used information from a total of 161 individuals spanning 4 generations from the FTLD-U part of the pedigree.

Statistical Analysis

The informativeness of the pedigree was established by simulation studies (FastSlink v2.51 program), which yielded a LOD score of 6.35. Three diagnostic thresholds were tested to maximize the linkage information. In model I, affected individuals were defined as those with a diagnosis of definite or probable HDDD. Family members with a diagnosis other than definite or probable HDDD and all asymptomatic individuals younger than 77 years were considered as phenotype unknown. The remaining family members were considered as unaffected (nondemented individuals older than 77 years). In model II, all individuals with a lifetime diagnosis...
Table 1. Main Features and Research Criteria Used for Definite, Probable, and Possible Hereditary Dysphasic Disinhibition Dementia Classification

<table>
<thead>
<tr>
<th>Signs and Symptoms of Hereditary Dysphasic Disinhibition Dementia</th>
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<tr>
<td><strong>A. Cognitive impairment of gradual onset and progressive course</strong></td>
</tr>
<tr>
<td><strong>B. Family history of dementia with frontal features</strong></td>
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<tr>
<td><strong>C. Early personality/behavior impairment: either a, b, c, or d are present and prominent within the first 3 years of noted cognitive impairment</strong></td>
</tr>
<tr>
<td>a. Change in personality</td>
</tr>
<tr>
<td>b. Impaired executive functions</td>
</tr>
<tr>
<td>c. Apathy</td>
</tr>
<tr>
<td>d. Repetitive/compulsive behavior</td>
</tr>
<tr>
<td><strong>D. Early language impairment: either a or b are present and prominent within the first 3 years of noted cognitive impairment</strong></td>
</tr>
<tr>
<td>a. Reduced language output: less spontaneous speech, inability to finish sentences (within 3 years), or mutism (within 5 years)</td>
</tr>
<tr>
<td>b. Verbal perseveration, stuttering, echolalia, pardysphasia</td>
</tr>
<tr>
<td><strong>E. Pyramidal/extrapyramidal signs</strong></td>
</tr>
<tr>
<td>a. Focal hyperreflexia or increased tone, hemiparesis</td>
</tr>
<tr>
<td>Babinski’s sign, without evidence of stroke</td>
</tr>
<tr>
<td>b. “Primitive” frontal reflexes</td>
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<tr>
<td>c. Bradykinesia/hypokinesia, resting tremor, parkinsonian gait, unilateral extremity neglect</td>
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**Research Criteria for Disease Classification**

I. **Definite HDDD:** Pathology (*macroscopic findings*: frontal lobe atrophy, enlarged lateral ventricles, thinning of the cortical ribbon, pale and gliotic underlying white matter; *microscopic findings*: neuronal loss, gliosis and superficial spongiosis in the frontal lobe, neuronal loss in the hippocampus, presence of ubiquitin-positive intraneuronal inclusions) plus criteria for probable/possible HDDD or DSM-IV for AD dementia

II. **Probable HDDD:** A, B, C, and D required; memory impairment is not the prominent feature at disease onset

III. Possible HDDD: A and B required; C or D required; memory impairment could be prominent at disease onset

HDDD = hereditary dysphasic disinhibition dementia; DSM-IV = Diagnostic and Statistical Manual, fourth edition; AD = Alzheimer’s disease.

of definite or probable HDDD were considered affected; remaining family members were considered as phenotype unknown. In model III, all individuals with HDDD (definite plus probable plus possible) were considered affected; remaining family members were considered as phenotype unknown.

**DNA Sequencing**

All the exons and the intron–exon boundaries of PGRN were amplified using gene-specific primers. Direct sequencing of the amplified fragments was performed using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Wellesley, MA) and standard protocols. For most of the fragments, the primers used for sequencing were the same as those used for polymerase chain reaction amplification (primer sequence available on request). Sequence variations were tested for segregation with the disease and screened in a set of 50 unrelated age-matched population control subjects. To test the effect of the mutation in other forms of dementia in this kindred, we screened DAT individuals from both within the FTLD-U branch and the extended pedigree.

**Neuropathological Procedures**

Brain tissue was obtained with the consent of the next of kin and with approval of Washington University Human Studies Committee. Autopsies were performed according to established Alzheimer’s Disease Research Center Neuropathology Core protocols. In brief, brain tissue was preserved in buffered 10% formalin, and when available, tissue was frozen at −80°C for biochemistry and molecular genetic studies. Tissue samples were obtained from the frontal, temporal, parietal, and occipital lobes, basal ganglia, thalamus, amygdala, hippocampus, midbrain, pons, medulla oblongata, and cerebellum. The spinal cord was unavailable for examination.

**Histology and Immunohistochemistry**

Routine stains included hematoxylin and eosin and a modified Bielschowsky silver impregnation. After antigen retrieval methods, immunohistochemistry was performed using antibodies to ubiquitin (Chemicon International, Temecula, CA), tau (PHF1; gift of Dr P. Davies), α-synuclein (Zymed Laboratories, San Francisco, CA), β-amyloid (4G8; Signet Laboratories, Dedham, MA), α-internexin (Zymed Laboratories, San Francisco, CA), valosin-containing protein (gift of Dr C.-C. Li), and progranulin (R&D Systems, Minneapolis, MN) on representative areas. Cases were diagnosed according to established and other neuropathological criteria where they exist. Alzheimer’s disease (AD)–type changes were assessed using the staging of Braak and Braak and the diagnostic criteria of Khachaturian, the CERAD, and the National Institute on Aging-Reagan Institute criteria.

**Results**

**Clinical Features**

Although the HDDD2 kindred originated in Central Europe, all of the subjects included in this study were born and reside in the United States. About 80% of these subjects live in three adjacent counties in the southern United States. The HDDD2 pedigree has 161 members, spanning 4 generations. Among the demented individuals, identified through clinical examination, medical report, or family report, 18 18 met criteria for probable or definite HDDD (8 were HDDD definite), 8 met criteria for possible HDDD, and 2 were diagnosed with DAT. Table 2 summarizes the clinical profile of the HDDD individuals.

**Genetic Analysis**

LINKAGE ANALYSIS OF 17q21. Significant evidence of linkage was observed with markers on 17q using a whole genome scan (data not shown). High-density screening was subsequently performed between D17S798 and D17S784 to identify the putative disease
locus. This interval included the region previously linked to HDDD2 and several other FTD families with ubiquitin-positive, tau-negative inclusions.9,10,11

The highest two-point LOD score was observed at D17S932 (LOD = 4.20, \( \theta = 0 \)) for model I. Several flanking markers also yielded conclusive evidence of linkage in the absence of recombination (Fig 1A). Under stringent criteria, model II yielded a similar LOD score for all markers, but slightly lower than model I. This decline in LOD score was even more pronounced at all loci when the analysis was performed using model III, except D17S932 and D17S930 (see Fig 1A). Multipoint analysis yielded evidence of linkage (LOD > 3.00) in the interval containing D17S932 and D17S930, suggesting the possible localization of the causal gene for FTLD-U.

**DIRECT DNA SEQUENCING.** The close proximity of \( \text{PGRN} \) to the most informative markers (see Fig 1B) and a recent report of null mutations in the \( \text{PGRN} \) gene led us to examine the gene together with other candidate genes in the region. Mutation detection was performed using the Sequencher package v4.6 (Gene codes pvt Ltd, Ann Arbor, MI). A novel heterozygous missense mutation Ala-9 Asp (A9D) was found in exon 1 (Fig 2A). The mutation was found to cosegregate with the disease phenotype and at-risk individuals with the disease haplotype (see Fig 2B). The mutation was not present in unaffected family members who did not carry the disease haplotype or in matched population control subjects, implying that the mutation is likely to be pathogenic. The mutation was also absent from individuals who had DAT within the FTLD-U branch and in the extended kindred (see Fig 2B).

**Table 2. Clinical Description of Individuals with Hereditary Dysphasic Disinhibition Dementia (HDDD2) Kindred**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HDDD (n = 26)</th>
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<tbody>
<tr>
<td>Sex, F/M</td>
<td>13/13</td>
</tr>
<tr>
<td>Mean onset of disease (range), yr</td>
<td>63.5 (52–77)</td>
</tr>
<tr>
<td>Mean duration of disease (range), yr</td>
<td>7.2 (6–11)</td>
</tr>
<tr>
<td>Mean age at death (range), yr</td>
<td>70.2 (59–86)</td>
</tr>
</tbody>
</table>

HDDD = hereditary dysphasic disinhibition dementia.
Neuropathological Findings

MACROSCOPY. The neuropathology of the first four cases of HDDD2 that came to autopsy has been reported previously. Here, we describe an additional four cases and review all eight cases for contemporary immunohistochemical and diagnostic criteria. The mean brain weight was 1,044 gm (range, 800–1,270 gm). Atrophy varied from moderate to severe and was most pronounced in the frontal lobe in all HDDD cases (Fig 3). The temporal lobe was severely affected in the majority of cases, as was the parietal lobe, but to a lesser degree. In about half of the cases, the ventricular dilatation and narrowing of gyri in the frontal and anterior temporal lobes was comparable with that seen in classic cases of Pick’s disease. There was variable atrophy of the hippocampus ranging from severe to relatively well preserved (see Fig 3). There was some atrophy of the basal ganglia in about half of the cases. The brainstem and cerebellum were macroscopically unremarkable in most cases, and the pigmented nuclei were generally well preserved.

MICROSCOPY. The stereotypical histological features of all FTLDs were present in all eight cases: severe neuronal loss, status spongiosus, and reactive astrogliosis in affected areas. The distribution and severity of pathology varied from case to case, but invariably, the frontal lobe was the most severely affected. There was also pronounced neuronal loss in neocortex of the temporal and, to a lesser degree, the parietal lobe. The degree of neuronal loss was variable, and in the most severe cases, both association and motor cortices were affected. In places, the cortical ribbon was completely devoid of neurons in the most severe cases. More typically, microvacuolation could be seen in laminae II and III (Fig 4). The hippocampus was, generally, less atrophied and showed less neuronal loss than is commonly seen in AD. Neuronal loss from the thalamus was observed in a minority of cases. The nuclei of the brainstem and cerebellum were generally unremarkable. The characteristic lesions of MND, ubiquitin-positive skein-like inclusions and Bunina bodies in motor neurons, and corticospinal tract degeneration were absent. The signature lesions of FTLD-U were present in seven of eight cases: ubiquitin-positive, tau-negative neuronal cytoplasmic inclusions (NCIs); dystrophic neurites (DNs); and neuronal intranuclear inclusions (NIIs) (see Figs 4B–E). In one case, after repeated antigen retrieval methods, no ubiquitin-positive inclusions were detected. These three types of inclusions were seen most readily in the frontal lobe, but their presence was also noted in the temporal and parietal neocortex and basal ganglia; the occipital lobe did not exhibit this pattern of pathology.

Progranulin immunohistochemistry showed a predominantly neuronal cytoplasmic pattern of expression in normal brain (Fig 5A). High levels of expression were also observed in microglial cells (see Fig 5D), and much lower levels in oligodendrocytes and astrocytes. In cases of HDDD2 with AD pathology, the DNs of
neuritic plaques were intensely labeled and were identical to those seen in a case of AD (see Fig 5B). Neurofibrillary tangles and diffuse β-amyloid plaques were not labeled by the anti-progranulin antibody (data not shown). Progranulin immunohistochemistry failed to demonstrate progranulin as a component of the ubiquitin-positive, tau-negative NCIs, NIIs, or DNs of familial FTLD-U with PRGN mutation (HDDD2) (see Figs 5C, D).

In none of the six cases where DNA was available was there an APOE4 genotype, yet a feature of the HDDD2 kindred is the presence of additional AD pathology in half of the cases, consistent with neurofibrillary tangle stage greater or equal to IV, according to Braak and Braak. In one patient who survived to 82 years, there was sufficient pathology for the diagnosis of AD according to Khachaturian’s criteria and “probable AD” by CERAD criteria. In those cases with AD pathology, the distribution was atypical. The
highest densities of lesions (neurofibrillary tangles and β-amyloid plaques) were seen in temporal, parietal, and occipital lobes, the frontal lobe being relatively devoid of this pathology. The relative absence of AD pathology from the frontal lobes indicates that this neurodegenerative disease occurs secondarily to FTLD-U and is probably age related, although synergistic effects of these two neurodegenerative processes cannot be excluded.

Discussion
This study details the clinical, pathological, and mutation analysis of the HDDD2 kindred. The HDDD syndrome resembles the so-called frontal variant of FTD with ubiquitin-positive intraneuronal inclusions. Variations of the signal recognition particle, which targets proteins to the endoplasmic reticulum membrane.

The clinicopathological criteria used to classify the patients into different groups (possible, probable, and definite HDDD) proved to be useful in our genetic analyses. Use of different phenotypic thresholds helped localize the candidate region on 17q21. The recently identified causal gene PGRN is located 0.2Mb in between D17S932 and D17S930. These two markers showed significant evidence of linkage under all three diagnostic thresholds tested, suggesting a common disease locus for them. In contrast, inclusion of the DAT individuals in the analysis did not improve the linkage information, implying a different cause. All affected members of the kindred are heterozygous for a novel missense mutation c.4068C to A in exon 1, resulting in an Ala-9 to Asp substitution, which introduces a charged amino acid into the signal peptide. Furthermore, none of the DAT individuals carried the mutation, confirming that the DAT observed in this kindred has a different cause than HDDD. The location of this mutation is in, or near, the binding site of the signal recognition particle, which targets proteins to the endoplasmic reticulum membrane. This putative mechanism introduces a change in the amino acid side chain, which could radically affect protein secretion whereas not affecting the level of the mRNA. However, in light of the functional null alleles it may be possible that the amino acid substitution may degrade the mutant RNA by nonsense-mediated decay, causing variation in protein expression like a null mutation.

We also confirm that HDDD2 has the characteristic features of all FTLDs: frontal and temporal lobe atrophy with severe neuronal loss, status spongiosus, and reactive astrogliosis in affected areas. In addition, the signature lesions of FTLD-U are also present: ubiquitin-positive, tau-negative NCIs and DNIs. As in most familial cases of FTLD-U and occasional sporadic cases, a third site of ubiquitin pathology was seen in HDDD2: NIs. Both NCIs and DNIs are readily seen in affected neocortex and basal ganglia, and NCIs may also be seen with varying density in the granule cells of the dentate fascia (see Fig 4E). The NCIs of FTLD-U are indistinguishable from those seen in some cases of MND and MND with dementia. In the majority of HDDD2 brains, ubiquitin-positive NIs were seen and, although less abundant than NCIs or DNIs, were found in affected cortex (see Figs 4C, D) and subcortical nuclei. NIs have been reported in a number of familial cases of FTLD-U, but they are not specific to familial cases because they are also seen in sporadic cases. Progranulin immunohistochemistry failed to detect the ubiquitin-positive inclusions of familial FTLD-U, an observation that is consistent with the
The biological function of PGRN in neurons is not well characterized; however, the basal gene expression of this growth factor suggests that it is multifunctional with important roles in several neuronal signaling pathways and neurodegeneration. The identification of PGRN as a cause of neurodegeneration also highlights the link between tumorigenesis and neurodegeneration. Overexpression of PGRN in vitro leads to uncontrolled cell growth in several cell types, whereas null alleles cause neurodegenerative disease. Similarly, mutations in the presenilins cause FAD, whereas knock-out of Presenilin (PS) in the skin leads to tumor formation. The heterozygous missense mutation we have identified could prevent normal insertion of progranulin into the endoplasmic reticulum membrane preventing secretion and leading to an accumulation of the precursor protein in the endoplasmic reticulum. The pathological mechanism could also include degradation of the mutant RNA through nonsense-mediated decay.

The search for the genetic basis of FTLD-U has been ongoing for more than a decade; this report, together with other positive findings, brings new insight to the disease pathogenesis and will help in the identification of therapeutic agents.

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References


