Molecular Pathogenesis of Frontotemporal Lobar Degeneration

Basic Science Seminar in Neurology

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Molecular genetic research provides a valuable tool to increase our understanding of diseases of the brain, which are otherwise difficult to study because of limited accessibility to the diseased tissue during life. One of the classic methods used in molecular genetics is linkage analysis. This is a technique designed to map disease genes in families by examining transmission of genes based on cosegregation of highly polymorphic genetic markers with disease. These genetic markers are mostly short tandem repeats of 2 to 4 nucleotides that can be genotyped at even spacing throughout the genome. Using a statistical algorithm, chromosome regions can be identified that are shared between patients more often than expected by chance. Shared regions are more likely to contain a disease gene. Findings from a single family can be extrapolated to larger groups of patients by exposing key genes and proteins in the pathologic cascade. Eventually, these insights may lead to development of diagnostic markers or therapeutic strategies.

A pertinent example is frontotemporal lobar degeneration (FTLD), a dementia disorder that brings about profound behavioral changes and impairment of language and executive dysfunction, with an age at onset ranging, on average, from 45 to 65 years. Up to 50% of patients have a positive family history, often with an autosomal dominant inheritance pattern in which multiple relatives across generations are affected.

Three clinical syndromes with FTLD pathologic characteristics can be discerned, coinciding with the topographic distribution of neurodegeneration. In approximately half of the patients with FTLD, the disease manifests as frontotemporal dementia, a disorder dominated by changes in personality and social conduct and associated with atrophy of the prefrontal and anterotemporal cortex. The other 2 FTLD syndromes, semantic dementia and progressive nonfluent aphasia, manifest clinically as language dysfunction. Patients with semantic dementia exhibit impaired word comprehension and an inability to recognize the meaning of other sensory stimuli. Bilateral atrophy of the middle and inferotemporal cortex is present. In contrast, patients with progressive nonfluent aphasia develop dysfunction of expressive language, with reduced spontaneous speech and increased phonologic and grammatical errors. Progressive nonfluent aphasia is associated with asymmetric atrophy of the left frontal and temporal cortices. The distinction between these 3 phenotypes is not absolute; patients often display a combination of symptoms, and several different FTLD syndromes may exist in families. Symptoms of motor neuron disease and parkinsonism further add to the clinical heterogeneity of FTLD.

HISTORICAL PERSPECTIVE: TAU OR NO TAU

Historically, patients with symptoms of FTLD were diagnosed as having Pick disease after Arnold Pick, who in 1892 first described a 71-year-old man who developed dementia with sensory aphasia and...
behavioral symptoms and demonstrated severe atrophy of the frontotemporal lobes at autopsy. Histopathologic examination of tissue samples revealed “ballooned” achromatic neurons and argyrophilic inclusions within frontal neurons, which were later referred to as Pick cells and Pick bodies, respectively, with the latter composed of insoluble filaments of the microtubule-associated protein tau. Autosomal dominant forms of Pick disease were reported, and linkage to chromosomal band 17q21 was reported by linkage analysis in families with autosomal dominant FTLD, resulting in the identification of mutations in the gene encoding the microtubule-associated protein tau (MAPT) (OMIM 157140), which is located at 17q21. At present, 42 mutations in MAPT have been described in 119 families with FTLD or related disorders (available at http://www.molgen.ua.ac.be/FTDMutations). Yet, in an estimated 60% to 70% of all patients with FTLD undergoing autopsy, no tau pathologic characteristics are demonstrated, which suggests that insoluble tau is not necessary for the clinical phenotype to develop. Moreover, 5 families with FTLD have been reported worldwide in which both tau pathologic conditions and MAPT mutations could not be demonstrated, although they showed linkage to 17q21. Instead, autopsies in these families showed FTLD with tau-negative neuronal intranuclear and cytoplasmic inclusions immunoreactive to ubiquitin (FTLDU), suggesting that different molecular mechanisms might lead to a similar clinical phenotype (Figure 1A, B, and E). This observation prompted a search for the genetic defect in these families having 17q21-linked FTLDU. The insight gained through molecular genetic research in these 5 families has widespread implications for FTLD and for other neurodegenerative diseases, of which we can see only the tip of the iceberg.

**MAPPING THE GENE FOR 17q21-LINKED FTLDU**

Successful disease gene mapping via linkage analysis relies on the process of genetic recombination that occurs during meiosis. During this process, physical breaks of the double DNA helix arise in paternal and maternal chromatids, which, when connected, result in genetically different gametes. If a parent transmits a disease mutation to a child, this child shares a DNA sequence around this disease mutation with the affected parent that is likely reduced in size because of genetic recombination. If multiple patients in a family exist, each meiosis may have resulted in a genetic recombination at a different position along the chromosome, which allows centromeric and telomeric delineation of the chromosomal region that is minimally shared between all patients (Figure 2A).

Careful analysis of the chromosomal region shared by patients in each family with FTLDU-17 revealed that the minimum region harboring the gene for FTLDU-17 was 6.2 million base pairs (bp), as delimited by a centromeric recombinant in Dutch family 1083 and by a telomeric recombinant in Canadian family UBC-17. Next, all genes within the shared region needed to be identified before they could be screened for mutations. Whereas this used to be an elaborate laboratory procedure, the completion of the draft sequence of the human genome via the Human Genome Project has now rapidly directed that process.

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**Figure 1.** Pathologic features of frontotemporal lobar degeneration with tau-negative neuronal intranuclear and cytoplasmic inclusions immunoreactive to ubiquitin. A, Severe cortical atrophy with narrowing of frontal lobe gyri in a typical patient. Ubiquitin immunostaining using a polyclonal antiubiquitin antibody (DAKO, Glostrup, Denmark) showed typical ubiquitin-immunoreactive neuronal intranuclear (B, arrow) and cytoplasmic (E, arrows) inclusions in the frontal cortical region of this patient. Note the typical “cat’s eye” intranuclear neuronal inclusion in B. Immunoreactivity with a polyclonal antibody against PGRN (progranulin) holoprotein (antihuman progranulin; R & D Systems, Minneapolis, Minnesota) showed PGRN immunostaining in neuronal perikaryon (C, arrows) and intense immunoreactivity in neuronal and cytoplasmic inclusions (G, double-headed arrow and inset). In neurons with inclusions, the normal nuclear staining of TDP-43 seems reduced. For immunohistochemistry, sections were antigen retrieved by microwaving in citrate buffer (pH 6) and were stained using avidin-biotin complex–horseradish peroxidase and diaminobenzidine (Roche, Nutley, New Jersey) as described previously. Scale bars represent 1 cm (A) and 20 µm (B-G).
Figure 2. From segregation analysis to disease-causing mutation. A, Segregation analysis of a 4-generation pedigree. Square represents male; circle, female; filled symbol, patient; diamond, number of unaffected relatives; and asterisk, individual for whom DNA was available. For each patient, haplotypes are shown in the region for which linkage was observed. For each genetic marker (listed to the left of the pedigree), both alleles are shown (coded by Arabic numerals). Markers in bold indicate the minimal shared haplotype. In patients for whom no DNA was available, haplotypes are reconstructed based on the genetic information of spouse and offspring (numbers in parentheses [? indicates that reconstruction of the haplotype was impossible]). Purple bar represents the haplotype that is shared by all patients. Green arrow denotes centromeric recombination; red arrow denotes telomeric recombination delineating the minimal shared region. PGRN is located in this region; N denotes normal allele, and D denotes disease allele. B, Assembly and annotation of the shared region on 17q21 (red bar in the upper panel) using public databases such as the University of California Santa Cruz (UCSC) genome browser (http://genome.ucsc.edu) identifies known genes in the region (a small number of which are shown in the middle panel). FISH indicates fluorescence in situ hybridization. Mutation analysis through direct sequencing of the identified genes results in the detection of a C to T transition (denoted by Y in the DNA sequence shown in the lower panel) in a coding DNA sequence of PGRN in a patient but not in the control individual.
leted.8 In parallel with the exclusion of the MAPT locus, other genes located in the shared region underwent a mutation screen- ing through direct sequence analysis, allowing computer-

assisted assembly and annotation of the DNA sequence in the candidate region based on genetic information in public databases (Figure 2B). Assembly and annotation of the minimal FTLDU-17 genomic sequence revealed 165 known genes, including MAPT. Although simple mutations in MAPT had been excluded after extensive DNA sequence analysis of a 138 500-bp MAPT genomic region,6 more complex mutations such as genomic re-arrangements (inversion of a chromosomal region) or copy number variations (duplication or deletion of the MAPT locus) might cause disease. With fluorescence in situ hybridization, a technique using fluorescently labeled DNA probes to visualize a given DNA sequence, it was proven that an inversion of the chromosomal region containing MAPT exists but that this inversion is not associated with affection status (ie, also occurs in healthy individuals).7 In addition, the presence of copy number variations causing disease was excluded via oligo-

based array comparative genome hybridization, a fluorescence in situ hybridization–based technique used to detect chromosomal regions that are amplified (eg, duplicated) or deleted.8 In parallel with the exclusion of complex mutations in MAPT, other genes located in the shared region underwent a mutation screening through direct sequence analysis, comparing base by base the DNA sequence of protein coding and regu-

latory parts of the genes between pa-

tients and healthy individuals (Figure 2B). After sequencing approximately 100 genes in the region, progranulin (GRN or PGRN [OMIM 138945]; 1.7 million bp centromeric of MAPT) was identified as the gene causing FTLDU-17 through the discovery of PGRN mutations in UBC-17, family 1083, and newly identified Belgian founder family DR8.8,9 PGRN encodes a growth factor involved in multiple processes, including development, wound repair, and inflammation. Although widely expressed in neurons, its function in brain is as yet un- known, but upregulation of PGRN in neurodegenerative diseases such as amyotrophic lateral sclerosis and Creutzfeldt-Jakob disease suggests a role in neuronal survival.

PGRN mutations identified in these 3 families and in an increasing number of other patients with FTLDU resulted in a reduction of PGRN protein (available at http://www.molgen.uu.ac.be/FTDMutations). Most PGRN loss-of-function mutations produced a premature stop codon, which if translated predicted a shorter (or C truncated) protein. However, in each case it was shown that the mutant transcript was degraded by nonsense-mediated decay, a surveillance mecha-

nism of the cell to avoid the produc-

tion of C-truncated proteins; other PGRN mutations caused loss of pro-

tein by preventing splicing and sub-sequent degradation of the un-

spliced transcript in the nucleus by destroying the translation initiation codon and preventing protein translation or by affecting the signal pep-
	
tide and leading to protein misloca-

tion and degradation.10,11 More recently identified in patients with FTLD are a whole gene deletion of PGRN and missense mutations11 that were shown to lead to reduced PGRN production and secretion.12

**RELEVANCE TO THE PRACTICE OF NEUROLOGY**

Although it is poorly understood why a partial loss of PGRN leads to FTLD, the marked reduction of PGRN protein in this neurodegenerative disease points toward a func-

tion of PGRN in neuronal survival. This implies that modulation of PGRN expression in the brain should be the focus of develop-

ment of a targeted therapeutic ap-

proach, which is lacking in FTLD. Unfortunately, this may not be as straightforward as it seems given that overexpression of PGRN is associ-

ated with tumorigenesis. However, continued research into the role of PGRN in neuronal survival will in the long run open new perspec-

tives for a cure for FTLD. Another more immediate clinical implication of the discovery of PGRN muta-

tions in FTLDU-17 lies in the possi-

bility of (presymptomatic) genetic diagnostic testing. PGRN muta-

tions are estimated to be at least as frequent as MAPT mutations.8 The

disease has been reported to manifest with features of other neurode-
genentative diseases such as Alzhei-

mer disease or Parkinson disease.13 So, diagnostic screening of PGRN should be considered in patients who have Alzheimer disease or Parkin-

son disease with a high familial concentra-

tion of dementia. In addition to genetic diagnostic testing, biomarker tests can be developed (eg, measuring the partial loss of PGRN in serum or in cerebrospinal fluid). In practice, no validated cerebrospinal fluid biomarkers for FTLD are available. Existing cerebrospinal fluid tests of total and phosphory-

lated tau produce widely varying re-

sults in studies on FTLD.14 This in part may be explained by the ge-

netic, pathologic, and clinical hetero-

geneity of the FTLD disorders, but even in patients with proven tauo-

pathy or MAPT mutation, cerebro-

spinal fluid tau measurements are inconsistent.

**RELEVANCE TO THE STUDY OF NEUROSCIENCE**

The discovery of PGRN loss-of-

function mutations causing FTLDU-17 marks the beginning of a new era of FTLD research. An obvious question that will be addressed is how a partial loss of this widely expressed protein causes such a specific disease. This insight may prove instrumental in the development of therapy. The age at onset of FTLD in families segregating the same PGRN mutation varies widely, suggesting the existence of modifying factors. Knowledge of these factors will aid in developing drugs that may delay onset of disease. The observation of clinical heterogeneity in families with a PGRN mutation (including clinical and pathologic diagnoses of Alzheimer disease or Parkinson disease15) and the role of PGRN in neuronal survival per se indicate that PGRN might be a genetic susceptibility factor for other neurodegenerative diseases. For example, evidence was found in amyotrophic lateral sclerosis of an asso-

ciation between common PGRN polymorphisms, age at onset, and survival, although PGRN-null mutations were absent.15 The patho-

logic features of FTLDU-17 are also
a hallmark of amyotrophic lateral sclerosis. TAR DNA-binding protein 43 (TDP-43) was shown to be a major constituent of the ubiquitin-reactive neuronal inclusions (Figure 1) in both disorders, suggesting that amyotrophic lateral sclerosis and FTLD share aspects of the same molecular pathogenesis. Elucidating the link between PGRN and TDP-43 in both disorders may have widespread implications far beyond FTLD.

Accepted for Publication: March 29, 2007.

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Financial Disclosure: None reported.

Funding/Support: This study was supported by the Special Research Fund of the University of Antwerp, the Fund for Scientific Research Flanders (FWO-F), the Medical Foundation Queen Elisabeth, the Stichting Alzheimer Onderzoek (Belgium), and a Zenith award of the Alzheimer’s Association (United States) (Dr Van Broeckhoven).

Dr Sleezers holds a postdoctoral fellowship of the FWO-F.

Additional Contributions: The Flanders Institute for Biotechnology Genetic Service Facility and the Biobank of the Institute Born-Bunge provided pathologic specimens. We thank the relatives of families 1083 and DR8 for their participation in the molecular genetic studies.

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