Pathological phenotype in familial neurodegenerative disease: implications for families and therapeutic constructs

Most major subtypes of neurodegenerative disease are comprised of common sporadic, age-related conditions, by definition lacking germline mutation, and rare familial autosomal dominant conditions, referred to by the same name owing to a similar phenotype. Familial cases are traditionally viewed as critically important to the understanding of disease pathogenesis, since singular genes and limited protein transcripts of presumed importance can be isolated and closely studied. For the same reason, animal models are more readily generated, either by overexpression of a germline mutation for example, or genetic manipulation to produce a construct with a number of mutations. Moreover, the underlying central purpose for such experimental constructs is to provide a homolog to human disease that can be studied in terms of pathogenesis, and, more importantly, therapeutic intervention. The fact that they are based on rare Mendelian conditions is never raised in terms of relevance to sporadic disease. In light of therapeutic failures in neurodegenerative diseases across the board, re-examination of the issue of familial disease, its accompanying pathology and its consistency with sporadic disease may be in order, as it may not be valid to design a new therapy based on a familial construct and then apply that therapy to the sporadic disease.

KEYWORDS: Alzheimer's disease ■ familial ■ frontotemporal dementia ■ Lewy body ■ mutation ■ pathology ■ prion ■ sporadic

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Since the majority of animal models for neurodegenerative diseases are based on germline mutations, and yet therapeutic constructs are ultimately aimed at treating the far more common sporadic condition, consistency of the genotype–phenotype relationship (especially pathological phenotype) between familial and sporadic disease is critical. Interestingly, however, this relationship is seldom examined. In this article, we discuss the issue of pathological phenotype and its relationship to germline mutations.

Pathology versus mutation: Alzheimer's disease

In the case of Alzheimer's disease (AD), detailed neuropathological studies of the rare familial cases have highlighted some differences from sporadic AD. Among *APP* mutation cases, unusually large amyloid plaques have been described in the Flemish variant (*A692G*) [1]. The Iowa variant (*D694N*) demonstrated an unusually heavy deposition of amyloid-β (Aβ)40, discrete cerebral atrophy, an abundance of cerebral amyloid angiopathy, as well as leukoencephalopathy and occipital calcifications [2]. Lewy bodies have been described in the *V717I* variant and other familial cases [3]. The Dutch type (*E693Q*) is dominated by cerebral amyloid angiopathy and focal symptoms related to recurrent strokes, although parenchymal pathology and progressive dementia also occur in a subset [4]. Unusual 'ring-like' amyloid plaques have been described in the Arctic variant (*E693G*) [5].

Studies on *PSEN2*-linked AD are few, as are the overall number of kindreds in the population, with only 22 families described to date worldwide [101]. One study demonstrated Aβ40-reactive ghost tangles and ectopic white matter neurons with neurofibrillary tangles in *M239V* mutation cases [6]. On the other hand, a 1998 study showed a relative abundance of cerebral amyloid angiopathy with minimal neuritic pathology or rare neurofibrillary tangles (*N141I*) [7]; an earlier study of the same kindred showed neurofibrillary tangles and granulovacuolar degeneration, abundant cerebral amyloid angiopathy and rarefaction of myelin, but no neuritic plaques [8].

The most striking differences between sporadic AD are generally seen in the *PSEN1* mutation cases. In these kindreds, there is extensive amyloid burden and neurofibrillary degeneration as expected, but also abundant white matter, deep gray matter, cerebellar deposits and an abundance of the peculiar, amorphous 'cotton wool' plaque that lacks Congo red positivity or a well developed amyloid core [9]. These patients

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may or may not present with focal signs including seizures, long tract signs including spastic paraparesis [10], Parkinsonism [11] and frontotemporal symptoms [12]. The pathology variably encompasses α-synuclein-positive Lewy bodies and Lewy neurites as well [13].

In a subset of so-called late onset familial AD linked to the ApoE ε4 allele, abundant cerebral amyloid angiopathy and a perivascular pattern of neurofibrillary degeneration was described [14], whereas other studies have shown little impact of the ApoE genotype on pathology burden [15].

It appears, therefore, that apart from anecdotal findings that have not been replicated, and the subset of *PSEN1* cases showing extensive pathology, abundant cotton wool plaques, early age at onset and unusual clinical signs such as spastic paraparesis or seizures, the pathological features of familial cases overall lack sufficient differences from sporadic AD for the neuropathologist to be able to confidently assign patients to the familial AD category in a prospective analysis. To be sure, the age of onset and family history are important clues to the possibility of germline mutation, and could raise the possibility of familial disease, but the question most commonly posed to the neuropathologist, that is, whether the brain findings by themselves rule in or rule out familial disease, is impossible to answer without genetic testing.

Pathology versus mutation: Lewy body dementia

Lewy body dementia (LBD) is generally regarded as the second most common form of dementia after AD, accounting for up to 30% of dementia cases. Nosology and classification continue to be problematic, as there is considerable pathological heterogeneity within the LBD category, and there is substantial overlap with AD on the one hand and Parkinson's disease on the other, on clinical, pathological and genetic grounds. Some features are worth noting, however, as they relate to the potential for familial disease. First, like other neurodegenerative disorders, genetic tendencies vary from sporadic presentation in some cases to evidence of autosomal dominant transmission in other cases. Indeed, as noted above, patients with germline *APP* and *PSEN* mutations occasionally show Lewy body pathology. Susceptibility based on ApoE polymorphism appears to be more in line with AD than with Parkinson's disease, although the literature is somewhat variable. One study did show that compared with AD, the likelihood of family disease is increased in LBD [16], a point of interest to living unaffected relatives, although the genetic basis for this increased susceptibility is uncharacterized. It might also be noted that considerable intrafamilial phenotypic variability exists, suggesting extra care is needed when discerning the meaning of a given family's neurological history. Interestingly, familial LBD has been described in association with mutations in α-synuclein, presenilin and even the prion protein gene (codon 232), emphasizing both an overlap with Parkinson's disease as well as significant genetic heterogeneity [17].

To summarize the issue of pathology predicting genetics in LBD, pathology once again falls short of being definitive. All things being equal, siblings of subjects with LBD are at increased risk of the disorder compared with AD, but this risk cannot be quantitated precisely, or assigned to a genetic locus. Once again, the family history is probably the best measure of the risk to unaffected relatives.

Pathology versus mutation: frontotemporal dementia

Frontotemporal dementia (FTD) is even more complicated, in that the pathological subtypes are heterogeneous and the heritability within those subtypes varies [18]. Overall, 30–50% of patients with the 'behavioral variant' of FTD have a positive family history, whereas semantic dementia and progressive nonfluent aphasia variants have a much lower frequency of positive family history [19]. The literature varies somewhat with respect to FTD/motor neuron disease and family history, ranging from 10 to 60%. An autosomal dominant pattern of inheritance can be demonstrated in 10–27% of FTD cases overall, and of these, germline mutations in *GRN* or *MAPT* can be demonstrated in approximately half of the cases. Mutations at other loci (e.g., valosin-containing protein, multivesicular body protein 2B, TAR-DNA binding protein and fused in sarcoma [FUS]) occur in less than 5% of cases. A subset of FTD–motor neuron disease cases has been linked to chromosome 9, although a specific genetic mutation at this locus has not been identified to date [20].

In terms of conclusions that may be drawn regarding genetic susceptibility based on a particular pathological presentation in FTD, it could be noted that: abundant dystrophic neurites are associated with semantic dementia, which in turn has a low frequency of germline mutation; numerous TDP-43- and ubiquitin-positive neuronal cytoplasmic inclusions in superficial and deep cortical laminae are seen in FTD–motor neuron disease, which has a variable familial tendency; numerous cytoplasmic

inclusions, dystrophic neurites and neuronal intranuclear inclusions raise the possibility of *GRN* mutation, particularly if there is a positive family history; and numerous intranuclear inclusions, along with few cytoplasmic inclusions and dystrophic neurites, raise the possibility of *VCP* mutation, and again especially if there is a positive family history. Also noteworthy is that FTD cases with ubiqutin-positive, TDP-43-negative inclusions may show immunoreactivity with the FUS antibody, but such cases lack *FUS* gene mutation [18]. FTD-FUS are also generally characterized by young age at onset, behavioral presentation, negative family history as noted and caudate atrophy on MRI. FUS-positive inclusions may be seen in cases where neuronal intermediate filament inclusion disease cases, which generally present with behavioral symptoms, negative family history, and accompanying pyramidal or extrapyramidal movement disorder [18].

Thus, aside perhaps from the presence of numerous intranuclear inclusions, the neuropathology in FTD does not predict with certainty the presence or absence of germline mutations. As with AD, a careful examination of the family history is the best determinant of the potential for familial disease. It should also be noted that the nosology and classification of FTD has changed considerably in recent years. It is therefore likely that additional changes to the classification and assessment will be necessary to accommodate new information, newly characterized genetic loci and new nosological FTD entities.

Pathology versus mutation: prion disease

Familial prion disease is somewhat different, in that familial prion diseases tend to differ from sporadic disease in terms of clinical progression, regional distribution of the pathology and, in some cases, characteristics of the protease resistant isoform of the prion protein by western blot. A number of PRNP mutations have also been identified, whereas the prion protein gene is small and easily sequenced. All things considered, detection of familial disease is relatively straightforward compared with other neurodegenerative conditions.

Familial prion disease comprises 10–15% of all prion disease cases, and exists in several phenotypes, including Creutzfeldt–Jakob disease (CJD), Gerstmann–Straussler–Scheinker syndrome (GSS) and fatal insomnia. The GSS phenotype progresses much slower than CJD and is characterized by large numbers of Congo-red and prion protein-positive amyloid plaques in the cerebral cortex and cerebellum. A notable subtype is the Indiana kindred in which prion protein plaques are accompanied by tau-positive dystrophic neurites, emphasizing similarities in pathogenesis with AD [21]. Regardless, GSS cases are very rare, and typically accompanied by a family history indicating autosomal dominant inheritance, although the recently described 'variably protease sensitive prionopathy' demonstrates pathological, immunohistochemical and protein biochemical features, suggesting that this latter condition may properly be classified as a sporadic form of GSS [22]. Fatal insomnia may either present sporadically or as an autosomal dominant familial condition [23]. In either case, the clinical and pathological phenotype is similar – progressive untreatable insomnia with EEG evidence of disrupted sleep patterns, autonomic overactivity, cognitive decline and death in about 1 year [24]. Pathologically, there is striking neuronal loss and gliosis (without spongiosis) in the medial thalamus, whereas spongiform degeneration is either mild or absent depending on disease duration. Thus, GSS pathology and fatal insomnia pathology are relatively stereotyped, such that the presence of either should raise the strong suspicion of familial disease. A notable exception to this rule is familial CJD, and in particular the more common *E200K* mutation.

In cases of CJD where spongiform degeneration occurs pathologically, approximately 85% represent sporadic cases, whereas most of the rest are due to germline mutations, in which case the pathology would be virtually indistinguishable between the two. Studies examining *E200K* familial CJD to provide further details of the clinical disease, pathological and immunhistochemical phenotype and protein chemistry characteristics are ongoing, and may show potential differences in the pathology of these cases versus sporadic disease [25]. It should also be noted that family history in familial CJD is often either lacking or poorly characterized due to misdiagnosis, such that any case that is otherwise typical CJD on the basis of the histopathology should have familial disease excluded by *PRNP* gene sequencing.

Conclusion

Familial neurodegenerative diseases are best viewed from the standpoint of the germline mutation that caused it. The pathological phenotype is notoriously variable, not only within the

setting of AD, but also within the spectrum of neurodegenerative conditions including LBD, FTD, and prion diseases. Attempts at disease modeling based on the pathological phenotype, as is customary, is problematic in this group of diseases for the same reason. From a practical standpoint, the best indicator of whether a given brain is affected by a familial disease is genetic analysis and then pedigree analysis if a given locus is uncharacterized. With only rare exceptions, pathology is a poor predictor of a germline mutation. By extension, neurodegenerative disease modeling based on known mutations that also endeavors to find a therapeutic construct for sporadic disease risks failure because of the *a priori* erroneous assumption that the familial and the sporadic condition is one and the same.

Future perspective

Alzheimer's disease genetics has given us the amyloid cascade hypothesis and the majority of transgenic models utilized for testing therapeutic strategies, however, copious and careful studies have now shown proof of concept. Aβ has been removed from the brains of patients with AD, and yet the disease continues unabated. Thus, the field is at a crossroad: will we follow the same paradigm and simply move the human therapeutic target closer to clinically unaffected individuals, and rely on so-called biomarkers to predict or identify success or failure? Or will we open our minds to alternative theories? To be sure, the former effort is already ongoing. It therefore seems unlikely that anything new or therapeutically meaningful will be produced from the amyloid cascade concept in the next

Executive summary

Background

- Whilst models and lesion-based therapies are based on familial disease, sporadic disease is targeted in clinical trials.
- The question of differences in sporadic and familial disease is seldom examined.

Pathology versus mutation: Alzheimer's disease

- *APP* mutation cases:
  - Unusually large plaques in *A692G*
  - A variety of unusual pathologies in *D694N*
  - Dominant vascular pathology and focal symptoms in *E693Q*
  - Lewy bodies reported in some kindreds
- *PSEN1* mutation cases:
  - Prodigious pathology and cotton wool plaques in many kindreds
  - Clinically heterogeneous including spastic paraparesis in some cases
- *PSEN2* mutation cases:
  - Too few cases for firm conclusions
  - Somewhat unusual findings noted in *M239V* and *N141I*
- ApoE:
  - ApoE genotype has little impact on pathological expression

Pathology versus mutation: Lewy body dementia

- Somewhat greater tendency for Lewy body dementia to 'run in families'.
- Kindreds with mutations in a variety of diverse proteins may be expressed as Lewy body dementia.
- Intrafamilial heterogeneity.

Pathology versus mutation: frontotemporal dementia

- Nosologically complex.
- 'Behavioral variant' with higher familial tendency compared with semantic dementia and progressive nonfluent aphasia.
- Reported frequency of family history varies from 10 to 60% in frontotemporal dementia cases with motor neuron disease.
- *GRN* and *MAPT* mutations demonstrated in approximately half of kindreds with autosomal dominant pattern.
- Numerous cytoplasmic inclusions, dystrophic neurites and neuronal intranuclear inclusions raise the possibility of *GRN* mutation.

Pathology versus mutation: prion disease

- More straightforward compared with other forms of dementia.
- Gene sequencing and excluding of familial disease is relatively easy.
- Germline mutation should not be excluded, even if there is no family history (codon 200 mutation Creutzfeldt–Jakob disease can present in a manner similar to sporadic Creutzfeldt–Jakob disease).
- Fatal insomnia can be familial or sporadic.
- Recently described protease-sensitive prionopathy may be a sporadic form of Gerstmann–Straussler–Schenker syndrome.

Conclusion

- Familial versus sporadic disease in neurodegeneration is complex.
- Designing a treatment based on familial disease, and applying that treatment to the putative sporadic condition may not be valid, as results to date tend to indicate.

5–10 years, given, at the very least, the length of time necessary to show cognitive benefit. The real question is whether anything new or useful will result from the study of alternative theories such as oxidative stress, inflammation or energy metabolism. While similarly unlikely to yield clinically meaningful data, a breakthrough in treatment in the near term is difficult to completely exclude, even if by accident, if the approach is broad based.

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Website

101 Alzheimer Disease and Frontotemporal Dementia Mutation Database www.molgen.ua.ac.be/admutations