In contrast to the common and genetically complex senile form of Alzheimer’s disease (AD), the molecular genetic dissection of inherited presenile dementias has given important mechanistic insights into the pathogenesis of degenerative brain disease. Here, we focus on recent genotype–phenotype correlative studies in presenile AD and the frontotemporal dementia (FTD) complex of disorders. Together, these studies suggest that AD and FTD are linked in a genetic spectrum of presenile degenerative brain disorders in which tau appears to be the central player.

Introduction

Through the genetic dissection of Alzheimer’s disease (AD), we hope to increase our mechanistic understanding of this prevalent and untreated disorder in which intra-and extraneuronal protein aggregates (known as tau tangles and amyloid plaques, respectively) accumulate in the degenerating brain. In particular, genetic studies of the rare and genetically simple early onset or presenile (onset before 65 years of age) forms of AD have led to the identification of several single gene lesions in the amyloid precursor protein (APP) [1] and the presenilins (PS) [2,3]. In addition, mutations in the gene encoding the microtubule associated protein tau (MAPT) cause autosomal dominant forms of frontotemporal dementia (FTD) [4,5], a degenerative brain disease that has overlapping features with AD (Box 1). Collectively, the discovery of these mutations has been highly instructive in delineating our current mechanistic understanding of AD and FTD. For detailed and up-to-date information on APP, PS and MAPT mutations, please see our interactive AD and FTD mutation database (http://www.molgen.ua.ac.be/ADMutations).

By contrast, similar to most frequent diseases, the genetic architecture of the common late-onset or senile form of AD (age of onset above 65 years; ~90% of all AD patients) is complex. So far the ε4 allele of the gene encoding apolipoprotein E (APOE) is the only well-established genetic risk-factor for late-onset AD, but the underlying mechanism remains poorly defined [6–8]. In addition, several studies have investigated numerous candidate loci and genes, but none of these has reached the established position of APOE. The current status of potential candidate risk genes and loci has been extensively reviewed recently [9]; for an update, see the Alzheimer’s Research Forum genetic database, ‘Alzgene’ (http://www.alzgene.org).

In this review, we emphasize that careful genotype–proteotype–phenotype correlative studies, including molecular genetic, biochemical, neuropathological and clinical investigations of inherited presenile forms of AD and FTD, are instrumental in defining the complete phenotypic spectrum associated with mutations in APP, PS and MAPT and will significantly advance our biological understanding of these diseases. More specifically, we review recent exciting evidence that AD-causing PS mutations have intrinsic loss-of-function properties and that PS loss-of-function has a role in FTD and amyloid-independent...
Box 1. Overlap between presenile AD and FTD

FTD and AD are primary degenerative dementias, meaning that a gradual loss of neurons is responsible for the progressive brain dysfunction. Within the group of presenile dementias (onset <65 years of age), FTD is the second most common form of neurodegenerative dementia after AD. Clinically, AD is primarily a disease of memory and cognition caused by a more generalized brain atrophy, starting in the medial temporal lobe. The hallmarks of FTD are behavior and/or language dysfunction caused by a more focal degeneration mainly affecting the frontal and temporal brain regions. Nevertheless and despite the existence of useful clinical diagnostic criteria for both disorders, the distinction between these mutations at the $\gamma$-secretase-cleavage site of APP, results in an extremely aggressive AD phenotype with an onset age of ~35 years and is characterized by tau tangles and extensive deposition of nonfibrillar ‘cotton wool’ amyloid plaques [17].

Overall, genotype–phenotype correlation studies of APP mutations strongly implicate various forms of Aβ phenotype.

Interestingly, the Austrian APP T714I mutation, which is located at the $\gamma$-secretase-cleavage site of APP, results in an extremely aggressive AD phenotype with an onset age of ~35 years and is characterized by tau tangles and extensive deposition of nonfibrillar ‘cotton wool’ amyloid plaques [17].

Together, genotype–phenotype correlation studies of APP mutations strongly implicate various forms of Aβ.

Figure 1. APP mutations. Schematic representation of the protein sequence encoded by exons 16 and 17 of APP. Each circle represents an amino acid; those with pathogenic missense mutations are shown in red and those with nonpathogenic missense mutations are in green. Pathogenic mutations are always at or close to sites that are cleaved by the $\alpha$, $\beta$, or $\gamma$-secretases. The figure was adapted with permission from the Alzheimer Research Forum (http://www.alzforum.org) and can also be found at our AD and FTD mutation database available at http://www.molgen.ua.ac.be/ADMutations.
deposition, ranging from vascular CAA and fibrillar core-containing plaques to non-fibrillar ‘cotton wool’ plaques, as an essential characteristic of AD (Figure 2). Interestingly, our own morphological studies of APP mutations in human and mouse have strongly implicated the vascular system in the formation of core-containing amyloid plaques suggesting that vascular damage might be an important contributing factor to AD pathogenesis [15,18]. Importantly and with the possible exception of the Dutch APP mutation, tau deposits in the form of tangles are a consistent but downstream consequence throughout the APP spectrum of disorders.

MAPT disorders: the ‘tauocentric’ view

In 1998, the first mutations in MAPT causing autosomal dominant FTD were identified [4,5] and 40 different causative MAPT mutations have now been reported (Figure 3 and FTD Mutation Database: http://www.molgen.ua.ac.be/FTDMutations) [19]. Interestingly, nearly all mutations are located in the C-terminus of the protein and include missense, silent and intronic variations in addition to two single codon deletions clustered in or near the microtubule-binding domains. Importantly and in sharp contrast to APP phenotypes, MAPT disorders are neuropathologically characterized by absence of Aβ deposits but share with AD the invariable presence of different forms of tau aggregates and are therefore called pure tauopathies [20] (Figure 2). Clinically, MAPT mutations most typically present with FTD. However, the spectrum of MAPT disease is surprisingly wide and ranges from phenotypes in which FTD is accompanied by severe parkinsonism and motor neuron disease to degenerative disorders that are, as in the case of the MAPT R406W mutation, clinically hardly distinguishable from AD [21,22].

More recently, several autosomal dominant FTD families have been described that lack visible tau positive lesions but are still conclusively linked to a chromosomal region that contains MAPT [23–25]. In these families, the neuropathological phenotype has been described as either ‘dementia lacking distinctive histopathology’ [23] or ‘FTD with tau-negative and ubiquitin-positive inclusions’ [24,25], although it is currently unclear if these represent distinct disease entities or are pathological manifestations of the same primary defect. Strikingly however and consistent with the absence of tau-positive lesions, no causative MAPT mutations have been found in these families, despite extensive sequencing of the whole genomic MAPT locus [26]. Although this might be explained by a defect in another gene in close proximity to MAPT [24], others have suggested that this FTD subtype is a ‘no tau tauopathy’ caused by a primary tau

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Figure 2. The genetic AD–FTD spectrum of degenerative brain disease. This figure integrates the genotype–proteotype–phenotype correlations and suggests the existence of a genetically interconnected spectrum of AD and FTD disorders. The FTD complex of disorders includes PSP and CBD, sporadic disorders with prominent parkinsonism neuropathologically characterized by tau deposits. Throughout the spectrum the involvement of tau either histopathologically or genetically appears to be a constant characteristic. Images in the histological phenotype panel represent prototypical examples of neuropathological dementia subtypes. CAA: Aβ-positive blood vessels in the temporal region of a patient with the Dutch APP E693Q mutation; CAA and AD: Aβ-positive blood vessels and dense core plaques in the temporal region of a patient with the Flemish APP A892G mutation; AD: Aβ-positive dense core plaques in the temporal region of a patient with the PS1 G183V mutation; Tau positive FTD: cytoplasmic and intranuclear ubiquitin (Ubi) positive inclusions in the temporal region of a patient of a family linked to the MAPT locus but without MAPT mutations. Scale bars represent 200 μm except in Tau negative FTD, where the scale bar corresponds to 50 μm. In the proteotype panel: +++ indicates highly abundant; ++ indicates abundant; + indicates present but inconsistently; ± indicates sometimes present and – means absent. In the genotype panel: arrows correspond to clearly established genotype-phenotype links, and dashed arrows correspond to probable links that need confirmation.
visible pathologic tau aggregates. Because FTD without
tau-mediated neurodegeneration can be dissociated from
[32] and mouse models [33] have shown that
Drosophila
the possible genetic involvement of
Moreover, absence of visible tauopathy does not exclude
worthwhile mentioning that we and others have recently
shown that
Figure 3. MAPT mutations (a) Schematic representation of MAPT exons nine to 13 encoding the four microtubule-binding domains and inter-repeat regions of the tau protein. Each circle represents an amino acid; those with pathogenic missense mutations are shown in red and those with non-pathogenic missense mutations are in green. (b) The 3' end of exon ten and 5' end of intron ten of MAPT, showing the pathogenic mutations in this region in red and how they destabilize the predicted stem-loop structure; thereby affecting the splicing out of exon ten, resulting in an altered ratio of three- to four microtubule-binding repeats and hence affects the microtubule-binding properties of the tau protein. This figure was reproduced with permission from the Alzheimer Research Forum (http://www.alzforum.org/) and can be found on our AD and FTD mutation database: http://www.molgen.ua.ac.be/ADMutations.

defect that leads to loss of brain tau but not tau mRNA
[27,28]. Nevertheless, this finding was not replicated and
remains controversial [29]. In this context however, it is
worthwhile mentioning that we and others have recently
shown that MAPT along with several other genes are
within a genomic 900-kb region flanked by inverted low-
copy repeats (LCRs) that through non-allelic homologous
recombination during primate evolution have induced a
 genomic inversion polymorphism called H1 and H2
[30,26]. These observations suggest that these LCRs
render the MAPT region susceptible to genomic rearrangements and that an as yet unidentified genomic mutation might be the cause of FTD in these families [31]. Moreover, absence of visible tauopathy does not exclude the possible genetic involvement of MAPT – studies in
Drosophila [32] and mouse models [33] have shown that
tau-mediated neurodegeneration can be dissociated from
visible pathologic tau aggregates. Because FTD without
detectable tau pathology is a frequently observed neuro-
pathological FTD subtype [34,35], the identification of the
underlying gene defect in MAPT or a neighboring gene is
of great importance and will significantly contribute to our
understanding of the neurodegenerative process in this
type of FTD.

Although a causative role for MAPT in the degenerative
process in these FTD families appears possible but
remains unproven, a primary genetic role of MAPT as a
susceptibility gene in sporadic pure tauopathies called
progressive supranuclear palsy (PSP) and corticobasal
degeneration (CBD) is likely. PSP and CBD are sporadic
disorders with prominent parkinsonism neuropathologi-
cally characterized by tau deposits and are part of the FTD
complex of disorders [36]. Interestingly, homozygosity of
MAPT polymorphisms that segregate on the extended H1
haplotype are consistently overrepresented in patients
with PSP [37,38] and CBD [39]. Although the genetic
mechanism explaining this well-replicated association remains unresolved, in vitro studies have suggested that the MAPT H1 haplotype might be more efficient at driving MAPT gene expression than the H2 haplotype [40]. To further understand the genetic mechanism explaining the association, we recently generated a high-density single nucleotide polymorphism (SNP) map by sequencing 138 kb of the genomic region of MAPT (R. Rademakers et al., personal communication) and identified a 22-kb PSP-risk-containing regulatory region in the large intron preceding the first coding exon of MAPT, which was fully explained by one SNP, htSNP167, creating a transcription factor CP2 (TFCP2) binding site. Recently, Pittman et al. [41] published SNP haplotype association data from a PSP patient–control sample that overlapped with the extended American sample we used in our study and identified a 56.3-kb risk-increasing interval that also contained htSNP167.

Together these data strongly indicate that MAPT, through a toxic gain-of-function mechanism, is capable of inducing neuronal death leading to a wide range of degenerative phenotypes that can be grouped under the FTD or Pick complex of degenerative brain disorders [36] (Figure 2). A toxic gain-of-function is also supported by several animal model studies in which overexpression of mutant and wild-type tau causes neurodegeneration [42,43,32].

In between the extremes: PS disorders?

Although the phenotypes induced by APP and MAPT lesions are strongly supportive for an ‘amyloidocentric’ and ‘tauocentric’ view of neurodegeneration, respectively, recent genetic evidence suggests that these pathways might converge at the level of PS (Figure 2).

First, strong evidence exists for a direct etiological link between PS and APP processing. Since the identification of mutations in PS [2,3], the most common cause of inherited presenile AD, 154 different PS mutations (144 in PS1 and ten in PS2), mostly of the missense type, have been identified (Figure 4 and AD Mutation Database: http://www.molgen.ua.ac.be/ADMutations). Similar to mutations at the β- or γ-secretase-cleavage site, PS mutations generally result in typical AD phenotypes with amyloid plaques and tau tangles. In addition, AD-causing PS mutations increase the in vitro ratio of Aβ42 to Aβ40? [44], and it is now well established that PS is a core component if not the catalytic subunit of the multimeric γ-secretase [45,46]. In general, patients with PS1

Figure 4. PS mutations. Schematic representation of PS1, the most frequently mutated gene in presenile AD. Each circle represents an amino acid; those with pathogenic missense mutations are shown in red and those with non-pathogenic missense mutations are in green. The predicted transmembrane regions of the protein (TM) are also shown. This figure was adapted with permission from the Alzheimer Research Forum (http://www.alzforum.org/). PS2 mutations are not shown but can also be found at our online AD and FTD mutation database available at http://www.molgen.ua.ac.be/ADMutations.
Do **APP** and **PS** mutations have gain- or loss-of-function properties?

An important but still unresolved issue, with respect to clinical **APP** and **PS** mutations, is whether they represent gain- or loss-of-function alleles. The answer to this question is important not only from a mechanistic point of view, but also with respect to the development of therapeutics targeting the γ-secretase complex. Indeed, if **APP** and **PS** mutations reduce γ-secretase cleavage of APP, the proposed use of γ-secretase inhibitors as therapeutic agents in AD might lead to an unwanted enhancement of the neuronal degeneration.

Because of their dominant mode of inheritance and ability to increase the ratio of Aβ42 to Aβ40 *in vitro* [44], **APP** and **PS** mutations are generally considered toxic gain-of-function alleles in the context of AD pathogenesis. However, when taking, for example, Notch signaling as a functional readout of PS or γ-secretase function, several studies suggest that AD-causing **PS** mutations are intrinsically at least partial loss-of-function mutations. Indeed, loss of Notch cleavage or signaling has been demonstrated in mammalian cell lines [60,61] and in *Caenorhabditis elegans* [62,63]. Further support for this idea comes from a strong loss-of-function mutation in the *C. elegans PS* homologue sel-12 (C60S), isolated in a forward genetic screen [64], which corresponds to the human PS1 C92S mutation and is known to cause AD [65] by increasing the ratio of Aβ42 to Aβ40 [66,67]. Nevertheless, the situation is not completely clear because, although all **PS** mutations and the partial reduction of normal PS1 activity [68] increase the ratio of Aβ42 to Aβ40, the total loss of PS results in loss of both Aβ40 and Aβ42 [45]. In addition, it is not well established if the increased Aβ42:Aβ40 ratio induced by the PS mutation is caused by a decrease in Aβ40, an increase in Aβ42 or a combination of both.

Interestingly, recent studies show that with respect to the generation of Aβ peptides from **APP**, AD-causing **PS** mutations have reliable loss-of-function properties. In a recently developed, highly reproducible cellular assay, we observed that all nine tested **PS** mutations consistently decreased Aβ40 and accumulated direct γ-secretase substrates in the form of **APP** C-terminal fragments, a sign of decreased PS activity (S. Kumar-Singh et al., unpublished). Although the Aβ42:Aβ40 ratio was significantly increased for all, in only four **PS** mutations a significant increase in Aβ42 was noted. A recent report on **PS2** mutations has also shown Aβ40 loss [69] and similar results were obtained using PS-deficient cells (B. De Strooper et al., unpublished). The interesting conclusion from these studies is that AD-related **PS** mutations are less efficient in cleaving several γ-secretase substrates including **APP** and therefore behave as intrinsic biological partial loss-of-function alleles regarding γ-secretase function. Consistent with decreased γ-secretase activity for clinical AD mutations, a recent study has shown that both **PS** and **APP** mutations located in the vicinity of the γ-secretase-cleavage site reduce γ-secretase-mediated liberation of the **APP** C-terminal fragment [70].

Together these results suggest that the consistently increased Aβ42:Aβ40 ratios induced by AD-causing **APP** and **PS** mutations are the consequence of reduced γ-secretase activity. However, the exact mechanism is not understood.

**Is there a role for loss of PS in amyloid-independent neurodegeneration?**

With respect to this gain- versus loss-of-function discussion, interesting results have come from several recent studies. Conditional knockout mice lacking both **PS** in the post-natal forebrain showed progressive synaptic impairments and, importantly, severe age-dependent neurodegeneration characterized by cytoplasmic accumulations of hyperphosphorylated tau [71,72] but no Aβ deposits [73]. Because these results show that complete loss of **PS** can lead to an amyloid-independent form of tau-positive
neurodegeneration, recent findings of PS mutations in FTD become highly intriguing. It was suggested that the PS1 G138V mutation, which is associated with FTD and with tauopathy in the form of Pick's disease but not with Aβ plaques, might also have loss-of-function properties because it affects the splice signal at the junction of the sixth exon and intron [57]. Interestingly, follow-up studies indeed revealed that this mutation, in addition to producing full-length PS1 G138V protein, also generates alternative transcripts that either lack exon six or exons six and seven leading to truncated proteins. In addition, cellular γ-secretase assays show that the truncated proteins behave as complete null alleles also suggesting a loss-of-function mechanism (Dermaut et al., unpublished). Strikingly, a loss-of-function mechanism has also been proposed for the PS1 insArg352 mutation that strongly inhibits γ-secretase cleavage of both Notch and APP [74] and is associated with FTD [56] without amyloid pathology (B. Boeve, personal communication).

Although additional confirmation is needed to establish a role of PS in amyloid-independent FTD, these studies suggest that throughout the PS spectrum of disorders, ranging from AD with a strong amyloid component to possibly tau-positive AD [57], partial PS loss-of-function might be the common theme, an idea that is also supported by studies suggesting that AD-risk-increasing alleles in the regulatory region of PS1 significantly decrease PS expression levels [75,76]. In addition, several recent cell biological studies have suggested that PS mutations through reduced PI3K-Akt signaling promote glycogen synthase kinase 3β (GSK3β) activity and hence tau hyperphosphorylation [77–79], further suggesting a direct mechanistic link between PS loss-of-function and tau pathology.

Another line of recent evidence has linked dysfunction of the endosomal-lysosomal degradative system to loss of PS function [80,81]. This is interesting, because neurodegeneration is a frequent observation in lysosomal disorders [82] and abnormalities in the endosomal-lysosomal system have long been thought to be an early and prominent feature in AD [83]. In addition, tauopathy in the form of tau tangles is a highly consistent feature in the lysosomal disorder Niemann-Pick Type C [84] that, in its adult onset form, can present with FTD-like dementia. Strikingly, the recent finding that mutations in the charged multivesicular body protein 2B (CHMBP2B) on chromosome 3 cause FTD [85] further implicates dysfunctional late-endosomal or lysosomal activity in neuronal degeneration.

Is tau the central molecule in the AD–FTD spectrum?
When placed in the context of previous genotype–phenotype correlation studies, recent findings show that FTD and AD not only share important clinical and neuropathological features but are also etiologically linked at the molecular genetic level, implying that these disorders are part of a genetically interconnected spectrum of presenile degenerative brain disorders. In addition, recent studies showing that: (i) genetic alterations at the level of MAPT are strongly associated with different types of tau-mediated neurodegeneration; (ii) AD-causing PS mutations are intrinsically loss-of-function alleles; and (iii) PS loss-of-function can lead to tau pathology, lead us to propose that tau is the major player throughout this AD–FTD spectrum (Figure 5). Within this framework, the further etiologic and mechanistic establishment of PS loss-of-function in amyloid-independent and tau-mediated neurodegeneration as well as the identification of the molecular defect leading to tau-negative FTD (caused by MAPT or another gene nearby) are the most exciting and important future research topics.

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