**γ-secretases: from cell biology to therapeutic strategies**

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Presenilins form the catalytic part of the γ-secretases, protein complexes that are responsible for the intramembranous cleavage of transmembrane proteins. The presenilins are involved in several biological functions, but are best known for their role in the generation of the β-amyloid (Aβ) peptide in Alzheimer’s disease and are therefore thought to be important drug targets for this disorder. Mutations in the presenilin genes cause early-onset familial Alzheimer’s disease, but mutation carriers have substantial phenotypic heterogeneity. Recent evidence implicating presenilin mutations in non-Alzheimer’s dementia, including frontotemporal dementia and Lewy body dementia, warrants further investigation. An increased understanding of the diversity of the molecular cell biology of the γ-secretase complex and the effects of clinical mutations in the presenilin genes might help pave the way for improved development of drugs that are designed to target γ-secretase enzymatic activity in Alzheimer’s disease and potentially in other neurological diseases.

**Introduction**

Presenilins first caught the attention of the scientific world with the identification of pathogenic mutations in the encoding gene PSEN1 in several families with early-onset Alzheimer’s disease. Soon after, mutations in a similar gene, PSEN2 (presenilin 2), were found. More than 170 mutations have since been identified in PSEN1, whereas mutations in PSEN2 are much rarer. Presenilin mutations are responsible for an important fraction of autosomal inherited Alzheimer’s disease, although estimates vary from 9% to 62%. On the basis of the latter percentage, presenilin mutations combined with mutations in the APP (amyloid precursor protein) gene could account for up to 82% of mutations that cause early-onset genetic Alzheimer’s disease. Since 1995, no new genes have been identified and it therefore seems reasonable to suggest that all major monogenic loci have been found and that other families with inherited early-onset Alzheimer’s disease most probably have a more complex, oligogenetic aetiology. However, as only about 2% of patients with Alzheimer’s disease have early onset, the total contribution of APP and the presenilin genes to the risk of Alzheimer’s disease in the general population remains low. Despite years of association studies and meta-analyses, until very recently the only firmly established risk factor for sporadic Alzheimer’s disease was variants in APOE, the gene that encodes apolipoprotein E, with two copies of the APOE ε4 allele leading to an eightfold increase in the risk for Alzheimer’s disease. However, three more genes that confer risk for sporadic Alzheimer’s disease have recently been uncovered in genome-wide association studies—CR1 (complement component receptor 1), CLU (clusterin), and PICALM (phosphatidylinositol binding clathrin assembly protein)—which all might directly or indirectly affect the β-amyloid (Aβ) pathway.

The importance of the identification of the presenilin mutations for research into Alzheimer’s disease should, however, not be underestimated. Their discovery has enhanced understanding of a crucial enzymatic process in the generation of the Aβ peptide, which is thought to be a key factor in the pathogenesis of the disease. Moreover, together with the other proteases involved in APP processing, the presenilins have become one of the few advanced drug targets in Alzheimer’s disease. In this Review, we update our current knowledge about presenilins in Alzheimer’s disease and critically evaluate evidence that implicates presenilins in other neurodegenerative diseases. We also discuss the potential of presenilins and γ-secretases as drug targets for the treatment of Alzheimer’s disease.

**Basic cell biology of presenilin**

The two presenilins are highly homologous (about 80%) nine-transmembrane domain proteins (figure 1). Presenilins provide the catalytic core to the multimeric γ-secretase enzymatic complexes, which also contain nicastrin, presenilin enhancer 2 (Pen-2), and anterior pharynx defective 1 (Aph1). γ-secretases cleave various type I transmembrane proteins, including the adhesion molecules N-cadherin and E-cadherin, the neurotrophin receptor p75, the regulatory β2 subunit of voltage-gated sodium channels, the axon guidance molecule DCC, and neuregulin (for a review see elsewhere). Neuregulin has an important role in myelin formation, and single nucleotide polymorphisms in the gene have been associated with an increased risk of neurodevelopmental disorders. A deficiency in a γ-secretase subtype (see below) in mice causes disturbed neuregulin processing in the brain, possibly causing prepulse inhibition, increased dopaminergic tonus, and behavioural symptoms relevant to neurodevelopmental disorders such as schizophrenia. The two substrates with the most far-reaching pathophysiological consequences are, however, APP and the Notch receptor, which we discuss in more detail below.

**APP processing**

Aβ peptides are generated through the processing of APP by β-secretase and γ-secretase in a stepwise fashion (figure 1). After the β-secretase enzyme cleaves the APP extracellular domain, γ-secretase cleaves the remaining segment to release the Aβ peptide. These Aβ peptides can aggregate into small neurotoxic oligomeric structures and eventually form the typical senile plaques seen in Alzheimer’s disease. γ-secretase cleaves APP at different positions, possibly in a consecutive way, resulting in the release of several Aβ peptides that contain between 37 and...
Presenilin mutations generally increase the relative amounts of $\beta_6$ to $\beta_8$, and this ratio is widely regarded as an important indicator of pathogenic mutations. The extent to which other long $\alpha$-peptides ($\beta_7$ to $\beta_8$) are secreted or degraded intracellularly and whether they also have pathological importance remain unclear. Shorter peptides ($\beta_7$ and shorter) might be less problematic, but this remains unproven. Novel drugs that are under investigation target $\gamma$-secretases with the aim of modulating, rather than inhibiting, their activity, so that more of the shorter $\alpha$-peptides are generated (see below).

**Notch processing**

After binding of its ligands, such as delta and serrate, Notch is first cleaved by a metalloproteinase, ADAM10 (a disintegrin and metallopeptidase); but see elsewhere for a contrasting view), in its extracellular domain, which triggers cleavage by $\gamma$-secretase in the transmembrane domain.$^{1,2}$ The Notch intracellular domain is then released and translocates to the nucleus to regulate the transcription of a set of target genes involved in development and differentiation. This makes Notch important for the differentiation of many cell types, not only during embryonic development, but also in adult life.$^{3,4}$ Interference with the Notch pathway therefore leads to many disturbances in embryogenesis, including disrupted somitogenesis and blood vessel formation.$^{5,6}$ The Notch pathway also regulates several oncogenes and oncogenic signalling pathways such as those that involve Myc and Akt.$^{7}$ These pathways are of relevance for cancer, which, as we briefly discuss, is why $\gamma$-secretase inhibitors are also considered for the treatment of some human cancers. Both genetic and pharmacological experiments in mouse, fish, and fly models indicate, however, that a major concern for $\gamma$-secretase inhibitors is the side-effects that result from interference with Notch cleavage and signalling.$^{8,9}$ In the context of Alzheimer’s disease, the inhibition of Notch signalling by $\gamma$-secretase inhibitors has to be considered as an intrinsic, mechanism-based cause of serious side-effects, including gastrointestinal bleedings, immune disturbances, and hair loss.$^{10,11}$

**Presenilins and intracellular calcium**

Recent studies have indicated that the function of presenilins extends beyond their role in $\gamma$-secretase cleavage. These proteins constitute calcium leak channels in the endoplasmic reticulum when not incorporated into the $\gamma$-secretase complexes.$^{12}$ Mutations seen in Alzheimer’s disease disturb this leak function and possibly also affect the regulation of ryanodine receptors and inositol 1,4,5-trisphosphate receptors,$^{13,14}$ leading to increased accumulation of calcium ions ($Ca^{2+}$) in the endoplasmic reticulum.$^{15}$ A conditional knockout model of the presenilins in the hippocampus showed disturbances in neurons in the CA3 region, but this finding was explained by an effect on ryanodine receptor-mediated release of $Ca^{2+}$ from the endoplasmic reticulum, which modulated glutamatergic neurotransmitter release and disturbed long-term potentiation via a presynaptic mechanism.$^{16}$ A conditional knockout mouse model of both presenilins in the forebrain showed marked neurodegeneration with increasing age.$^{17}$ It is unclear whether the neurodegeneration in this model is linked to the early presynaptic deficits and whether impaired $\gamma$-secretase (proteolytic) function or disturbed $Ca^{2+}$ homeostasis underlie this pathology. Therefore, the extent to which these calcium effects are relevant to the pathogenesis of Alzheimer’s disease in human beings remains to be determined.

**Loss-of-function or gain-of-function of presenilin in Alzheimer’s disease**

The question of whether the presenilin clinical mutations confer gain-of-function or loss-of-function effects has
been much debated in recent years. It is becoming increasingly clear that, from a genetic point of view, mutations act in a “dominant” fashion, whereas from a biochemical point of view, most have loss-of-function effects, both in enzymatic assays or when measuring Ca²⁺ leakage channel activity. Many clinical mutations clearly have loss-of-function effects on some substrates such as syndecan, N-cadherin, and Notch. The effects on APP processing are, however, more varied. Most mutations tested lead to a decrease in total Aβ production (biochemical loss-of-function), whereas a few mutations give rise to an increase in Aβ₄₂,⁶⁶–⁶⁹ The net result is that, even if the total amount of Aβ peptides decreases, there is an increase in the Aβ₄₂: Aβ₄₀ ratio, which might affect the formation of toxic Aβ oligomers.

An important conclusion, therefore, is that the absolute amount of Aβ peptides generated seems to be less crucial than the particular type of Aβ peptide, at least in familial Alzheimer’s disease linked to presenilin mutations. The recent insight that amyloid plaques or single Aβ peptides are not extremely toxic, but that an ill-defined oligomeric state of Aβ peptides affects synapses and neurons, might provide an explanation for this apparent paradox.⁷⁰ Although not proven, the ratio of longer or more hydrophobic Aβ peptides (Aβ₄₂, but also longer or truncated species) to Aβ₄₀ and possibly other shorter species might determine a complex equilibrium between monomers, oligomers, and fibrils. If true, the quality rather than the quantity of the Aβ peptides could drive oligomer conformation and disease. The situation is not straightforward, because increasing the amount of Aβ peptides without a change in ratio can also cause Alzheimer’s disease, as seen with the APP mutation in a Swedish family. The question is thus whether we need a quantitative or a qualitative decrease in Aβ to prevent or treat Alzheimer’s disease. Both approaches—blocking and modulating γ-secretase activity—are currently being considered, as discussed below. In this regard, the finding that a global decrease in total Aβ (rather than a specific reduction in Aβ₄₀) diminishes amyloid plaque load and memory deficits in a mouse model suggests that a total Aβ decrease might still be a valuable therapeutic option. However, the available transgenic Alzheimer’s disease mouse models come with their own limitations: the pathology in these models is generally driven by high Aβ production, which is not necessarily the situation for all cases of human Alzheimer’s disease.

The heterogeneity of the clinical phenotype caused by presenilin mutations

Mutations in the presenilin genes cause autosomal dominant early-onset Alzheimer’s disease but in recent years the presenilins have also been implicated in other clinical disorders such as spastic paraparesis, frontotemporal dementia, and Lewy body dementia. Although the link to Alzheimer’s disease is undisputable, mutation carriers have a high phenotypic heterogeneity, most notably in the age at onset. This feature remains insufficiently investigated and could provide clues to a better understanding of genetic and environmental modulators of the disease.

Age at onset

The first symptoms associated with Alzheimer’s disease in patients with PSEN1 mutations are generally observed in the fifth decade of life, although can be seen in the third decade in some families. Alzheimer’s disease related to PSEN2 mutations has a later and more variable age of onset, between the ages of 45 and 88 years (on average in the sixth decade); patients with Alzheimer’s disease related to these mutations live longer and have a longer disease duration than do patients with PSEN1 mutations. The reason why PSEN2 mutations manifest at an older age than do PSEN1 mutations is unclear, and further work on the differential tissue distribution or the reported differences in molecular properties of PSEN2 or γ-secretase might yield an explanation.

In contrast to sporadic Alzheimer’s disease, no major modifiers of age at onset have been identified in patients with early-onset familial disease. The only modifier suggested in families with early-onset disease is the HLA-2 genotype, but the precise mechanism is unclear.

Spastic paraparesis

After the original suggestion of an association between early-onset Alzheimer’s disease and spastic paraparesis and the confirmation in a large Finnish family, more PSEN1 mutations have been linked to this variant form of Alzheimer’s disease in some individuals ( these mutations are reviewed in the table). Autopsy reports of this variant of Alzheimer’s disease note corticospinal tract degeneration at the level of the medulla and the spinal cord. Hypometabolism on fluorodeoxyglucose-PET in the motor cortex, a sign of malfunction, has also been reported. There is widespread presence of “cotton wool” plaques in the hippocampus and the primary and association cortices in these patients. These plaques do not have the congophilic staining typical of the “canonical” Alzheimer’s disease plaques, and these plaques do not retain immunoreactivity for the Aβ peptide (figure 2). It is unclear whether these cotton wool plaques have a particular effect on the neurons. The canonical-core plaques with tau-positive dystrophic neurites also occur in these patients.

Spastic paraparesis occurs in only a few of the family members studied, while other family members present with a typical picture of Alzheimer’s disease dementia. This indicates the occurrence of gene modifiers that increase the sensitivity of these particular patients to damage to neurological systems outside the cognitive system. In one study, mutations in known spastic paraplegia genes (spastin [SPAST], paraplegin [SPG], and atlastin [ATL], which cause more than 50% of cases of
hereditary spastic paraplegia) and other Alzheimer’s disease genes were excluded as gene modifiers of the disease phenotype, indicating that further work is needed to explain this particular variant of Alzheimer’s disease.105

Apart from their effect on Aβ generation, there are several hypotheses to explain why presenilin mutations could interfere with neuronal function. Their involvement in Ca²⁺ homeostasis at several levels and their effects on synaptic transmission might have a role. Data from a recent study suggest that presenilin could be involved in motor neuron fast axonal trafficking. In mice that expressed a mutant form of presenilin with exon 9 deleted, functional motor deficits were observed.113 However, the precise molecular mechanism by which presenilin mutations might sometimes cause additional motor neuron degeneration requires further investigation.

### Other neurodegenerative diseases

Several case reports have suggested a link between presenilin mutations and Lewy body dementia,

<table>
<thead>
<tr>
<th>Domain</th>
<th>Exon</th>
<th>Population</th>
<th>Unusual clinical features</th>
<th>Unusual pathology</th>
<th>Functional implications/effect on Aβ</th>
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<tbody>
<tr>
<td>Del88/Glu91</td>
<td>TM1</td>
<td>Scotland</td>
<td>None</td>
<td>CWP, AA</td>
<td>↑ Aβ₄₂ in cell lines</td>
</tr>
<tr>
<td>Leu89Pro</td>
<td>TM1</td>
<td>Japan</td>
<td>In combination with visual variant of Alzheimer’s disease</td>
<td>No autopsy data available</td>
<td>↑ Aβ₄₀ in cell lines</td>
</tr>
<tr>
<td>Aon135Ser</td>
<td>TM2</td>
<td>Greece</td>
<td>Seizures</td>
<td>Corticospinal tract degeneration</td>
<td>Not tested</td>
</tr>
<tr>
<td>Met139Val</td>
<td>TM2</td>
<td>USA, UK, Germany</td>
<td>Seizures, myoclonus, cerebellar ataxia</td>
<td>Pathology in basal ganglia and cerebellum</td>
<td>Not tested</td>
</tr>
<tr>
<td>Tyr154Asn</td>
<td>TM2</td>
<td>Japan</td>
<td>None</td>
<td>No autopsy data available</td>
<td>↑ Aβ₄₀ in cell lines</td>
</tr>
<tr>
<td>InsPhet157/158</td>
<td>Loop 2</td>
<td>USA</td>
<td>Apraxia, dystonia</td>
<td>CWP</td>
<td>CWP</td>
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<tr>
<td>Leu166Pro</td>
<td>TM3</td>
<td>Germany</td>
<td>Seizures, cerebellar ataxia, onset in adolescence</td>
<td>CWP including in the cerebellum</td>
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</tr>
<tr>
<td>Phe237Il</td>
<td>TM5</td>
<td>Japan</td>
<td>Seizures, myoclonus, dystonia</td>
<td>No autopsy data available</td>
<td>↑ Aβ₄₀ in cell lines</td>
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<tr>
<td>Tyr256Ser</td>
<td>TM6</td>
<td>Australia</td>
<td>Extrapyramidal signs, cerebellar ataxia</td>
<td>CWP</td>
<td>↑ Aβ₄₀ in brain</td>
</tr>
<tr>
<td>Val261Leu</td>
<td>TM6</td>
<td>Spain</td>
<td>None</td>
<td>No autopsy data available</td>
<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
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<tr>
<td>Val261Pro</td>
<td>TM6</td>
<td>USA, UK, Germany</td>
<td>Seizures, myoclonus, cerebellar ataxia</td>
<td>Pathology in basal ganglia and cerebellum</td>
<td>Not tested</td>
</tr>
<tr>
<td>Phe237Ile</td>
<td>TM6</td>
<td>Japan</td>
<td>None</td>
<td>No autopsy data available</td>
<td>↑ Aβ₄₀ in cell lines</td>
</tr>
<tr>
<td>Tyr256Ser</td>
<td>TM6</td>
<td>Greece</td>
<td>Seizures and myoclonus, visuospatial impairment</td>
<td>CWP</td>
<td>↑ Aβ₄₀ in brain</td>
</tr>
<tr>
<td>Gly266Ser</td>
<td>Loop 6</td>
<td>Japan</td>
<td>Apraxia</td>
<td>No autopsy data available</td>
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</tr>
<tr>
<td>Arg278Lys</td>
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<td>Italy</td>
<td>1 of 3 patients in this kindred had spastic paraparesis without dementia</td>
<td>No autopsy data available</td>
<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
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<td>UK</td>
<td>Visuospatial impairment</td>
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<td>Arg278Thr</td>
<td>Loop 6</td>
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<td>None</td>
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<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
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<td>Glu280Gly</td>
<td>Loop 6</td>
<td>Ireland</td>
<td>Deep white matter abnormalities on MRI</td>
<td>CWP, AA</td>
<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
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<tr>
<td>Glu280Glu</td>
<td>Loop 6</td>
<td>Canada</td>
<td>None</td>
<td>CWP, Lewy bodies, corticospinal tract degeneration</td>
<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
</tr>
<tr>
<td>Pro284Leu</td>
<td>Loop 6</td>
<td>Japan</td>
<td>Extrapyramidal signs</td>
<td>CWP</td>
<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
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<tr>
<td>Pro284Ser</td>
<td>Loop 6</td>
<td>Italy</td>
<td>Frontal and temporal white matter abnormalities on MRI</td>
<td>No autopsy data available</td>
<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
</tr>
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<td>G to T splice acceptor site substitution</td>
<td>Loop 6</td>
<td>Japan</td>
<td>None</td>
<td>No autopsy data available</td>
<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
</tr>
<tr>
<td>G to A splice acceptor site substitution</td>
<td>Loop 6</td>
<td>Australia</td>
<td>None</td>
<td>CWP, AA</td>
<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
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<tr>
<td>DelExon9</td>
<td>Loop 6</td>
<td>Finland</td>
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<td>CWP</td>
<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
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<tr>
<td>DelExon9</td>
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<td>Australia</td>
<td>None</td>
<td>CWP</td>
<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
</tr>
<tr>
<td>Leu381Val</td>
<td>TM7</td>
<td>Bulgaria</td>
<td>Extrapyramidal signs</td>
<td>No autopsy data available</td>
<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
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<tr>
<td>Aon405Ser</td>
<td>Loop 7</td>
<td>Japan</td>
<td>None</td>
<td>Some AA</td>
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<tr>
<td>Pro436Gln</td>
<td>TM9</td>
<td>UK</td>
<td>None</td>
<td>CWP</td>
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<td>DelThr440</td>
<td>TM9</td>
<td>Japan</td>
<td>Extrapyramidal signs</td>
<td>CWP, AA, Lewy bodies, corticospinal tract degeneration</td>
<td>↑ Aβ₄₀:Aβ₄₀ ratio in cell lines</td>
</tr>
</tbody>
</table>

For a comprehensive list of studies of clinical heterogeneity associated with PSEN1 mutations in Alzheimer’s disease, see elsewhere.113 AA=amyloid angiopathy, Aβ=β-amyloid. CWP=cotton-wool plaques. PSEN1=presenilin 1 gene. TM=transmembrane domain.

Table: Overview of PSEN1 mutations associated with early-onset Alzheimer’s disease and spastic paraparesis
frontotemporal dementia, and cerebral amyloid angiopathy, indicating that presenilin mutations could be implicated in an even broader clinical range of diseases than originally thought.

Three independent reports have been published linking particular mutations in \textit{PSEN1} to frontotemporal dementia: Leu113Pro, insArg352, and Gly184Val.\textsuperscript{117–119} Patients with the Leu113Pro mutations\textsuperscript{118} had typical Alzheimer's disease neuropathology (with plaques and tangles), but had prominent involvement of the frontal cortex. Therefore, these patients seemed to have a frontal variant of Alzheimer’s disease, rather than frontotemporal dementia (Campion D, personal communication). In the insArg352 case report,\textsuperscript{117} the mutation affected APP processing by decreasing A\textsubscript{β} production. On neuropathological examination of the proband, however, frontotemporal lobar degeneration with ubiquitin-positive inclusions was found, suggestive of a progranulin mutation. In a follow-up report,\textsuperscript{120} this patient was also found to have a progranulin mutation, which is probably the real cause of the observed pathology.\textsuperscript{120} The relevance of the presenilin mutation or polymorphism remains unclear. Finally, the Gly184Val case had a Pick-type tauopathy without extracellular A\textsubscript{β} plaques.\textsuperscript{119} In the meantime, progranulin and MAPT (microtubule-associated protein tau; Tau) mutations in this family have been excluded,\textsuperscript{121} leaving the possibility that the Gly184Val mutation could be pathological; however, in the absence of further published pathological investigation, it remains unclear whether and to what extent the other four reported carriers in the family had a similar Pick's disease phenotype.\textsuperscript{119} Such work is essential to begin to establish a causal relationship.

Lewy bodies are also frequently observed in patients with both familial and sporadic Alzheimer’s disease, but these are fewer and less widespread than seen in patients with typical Lewy body disease.\textsuperscript{122} One case report of a patient with a \textit{PSEN2} mutation presenting with widespread Lewy bodies and a full clinical picture of Lewy body dementia has been published.\textsuperscript{123} However, only one of the five patients in this family had this clinical presentation, whereas other family members presented with more typical symptoms of Alzheimer’s disease.

In conclusion, the evidence linking presenilin mutations to other neurodegenerative diseases apart from Alzheimer’s disease seems to be weak. The range of clinical symptoms caused by the different presenilin mutations is quite heterogeneous, and further work is needed to understand this. Given the complex cell biology and the various functions in which the γ-secretase complex is involved, the different mutations in presenilin, apart from their effect on A\textsubscript{β} processing,\textsuperscript{66} might have a heterogeneous effect on other aspects of its biology. Such effects might also partly contribute to the clinical variability. Moreover, the two presenilin proteins can combine into different γ-secretase complexes. Whether these different complexes are

\textbf{γ-secretases: therapeutic opportunities and challenges}

As γ-secretase is the final protease involved in the production of A\textsubscript{β} peptides, it is, at least with regard to the amyloid hypothesis of Alzheimer’s disease pathogenesis, a very attractive drug target. Given its central role in Notch cleavage, the protease is also increasingly considered as a drug target for cancer. Therefore, compounds that efficiently target γ-secretase could have a wide range of therapeutic uses. Here, we briefly discuss the therapeutic potential and challenges of γ-secretase inhibitors and γ-secretase modulators. Insights into the
heterogeneity of the γ-secretase complex and the regulatory role of G-protein-coupled receptors might also provide opportunities for the development of novel therapies.

γ-secretase inhibitors
Several γ-secretase inhibitors have been developed and tested preclinically and clinically. The first results in animal models were promising, and brain Aβ concentrations were reduced in a mouse model of Alzheimer’s disease. The first reports raised hope for cancer as well; for example, γ-secretase inhibitors increased survival in mouse models of leukaemia. These inhibitors also reduced osteosarcomas in vivo in mice and had a synergistic effect with other chemotherapy approaches in human colon adenocarcinoma cell lines. γ-secretase inhibitors also seemed to have beneficial effects in a model of focal ischaemic stroke, probably via inhibition of Notch signalling. In an experimental model of multiple sclerosis, γ-secretase inhibitors have enhanced remyelination and reduced axonal damage. In these models, the drugs could be given over a short time period, which reduces the likelihood of severe adverse events, as discussed below.

The results of the first clinical trials have raised important questions about the therapeutic potential and possible complications of γ-secretase inhibitors. For example, trials of γ-secretase inhibitors in patients with T-cell acute lymphoblastic leukaemia resulted in serious gastrointestinal side-effects, and their anti-leukaemic effects seemed very limited. γ-secretase inhibitors also have a limited effect on Notch signalling. In an experimental model of multiple sclerosis, γ-secretase inhibitors have enhanced remyelination and reduced axonal damage. In these models, the drugs could be given over a short time period, which reduces the likelihood of severe adverse events, as discussed below.

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So far, owing to their inhibition of Notch cleavage as well as their inhibition of APP cleavage, all the tested γ-secretase inhibitors have led to serious gastrointestinal side-effects in animals because of the transformation of proliferative intestinal crypt cells into postmitotic goblet cells. In mice, they also affected the maturation of B lymphocytes and T lymphocytes, causing immunosuppression. By contrast, the side-effects of semagacestat reported in a phase 2 trial were relatively mild, possibly because γ-secretase was not completely inhibited. Three patients had to stop the trial because of gastrointestinal side-effects, but these adverse events did not seem to be caused by immediate Notch-related toxicity. At a high dosage, prolonged QT intervals were observed on electrocardiogram (ECG) scans. Other notable side-effects included skin rashes and changes to hair colour: on withdrawal of the drug, these were reversible. As the drug has to be carefully dosed to provide information on the clinical correlates of chronically decreasing Aβ production in the brain.

To avoid Notch-related toxicity, inhibitors such as begacestat have recently been developed that apparently have a limited effect on Notch signalling pathways, while inhibiting APP cleavage. So far, BMS-708163 is the most advanced of these compounds in clinical trials.

An alternative way to avoid the gastrointestinal toxicity of γ-secretase inhibitors came from work in cancer research. Glucocorticoids are normally part of the therapeutic armamentarium against T-cell acute lymphoblastic leukaemia. However, some patients develop glucocorticoid resistance owing to Notch mutations. In a recent study, a twofold benefit of combination therapy with a γ-secretase inhibitor and glucocorticoids was found. The secretase inhibitor reversed the glucocorticoid resistance; glucocorticoids also antagonised the effects of Notch inhibition on the intestinal epithelium and thus protected against gastrointestinal toxicity. These results, done both in vitro on T-cell acute lymphoblastic leukaemia cells and in vivo in mice, open the door to new therapeutic trials of γ-secretase inhibitors in cancer. A better understanding of the mechanisms through which these inhibitors cause gastrointestinal toxicity might aid the development of other strategies to reverse this toxicity. However, treatment with glucocorticoids can have its own severe side-effects, particularly in the long term, and therefore this combination therapy is unlikely to be used in the chronic treatment of Alzheimer’s disease.

γ-secretase modulators
Although γ-secretase inhibitors are directed towards the quantitative modulation of Aβ production, a fortuitous observation several years ago suggested the possibility that γ-secretase activity could be modulated to obtain a qualitative effect on Aβ generation. Substantial progress has been made recently, although a real clinical breakthrough has not yet been achieved.

So far, two types of compounds have been identified as potentially clinically useful γ-secretase modulators: non-steroidal anti-inflammatory drugs (NSAIDs) and some kinase inhibitors. One of the NSAIDs, ibuprofen, reduced plaque load and brain inflammation in an Alzheimer’s disease mouse model and decreased the production of the aggregation-prone Aβ peptide in vitro, favouring instead the production of the shorter Aβ peptide. No substantial effect on Notch cleavage was observed. This finding in 2001 led to a large amount of research into the clinical effects of NSAIDs on Alzheimer’s disease. Several small clinical trials with NSAIDs in Alzheimer’s disease resulted in inconsistent clinical benefits, and tolerability of sustained treatment was of concern because of well-known severe gastrointestinal side-effects owing to inhibition of the cyclo-oxygenase (COX1) enzyme. Another trial with a COX2-selective NSAID, rofecoxib, led to no significant clinical improvement.

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To avoid the gastrointestinal side-effects of NSAIDs, a derivative molecule without COX-inhibitory activity, tarenflurbil (also known as R-flurbiprofen), was developed. This drug belongs to a new class of molecules termed selective Aβ40-lowering agents (SALA), because these compounds modulate γ-secretase activity to reduce the production of Aβ40 without affecting the production of Aβ42 or Notch cleavage. Results from photocrosslinking experiments have shown that these γ-secretase modulators bind in the N-terminal region of the transmembrane domain of APP to modulate its cleavage by γ-secretase. This finding can help explain how APP cleavage is specifically modulated without interfering with Notch cleavage. In mice, tarenflurbil reduced the brain concentrations of Aβ42 and prevented learning and memory deficits.

In the 24-month randomised, controlled phase 2 trial of patients with mild to moderate Alzheimer’s disease, no benefit of treatment was seen on the rate of cognitive decline versus placebo. However, patients with mild Alzheimer’s disease who received tarenflurbil had a significantly lower rate of decline in activities of daily living and global function than did those who received placebo. Unfortunately, however, this compound did not show any clinical benefit in a recent phase 3 clinical trial. As expected, no severe gastrointestinal side-effects were observed. Penetration of the blood–brain barrier seems to be a major problem for this drug and paucity of data prevents evaluation of whether effective lowering or modulation of Aβ production was ever attained in the trial. Therefore, as for γ-secretase inhibitors, one of the major hurdles is the need to design a targeted γ-secretase modulator that can reach therapeutic concentrations in the brain.

A tarenflurbil analogue, CHF5074, was recently shown to reduce amyloid plaque load and attenuate the spatial memory deficit in transgenic mouse models of Alzheimer’s disease, indicating that the use of NSAID analogues should still be considered as a potential therapeutic strategy for Alzheimer’s disease.

γ-secretase heterogeneity

As described above, the γ-secretase enzyme is assembled from four components. However, two different genes exist for two of these proteins: PSEN1 and PSEN2, and Aph1A and Aph1B. The complexity is further increased by alternative splicing of the different mRNAs encoded by these genes. At least four different combinations are therefore possible in human beings, and even more if the alternatively spliced variants are taken into account. The Aph1A-containing complex is essential for Notch processing during embryogenesis. Aph1B is expressed in mouse and human brain, but Aph1B-knockout mice mainly develop as normal. By contrast, knockout of Aph1B in a mouse model of Alzheimer’s disease unexpectedly rescued several phenotypes associated with Alzheimer’s disease. These Aph1B-knockout mice had decreased Aβ production, a much-reduced plaque load, and normal memory function. These events occurred without significant Notch-related side-effects, as neither intestinal metaplasia nor involution of spleen and thymus were observed, and T-lymphocyte ratios and pancreas histopathology were normal.

Both Aph1A and Aph1B γ-secretase complexes are equally competent in cleaving synthetic APP and Notch substrates. Although the Aph1B-containing complexes produced more longer Aβ peptides (Aβ42 and longer) in cell-free assays, the total ratio of Aβ42:Aβ40 in brain extracts from transgenic and wild-type mice was not significantly changed. The longer Aβ peptides might therefore be degraded in the cell before secretion. The total amount of Aβ peptides in Aph1B-knockout mouse and human brain material from which Aph1B complexes were removed was, however, decreased, emphasising the crucial contribution of Aph1B to the total γ-secretase activity in the brain.

This γ-secretase heterogeneity provides new possibilities for targeted therapies. As Aph1B is strongly expressed in the brain, and apparently does not have a substantial role in Notch signalling in peripheral tissues, it is theoretically attractive to try to specifically target the Aph1B complex. If such a drug could be developed, this inhibitor could be given to patients, or even as a preventive therapy for Alzheimer’s disease, without concerns about potential Notch-related side-effects. Whether PSEN2-containing complexes provide similar opportunities for less toxic drug development remains to be investigated. PSEN2-knockout animals have similar phenotypes to wild-type animals, with only minimum apoptotic changes in the lung. Furthermore, there is evidence that compounds can target PSEN1-containing or PSEN2-containing complexes specifically in vitro. However, to date there have been no published reports to suggest that selectively blocking PSEN2 function in an Alzheimer’s disease mouse model improves Alzheimer-related symptoms.

Regulation of γ-secretase by G-protein-coupled receptors

Research on the physiological control mechanisms of γ-secretase activity might yield novel drug targets. This type of work is in its early stages, but two independent reports indicate that γ-secretase subcellular localisation and proteolytic activity might be regulated by G-protein-coupled receptors, in particular the β2-adrenergic receptor and G-protein-coupled receptor 3 (GPR3). GPR3 is an orphan receptor, but is expressed in the hippocampus and cortex. This receptor seems to promote assembly of the γ-secretase complex, resulting in increased trafficking of the γ-secretase components and mature γ-secretase complex to the cell surface, which eventually leads to an increase in Aβ generation. Activation-induced receptor-mediated endocytosis of the β2-adrenergic receptor with the γ-secretase complex also results in an increase in Aβ production. Therefore, drugs...
that modulate the activity of these receptors might become useful as alternatives to γ-secretase inhibitors and modulators. The differential effect of GPR3 on APP versus Notch processing, indicating that Aβ generation can be blocked independently of any effects on Notch, makes GPR3 a particularly attractive target.

Conclusions and future directions

As the catalytic core of the γ-secretase enzymes, presenilins are key players in the production of aggregation-prone Aβ peptides. The many mutations in the presenilin and APP genes have provided strong evidence for the amyloid hypothesis of Alzheimer’s disease pathogenesis. Although patients with autosomal dominant early-onset familial Alzheimer’s disease account for less than 0.5% of all patients with Alzheimer’s disease, they share a similar neuropathological profile with patients who have late-onset sporadic disease. Hence, much has been learned from studying the early-onset mutations. Given their wide expression and important biological role, one might expect presenilins to be involved in a large range of human diseases. So far, however, there is little evidence to support associations with diseases other than Alzheimer’s disease. Despite more than a decade of research into γ-secretase inhibitors, no therapeutic drugs have yet reached the clinic. A major obstacle is the toxic Notch-related side-effects. If γ-secretase inhibitors that inhibit the processing of APP while sparing Notch can be further developed, this would provide a major breakthrough. Recent insights into the heterogeneity of the γ-secretase complexes might help the rational design of such compounds. However, even if effective γ-secretase inhibitors or other Notch-sparing γ-secretase therapies can be developed, the blood–brain barrier still poses a major problem.

Contributors

BAB and BDS planned the Review. BAB undertook the literature search and wrote the first draft. BDS rewrote, edited, and finalised the text.

Conflicts of interest

BDS has been a consultant for Envivo Pharmaceuticals, Johnson & Johnson, Esai, Eli-Lilly, Galapagos, and Remynd in the past 3 years. He has received research support from Envivo Pharmaceuticals, Eli-Lilly, and Movetis, and a freedom to discover award from Bristol-Myers Squibb. He holds several patents related to γ-secretases and β-secretases. BAB has no conflicts of interest.

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