γ-secretases: from cell biology to therapeutic strategies

Bruno A Bergmans, Bart De Strooper

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 dementias, 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.

Lancet Neurol 2010; 9: 215–26

Department of Molecular and Developmental Genetics, VIB, Leuven, Belgium (B A Bergmans MD, B De Strooper MD)**; and Center for Human Genetics, Katholieke Universiteit Leuven, Leuven, Belgium** (B A Bergmans, B De Strooper)

Correspondence to: Bart De Strooper, Center for Human Genetics, Herestraat 49, 3000 Leuven, Belgium **bart.destrooper@vib-cme.kuleuven.be**

For more on ***PSEN2*** see http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=600759

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.[1] Soon after, mutations in a similar gene, *PSEN2* (presenilin 2), were found.[2–4] More than 170 mutations have since been identified in *PSEN1*, whereas mutations in *PSEN2* are much rarer.[5] Presenilin mutations are responsible for an important fraction of autosomal inherited Alzheimer's disease, although estimates vary from 9%[6] to 62%.[7–9] 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[8,9] 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.[10] However, as only about 2% of patients with Alzheimer's disease have early onset,[11] the total contribution of *APP* and the presenilin genes to the risk of Alzheimer's disease in the general population remains low.[12] 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.[13–15] 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.[16,17]

The importance of the identification of the presenilin mutations for research into Alzheimer's disease should, however, not be underestimated. Their disovery has enhanced understanding of a crucial enzymatic process in the generation of the Aβ peptide,[18] which is thought to be a key factor in the pathogenesis of the disease.[19] 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,[22,23] which also contain nicastrin, presenilin enhancer 2 (Pen-2), and anterior pharynx defective 1 (Aph1).[20] γ-secretases cleave various type I transmembrane proteins, including the adhesion molecules N-cadherin and E-cadherin,[24,25] the neurotrophin receptor p75,[26] the regulatory β2 subunit of voltage-gated sodium channels,[27] the axon guidance molecule DCC,[28] and neuregulin[29] (for a review see elsewhere[30]). Neuregulin has an important role in myelin formation,[31] and single nucleotide polymorphisms in the gene have been associated with an increased risk of neurodevelopmental disorders.[32–34] 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.[29] 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.[22,23,35] 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

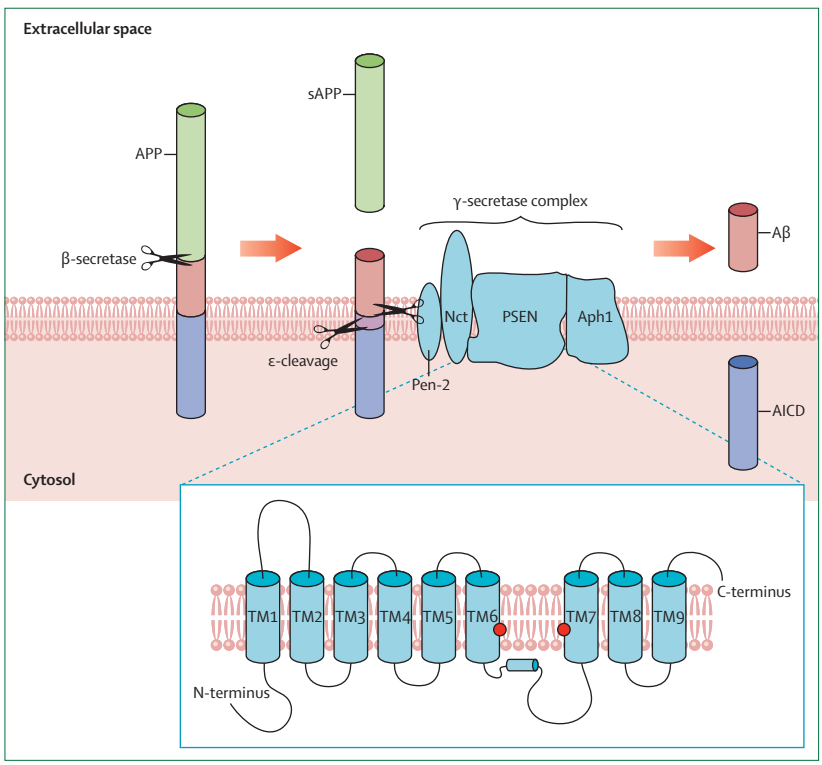

Figure 1: **Presenilins, the γ-secretase complex, and APP processing**
After release of sAPP by β-secretase, the remaining APP segment is further cleaved by γ-secretase. First, ε-cleavage by γ-secretase releases the AICD. Second, γ-secretase cleavage releases the Aβ peptide, of which there can be longer and shorter forms (eg, $Aβ_{40}$ and $Aβ_{42}$), depending on the exact cleavage site by γ-secretase. The γ-secretase enzyme consists of four components: PSEN, Aph1, Nct, and Pen-2.[20] The magnified area shows the nine TM domains of PSEN.[21] The two red circles indicate the catalytic aspartates. Aβ=β-amyloid. AICD=APP intracellular domain. Aph1=anterior pharynx defective. APP=amyloid precursor protein. Nct=nicastrin. Pen-2=presenilin 2 enhancer. PSEN=presenilin. sAPP=soluble APP. TM=transmembrane.

49 amino-acid residues ($Aβ_{37}$ to $Aβ_{49}$). $Aβ_{40}$ is the most abundant product cleaved and is found in the CSF and the plasma. $Aβ_{42}$ is less common, occurring in about a tenth of the amounts of $Aβ_{40}$, but is considered to be pathogenic. This peptide aggregates faster than $Aβ_{40}$ and is apparently also more toxic in cell culture assays.[36–38] The presenilin mutations generally increase the relative amounts of $Aβ_{42}$ to $Aβ_{40}$, and this ratio is widely regarded as an important indicator of pathogenic mutations.[39] The extent to which other long Aβ peptides ($Aβ_{43}$ to $Aβ_{49}$) are secreted or degraded intracellularly and whether they also have pathological importance remain unclear. Shorter peptides ($Aβ_{38}$ and shorter) might be less problematic because they are more soluble, but this remains unproven. Novel drugs that are under investigation target γ-secretases with the aim of modulating, rather than inhibiting, their activity, so that more of the shorter Aβ 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;[40–42] but see elsewhere for a contrasting view[43]), in its extracellular domain, which triggers cleavage by γ-secretase in the transmembrane domain.[18,44] 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.[45–47] Interference with the Notch pathway therefore leads to many disturbances in embryogenesis, including disrupted somitogenesis and blood vessel formation.[45,47] The Notch pathway also regulates several oncogenes and oncogenic signalling pathways such as those that involve Myc and Akt.[48] These pathways are of relevance for cancer, which, as we briefly discuss, is why γ-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 γ-secretase inhibitors is the side-effects that result from interference with Notch cleavage and signalling.[49,50] In the context of Alzheimer's disease, the inhibition of Notch signalling by γ-secretase inhibitors has to be considered as an intrinsic, mechanism-based cause of serious side-effects, including gastrointestinal bleedings, immune disturbances, and hair loss.[18,51–54]

Presenilins and intracellular calcium

Recent studies have indicated that the function of presenilins extends beyond their role in γ-secretase cleavage. These proteins constitute calcium leak channels in the endoplasmic reticulum when not incorporated into the γ-secretase complexes.[55] 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,[56–60] leading to increased accumulation of calcium ions (Ca^{2+}) in the endoplasmic reticulum.[61] 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.[62] A conditional knockout mouse model of both presenilins in the forebrain showed marked neurodegeneration with increasing age.[63] It is unclear whether the neurodegeneration in this model is linked to the early presynaptic deficits and whether impaired γ-secretase (proteolytic) function or disturbed Ca^{2+} homoeostasis 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.[64,65] It is becoming increasingly clear that, from a genetic point of view, mutations act in a "dominant" fashion, whereas from a biochemical view, most have loss-of-function effects, both in enzymatic assays[66] or when measuring Ca^{2+} leakage channel activity.[61] Many clinical mutations clearly have loss-of-function effects on some substrates such as syndecan, N-cadherin,[66] and Notch.[66–68] 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 $Aβ_{42}$.[66,69] The net result is that, even if the total amount of Aβ peptides decreases, there is an increase in the $Aβ_{42}$:$Aβ_{40}$ 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,[70] but that an ill-defined oligomeric state of Aβ peptides affects synapses and neurons, might provide an explanation for this apparent paradox.[71,72] Although not proven, the ratio of longer or more hydrophobic Aβ peptides ($Aβ_{42}$, but also longer or truncated species) to $Aβ_{40}$ 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.[73] 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 $Aβ_{42}$) diminishes amyloid plaque load and memory deficits in a mouse model[74–76] 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.[77] 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.[77,78] 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[79,80] 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.[81,82] The only modifier suggested in families with early-onset disease is the HLA-2 genotype, but the precise mechanism is unclear.[83,84]

Spastic paraparesis

After the original suggestion of an association between early-onset Alzheimer's disease and spastic paraparesis[85] and the confirmation in a large Finnish family,[86,87] 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.[91,114] Hypometabolism on fluorodeoxyglucose-PET in the motor cortex, a sign of malfunction, has also been reported.[94] There is widespread presence of "cotton wool" plaques in the hippocampus and the primary and association cortices in these patients.[114] These plaques do not have the congophilic staining typical of the "canonical" Alzheimer's disease plaques, and they also have very few dystrophic neurites, but they 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-cored 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

	Domain	Exon	Population	Unusual clinical features	Unusual pathology	Functional implications/ effect on Aβ
Del83/Met84[88,89]	TM1	4	Scotland	None	CWP, AA	↑ $Aβ_{42}$ in cell lines
Leu85Pro[90]	TM1	4	Japan	In combination with visual variant of Alzheimer's disease	No autopsy data available	↑ $Aβ_{42}$ in cell lines
Asn135Ser[91]	TM2	5	Greece	Seizures	Corticospinal tract degeneration	Not tested
Met139Val[92]	TM2	5	USA, UK, Germany	Seizures, myoclonus, cerebellar ataxia	Pathology in basal ganglia and cerebellum	Not tested
Tyr154Asn[93]	TM2	5	Japan	None	No autopsy data available	Not tested
insPhe157I/158[94]	Loop 2	5	USA	Apraxia, dystonia	CWP	Not tested
Leu166Pro[95]	TM3	6	Germany	Seizures, cerebellar ataxia, onset in adolescence	CWP including in the cerebellum	↑ $Aβ_{42}$ in cell lines
Phe237Ile[96]	TM5	7	Japan	Seizures, myoclonus, dystonia	No autopsy data available	Not tested
Tyr256Ser[97]	TM6	7	Australia	Extrapyramidal signs, cerebellar ataxia	CWP	↑ $Aβ_{42}$ in brain
Val261Leu[98]	TM6	8	Spain	None	No autopsy data available	Not tested
Val261Phe[99]	TM6	8	USA	None	No autopsy data available	Not tested
Pro264Leu[100]	Loop 6	8	France	Seizures and myoclonus, visuospatial impairment	No autopsy data available	Not tested
Gly266Ser[101]	Loop 6	8	Japan	Apraxia	No autopsy data available	Not tested
Arg278Lys[102]	Loop 6	8	Italy	1 of 3 patients in this kindred had spastic paraparesis without dementia	No autopsy data available	↑ $Aβ_{42}$:$Aβ_{40}$ ratio in cell lines
Arg278Ser[103]	Loop 6	8	UK	Visuospatial impairment	No autopsy data available	Not tested
Arg278Thr[86]	Loop 6	8	Australia	None	No autopsy data available	Not tested
Glu280Gly[104]	Loop 6	8	Ireland	Deep white matter abnormalities on MRI	CWP, AA	Not tested
Glu280Gln[105]	Loop 6	8	Canada	None	CWP, Lewy bodies, corticospinal tract degeneration	Not tested
Pro284Leu[106]	Loop 6	8	Japan	Extrapyramidal signs	CWP	Not tested
Pro284Ser[107]	Loop 6	8	Italy	Frontal and temporal white matter abnormalities on MRI	No autopsy data available	Not tested
G to T splice acceptor site substitution[86]	Loop 6	9	Japan	None	No autopsy data available	Functional del290-319
G to A splice acceptor site substitution[108]	Loop 6	9	Australia	None	CWP, AA	Functional del290-319
DelExon9[87,88]	Loop 6	9	Finland	None	CWP	↑ $Aβ_{42}$ in cell lines and in brain
DelExon9[109]	Loop 6	9	Australia	None	CWP	Not tested
Leu381Val[110]	TM7	11	Bulgaria	Extrapyramidal signs	No autopsy data available	Not tested
Asn405Ser[111]	Loop 7	11	Japan	None	Some AA	Not tested
Pro436Gln[88]	TM9	12	UK	None	CWP	↑ $Aβ_{42}$ in cell lines
DelThr440[112]	TM9	12	Japan	Extrapyramidal signs	CWP, AA, Lewy bodies, corticospinal tract degeneration	Not tested

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**

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 Ca^{2+} homoeostasis 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.[115,116] 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,

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 *PSEN1* to frontotemporal dementia: Leu113Pro, insArg352, and Gly184Val.[117–119] Patients with the Leu113Pro mutations[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,[117] the mutation affected APP processing by decreasing Aβ 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, this patient was also found to have a progranulin mutation, which is probably the real cause of the observed pathology.[120] The relevance of the presenilin mutation or polymorphism remains unclear. Finally, the Gly184Val case had a Pick-type tauopathy without extracellular Aβ plaques.[119] In the meantime, progranulin and MAPT (microtubule-associated protein tau; Tau) mutations in this family have been excluded,[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.[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.[122] One case report of a patient with a *PSEN2* mutation presenting with widespread Lewy bodies and a full clinical picture of Lewy body dementia has been published.[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 Aβ processing,[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 involved in different aspects of the pathology remains to be fully investigated. In the next section, we discuss how this heterogeneity might affect therapeutic options in Alzheimer's disease and beyond.

γ-secretases: therapeutic opportunities and challenges

As γ-secretase is the final protease involved in the production of Aβ 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

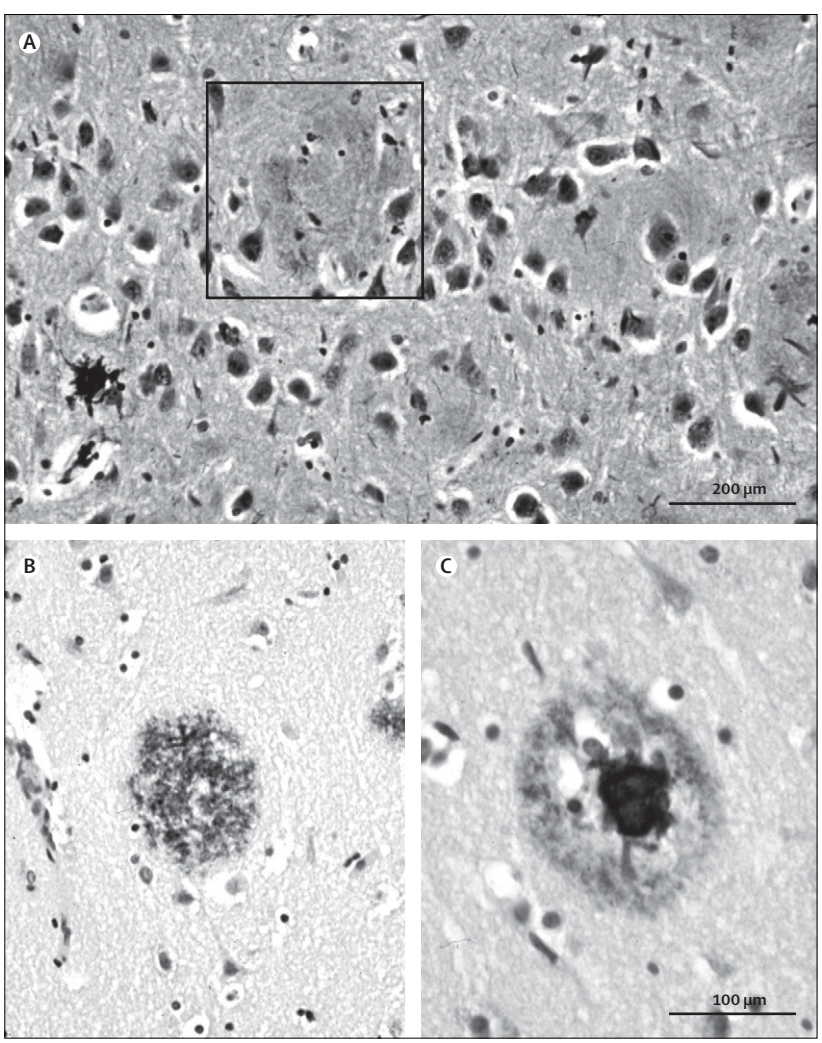

Figure 2: **Plaque pathology in Alzheimer's disease**
Cotton-wool plaques are typically seen in the brains of patients with Alzheimer's disease and spastic paraparesis. (A) Entorhinal cortex showing cotton wool plaques (modified Bielschowsky silver stain, 200×). (B) Cortex showing Aβ staining of a diffuse plaque (Aβ stain, 400×). (C) Cortex showing a classic plaque (Aβ stain, 400×). Aβ=β-amyloid. Reproduced from Brooks and colleagues,[108] with permission from Oxford University Press.

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.[124] The first reports raised hope for cancer as well; for example, γ-secretase inhibitors increased survival in mouse models of leukaemia.[125] These inhibitors also reduced osteosarcomas in vivo in mice[126] and had a synergistic effect with other chemotherapy approaches in human colon adenocarcinoma cell lines.[127] γ-secretase inhibitors also seemed to have beneficial effects in a model of focal ischaemic stroke,[128] probably via inhibition of Notch signalling. In an experimental model of multiple sclerosis, γ-secretase inhibitors have enhanced remyelination and reduced axonal damage.[129] 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,[52,54,130,131] and their anti-leukaemic effects seemed very limited.[52,132–134] LY450139 (semagacestat) is currently being studied for Alzheimer's disease in a phase 3 clinical trial.[135] In the phase 2 trial, this γ-secretase inhibitor decreased plasma $A\beta_{40}$ concentrations by more than 50%.[136] However, this response was biphasic with an increase in Aβ concentrations after the initial reduction. Reductions in Aβ in the CSF were not significant.

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.[54,130,131] In mice, they also affected the maturation of B lymphocytes and T lymphocytes, causing immunosuppression.[53] By contrast, the side-effects of semagacestat reported in a phase 2 trial[136–138] 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.[136–138] As the drug has to be carefully dosed to avoid Notch-related toxicity, the changes in Aβ concentrations in the phase 3 trial might not be sufficient 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.[139,140] So far, BMS-708163 is the most advanced of these compounds in clinical trials.[141,142]

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.[143] 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.[144] 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.[134,145] 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[146] 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.[147,148] One of the NSAIDs, ibuprofen, reduced plaque load and brain inflammation in an Alzheimer's disease mouse model[149] and decreased the production of the aggregation-prone $A\beta_{42}$ in vitro, favouring instead the production of the shorter Aβ peptide $A\beta_{38}$.[146] 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.[150] 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.[151] Another trial with a COX2-selective NSAID, rofecoxib, led to no significant clinical improvement.[152,153]

To avoid the gastrointestinal side-effects of NSAIDs, a derivative molecule without COX-inhibitory activity, tarenflurbil (also known as *R*-flurbiprofen), was developed.[154] This drug belongs to a new class of molecules termed selective $A\beta_{42}$-lowering agents (SALA), because these compounds modulate γ-secretase activity to reduce the production of $A\beta_{42}$ without affecting the production of $A\beta_{40}$ or Notch cleavage.[155,156] 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.[150,157] This finding can help explain how APP cleavage is specifically modulated without interfering with Notch cleavage. In mice, tarenflurbil reduced the brain concentrations of $A\beta_{42}$ and prevented learning and memory deficits.[158]

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.[159] 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.[160] As expected, no severe gastrointestinal side-effects were observed.[159] Penetration of the blood–brain barrier seems to be a major problem for this drug[36] 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.[161,162]

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*.[20] 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.[163–166] The Aph1A-containing complex is essential for Notch processing during embryogenesis.[167,168] 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.[74] 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 ($A\beta_{42}$ and longer) in cell-free assays, the total ratio of $A\beta_{42}$:$A\beta_{40}$ in brain extracts from transgenic and wild-type mice was not significantly changed.[74] 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. PS2-knockout animals have similar phenotypes to wild-type animals, with only minimum apoptotic changes in the lung.[169] Furthermore, there is evidence that compounds can target PSEN1-containing or PSEN2-containing complexes specifically in vitro.[80] 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[170] and G-protein-coupled receptor 3 (GPR3).[171] 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

Search strategy and selection criteria

References for this Review were identified through searches of PubMed with the search terms "presenilin and neurological disease", "presenilin and Alzheimer's disease", "presenilin and spastic paraparesis", "presenilin and cancer", "gamma-secretase and neurological disease", "gamma-secretase and Alzheimer's disease", "gamma-secretase and spastic paraparesis", and "gamma-secretase and cancer" from January 1990 to November 7, 2009. Several relevant articles were also identified by screening the bibliographies of published work. Only articles published in English were reviewed.

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.

Acknowledgments

BAB is supported by the Fund for Scientific Research Flanders (FWO-Vlaanderen). BDS is supported by the Fund for Scientific Research Flanders, KULeuven, Federal Office for Scientific Affairs, Belgium (IUAP P6/43/), a Methusalem grant of the KULeuven, and the Flemish Government and Memosad (FZ-2007-200611) of the European Union.

References

1 Sherrington R, Rogaev EI, Liang Y, et al. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* 1995; **375:** 754–60.

2 Levy-Lahad E, Wasco W, Poorkaj P, et al. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* 1995; **269:** 973–77.

3 Rogaev EI, Sherrington R, Rogaeva EA, et al. Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* 1995; **376:** 775–78.

4 Clark RF, Hutton M, Fuldner M, et al. The structure of the presenilin 1 (S182) gene and identification of six novel mutations in early onset AD families. *Nat Genet* 1995; **11:** 219–22.

5 Alzheimer Research Forum. The presenilin-1 mutations. http://www.alzforum.org/res/com/mut/pre/table1.asp (accessed Nov 7, 2009).

6 Cruts M, van Duijn CM, Backhovens H, et al. Estimation of the genetic contribution of presenilin-1 and -2 mutations in a population-based study of presenile Alzheimer disease. *Hum Mol Genet* 1998; **7:** 43–51.

7 Raux G, Guyant-Marechal L, Martin C, et al. Molecular diagnosis of autosomal dominant early onset Alzheimer's disease: an update. *J Med Genet* 2005; **42:** 793–95.

8 Lleo A, Blesa R, Queralt R, et al. Frequency of mutations in the presenilin and amyloid precursor protein genes in early-onset Alzheimer disease in Spain. *Arch Neurol* 2002; **59:** 1759–63.

9 Janssen JC, Beck JA, Campbell TA, et al. Early onset familial Alzheimer's disease: mutation frequency in 31 families. *Neurology* 2003; **60:** 235–39.

10 Alzheimer Research Forum. Presenilin mutations directory. http://www.alzforum.org/res/com/mut/pre/default.asp (accessed Nov 7, 2009).

11 Ott A, Breteler MM, van Harskamp F, et al. Prevalence of Alzheimer's disease and vascular dementia: association with education. The Rotterdam study. *Am J Epidemiol* 1998; **147:** 574–80.

12 Cruts M, Van Broeckhoven C. Presenilin mutations in Alzheimer's disease. *Hum Mutat* 1998; **11:** 183–90.

13 Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci USA* 1993; **90:** 1977–81.

14 Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993; **261:** 921–23.

15 Saunders A, Strittmatter WJ, Schmechel D, et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 1993; **43:** 1467–72.

16 Lambert J-C, Heath S, Even G, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet* 2009; **41:** 1094–99.

17 Harold D, Abraham R, Hollingworth P, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet* 2009; **41:** 1088–93.

18 De Strooper B, Annaert W, Cupers P, et al. A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature* 1999; **398:** 518–22.

19 Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002; **297:** 353–56.

20 De Strooper B. Aph-1, Pen-2, and nicastrin with presenilin generate an active gamma-secretase complex. *Neuron* 2003; **38:** 9–12.

21 Spasic D, Tolia A, Dillen K, et al. Presenilin-1 maintains a nine-transmembrane topology throughout the secretory pathway. *J Biol Chem* 2006; **281:** 26569–77.

22 De Strooper B, Saftig P, Craessaerts K, et al. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* 1998; **391:** 387–90.

23 Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, Selkoe DJ. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature* 1999; **398:** 513–17.

24 Struhl G, Adachi A. Requirements for presenilin-dependent cleavage of notch and other transmembrane proteins. *Mol Cell* 2000; **6:** 625–63.

25 Golde TE, Eckman CB. Physiologic and pathologic events mediated by intramembranous and juxtamembranous proteolysis. *Sci STKE* 2003; **2003:** RE4.

26 Jung KM. Regulated intramembrane proteolysis of the p75 neurotrophin receptor modulates its association with the TrkA receptor. *J Biol Chem* 2003; **278:** 42161–69.

27 Kim DY, Ingano LAM, Carey BW, Pettingell WH, Kovacs DM. Presenilin/gamma-secretase-mediated cleavage of the voltage-gated sodium channel beta2-subunit regulates cell adhesion and migration. *J Biol Chem* 2005; **280:** 23251–61.

28 Taniguchi Y, Kim SH, Sisodia SS. Presenilin-dependent gamma-secretase processing of deleted in colorectal cancer (DCC). *J Biol Chem* 2003; **278:** 30425–28.

29 Dejaegere T, Serneels L, Schäfer MK, et al. Deficiency of Aph1B/C-gamma-secretase disturbs Nrg1 cleavage and sensorimotor gating that can be reversed with antipsychotic treatment. *Proc Natl Acad Sci USA* 2008; **105:** 9775–80.

30 Kopan R, Ilagan MX. Gamma-secretase: proteasome of the membrane? *Nat Rev Mol Cell Biol* 2004; **5:** 499–504.

31 Willem M, Garratt AN, Novak B, et al. Control of peripheral nerve myelination by the beta-secretase BACE1. *Science* 2006; **314:** 664–66.

32 Consuelo W-B, Wei L, Debbie FL, et al. A novel missense mutation in the transmembrane domain of neuregulin 1 is associated with schizophrenia. *Biol Psychiatry* 2006; **60:** 548–53.

33 Stefansson H, Sigurdsson E, Steinthorsdottir V, et al. Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet* 2002; **71:** 877–92.

34 Rodney CPG, Rodney TP, Howard W, et al. Neuregulin-1 polymorphism in late onset Alzheimer's disease families with psychoses. *Am J Med Genet B Neuropsychiatric Genet* 2005; **139B:** 28–32.

35 Vassar R, Bennett BD, Babu-Khan S, et al. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 1999; **286:** 735–41.

36 Evin G. Gamma-secretase modulators: hopes and setbacks for the future of Alzheimer's treatment. *Exp Rev Neurother* 2008; **8:** 1611–13.

37 Wolfe MS. The gamma-secretase complex: membrane-embedded proteolytic ensemble. *Biochemistry* 2006; **45:** 7931–39.

38 Selkoe DJ, Wolfe MS. Presenilin: running with scissors in the membrane. *Cell* 2007; **131:** 215–21.

39 Scheuner D, Eckman C, Jensen M, et al. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* 1996; **2:** 864–70.

40 Pan D, Rubin GM. Kuzbanian controls proteolytic processing of Notch and mediates lateral inhibition during Drosophila and vertebrate neurogenesis. *Cell* 1997; **90:** 271–80.

41 Wen C, Metzstein MM, Greenwald I. SUP-17, a *Caenorhabditis elegans* ADAM protein related to Drosophila KUZBANIAN, and its role in LIN-12/NOTCH signalling. *Development* 1997; **124:** 4759–67.

42 Hartmann D, de Strooper B, Serneels L, et al. The disintegrin/metalloprotease ADAM 10 is essential for Notch signalling but not for alpha-secretase activity in fibroblasts. *Hum Mol Genet* 2002; **11:** 2615–24.

43 Brou C, Logeat F, Gupta N, et al. A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. *Mol Cell* 2000; **5:** 207–16.

44 Struhl G, Greenwald I. Presenilin is required for activity and nuclear access of Notch in Drosophila. *Nature* 1999; **398:** 522–25.

45 Kopan R, Goate A. A common enzyme connects notch signaling and Alzheimer's disease. *Genes Dev* 2000; **14:** 2799–806.

46 Kopan R, Ilagan MXG. Gamma-secretase: proteasome of the membrane? *Nat Rev Mol Cell Biol* 2004; **5:** 499–504.

47 Louvi A, Artavanis-Tsakonas S. Notch signalling in vertebrate neural development. *Nat Rev Neurosci* 2006; **7:** 93–102.

48 Palomero T, Ferrando A. Oncogenic NOTCH1 control of MYC and PI3K: challenges and opportunities for anti-NOTCH1 therapy in T cell acute lymphoblastic leukemias and lymphomas. *Clin Cancer Res* 2008; **14:** 5314–17.

49 Geling A, Steiner H, Willem M, Bally-Cuif L, Haass C. A gamma-secretase inhibitor blocks Notch signaling in vivo and causes a severe neurogenic phenotype in zebrafish. *EMBO Rep* 2002; **3:** 688–94.

50 Micchelli CA, Esler WP, Kimberly WT, et al. G-secretase/presenilin inhibitors for Alzheimer's disease phenocopy Notch mutations in Drosophila. *FASEB J* (November 1, 2002) 10.1096/fj.02– 0394fje.

51 Tournoy J, Bossuyt X, Snellinx A, et al. Partial loss of presenilins causes seborrheic keratosis and autoimmune disease in mice. *Hum Mol Genet* 2004; **13:** 1321–31.

52 DeAngelo D. A phase I clinical trial of the notch inhibitor MK-0752 in patients with T cell acute lymphoblastic leukemia/lymphoma (T-ALL) and other leukemias. *J Clin Oncol* 2006; **24:** 6585.

53 Wong GT, Manfra D, Poulet FM, et al. Chronic treatment with the gamma-secretase inhibitor LY-411,575 inhibits beta-amyloid peptide production and alters lymphopoiesis and intestinal cell differentiation. *J Biol Chem* 2004; **279:** 12876–82.

54 van Es JH. Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature* 2005; **435:** 959–63.

55 Tu H, Nelson O, Bezprozvanny A, et al. Presenilins form ER Ca^{2+} leak channels, a function disrupted by familial Alzheimer's disease-linked mutations. *Cell* 2006; **126:** 981–93.

56 Chan SL, Mayne M, Holden CP, Geiger JD, Mattson MP. Presenilin-1 mutations increase levels of ryanodine receptors and calcium release in PC12 cells and cortical neurons. *J Biol Chem* 2000; **275:** 18195–200.

57 Chakroborty S, Goussakov I, Miller MB, Stutzmann GE. Deviant ryanodine receptor-mediated calcium release resets synaptic homeostasis in presymptomatic 3xTg-AD mice. *J Neurosci* 2009; **29:** 9458–70.

58 Cheung K-H, Shineman D, Müller M, et al. Mechanism of Ca^{2+} disruption in Alzheimer's disease by presenilin regulation of InsP3 receptor channel gating. *Neuron* 2008; **58:** 871–83.

59 Stutzmann GE, Smith I, Caccamo A, Oddo S, LaFerla FM, Parker I. Enhanced ryanodine receptor recruitment contributes to Ca^{2+} disruptions in young, adult, and aged Alzheimer's disease mice. *J Neurosci* 2006; **26:** 5180–89.

60 Bezprozvanny I, Mattson MP. Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci* 2008; **31:** 454–63.

61 Nelson O. Familial Alzheimer disease linked mutations specifically disrupt Ca^{2+} leak function of presenilin 1. *J Clin Investig* 2007; **117:** 1230–39.

62 Zhang C, Wu B, Beglopoulos V, et al. Presenilins are essential for regulating neurotransmitter release. *Nature* 2009; **460:** 632–36.

63 Saura C, Choi S-Y, Beglopoulos V, et al. Loss of presenilin function causes impairments of memory and synaptic plasticity followed by age-dependent neurodegeneration. *Neuron* 2004; **42:** 23–36.

64 De Strooper B. Loss-of-function presenilin mutations in Alzheimer disease. Talking point on the role of presenilin mutations in Alzheimer disease. *EMBO Rep* 2007; **8:** 141–46.

65 Wolfe MS. When loss is gain: reduced presenilin proteolytic function leads to increased Abeta42/Abeta40. Talking point on the role of presenilin mutations in Alzheimer disease. *EMBO Rep* 2007; **8:** 136–40.

66 Bentahir M, Nyabi O, Jan V, et al. Presenilin clinical mutations can affect gamma-secretase activity by different mechanisms. *J Neurochem* 2006; **96:** 732–42.

67 Song W, Nadeau P, Yuan M, Yang X, Shen J, Yankner BA. Proteolytic release and nuclear translocation of Notch-1 are induced by presenilin-1 and impaired by pathogenic presenilin-1 mutations. *Proc Natl Acad Sci USA* 1999; **96:** 6959–63.

68 Schroeter EH, Ilagan MX, Brunkan AL, et al. A presenilin dimer at the core of the gamma-secretase enzyme: insights from parallel analysis of Notch 1 and APP proteolysis. *Proc Natl Acad Sci USA* 2003; **100:** 13075–80.

69 Kumar-Singh S, Theuns J, van Broeck B, et al. Mean age-of-onset of familial Alzheimer disease caused by presenilin mutations correlates with both increased Abeta42 and decreased Abeta40. *Hum Mutat* 2006; **27:** 686–95.

70 Galvan V, Gorostiza OF, Banwait S, et al. Reversal of Alzheimer's-like pathology and behavior in human APP transgenic mice by mutation of Asp664. *Proc Natl Acad Sci USA* 2006; **103:** 7130–35.

71 Walsh DM, Klyubin I, Fadeeva JV, et al. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 2002; **416:** 535–39.

72 Lesné S, Koh MT, Kotilinek L, et al. A specific amyloid-beta protein assembly in the brain impairs memory. *Nature* 2006; **440:** 352–57.

73 Citron M, Oltersdorf T, Haass C, et al. Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. *Nature* 1992; **360:** 672–74.

74 Serneels L, Van Biervliet J, Craessaerts K, et al. Gamma-secretase heterogeneity in the Aph1 subunit: relevance for Alzheimer's disease. *Science* 2009; **324:** 639–42.

75 Li T, Wen H, Brayton C, et al. Moderate reduction of gamma-secretase attenuates amyloid burden and limits mechanism-based liabilities. *J Neurosci* 2007; **27:** 10849–59.

76 Nishitomi K, Sakaguchi G, Horikoshi Y, et al. BACE1 inhibition reduces endogenous Abeta and alters APP processing in wild-type mice. *J Neurochem* 2006; **99:** 1555–63.

77 Bernardi L, Tomaino C, Anfossi M, et al. Late onset familial Alzheimer's disease: novel presenilin 2 mutation and PS1 E318G polymorphism. *J Neurol* 2008; **255:** 604–06.

78 Sherrington R, Froelich S, Sorbi S, et al. Alzheimer's disease associated with mutations in presenilin 2 is rare and variably penetrant. *Hum Mol Genet* 1996; **5:** 985–88.

79 Lai MT, Chen E, Crouthamel MC, et al. Presenilin-1 and presenilin-2 exhibit distinct yet overlapping gamma-secretase activities. *J Biol Chem* 2003; **8:** 8.

80 Zhao B, Yu M, Neitzel M, et al. Identification of gamma-secretase inhibitor potency determinants on presenilin. *J Biol Chem* 2008; **283:** 2927–38.

81 Van Broeckhoven C, Backhovens H, Cruts M, et al. APOE genotype does not modulate age of onset in families with chromosome 14 encoded Alzheimer's disease. *Neurosci Lett* 1994; **169:** 179–80.

82 Lendon CL, Martinez A, Behrens IM, et al. E280A PS-1 mutation causes Alzheimer's disease but age of onset is not modified by ApoE alleles. *Hum Mutat* 1997; **10:** 186–95.

83 Portet F, Dauvilliers Y, Campion D, et al. Very early onset AD with a de novo mutation in the presenilin 1 gene (Met 233 Leu). *Neurology* 2003; **61:** 1136–37.

84 Payami HP, Schellenberg GDP, Zareparsi SM, et al. Evidence for association of HLA-A2 allele with onset age of Alzheimer's disease. *Neurology* 1997; **49:** 512–18.

85 Kwok JBJ, Taddei K, Hallupp M, et al. Two novel (M233T and [rho]278T) presenilin-1 mutations in early-onset Alzheimer's disease pedigrees and preliminary evidence for association of presenilin-1 mutations with a novel phenotype. *Neuroreport* 1997; **8:** 1537–42.

86 Verkkoniemi A, Somer M, Rinne JO, et al. Variant Alzheimer's disease with spastic paraparesis: clinical characterization. *Neurology* 2000; **54:** 1103–09.

87 Crook R, Verkkoniemi A, Perez-Tur J, et al. A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin 1. *Nat Med* 1998; **4:** 452–55.

88 Houlden H, Baker M, McGowan E, et al. Variant Alzheimer's disease with spastic paraparesis and cotton wool plaques is caused by PS-1 mutations that lead to exceptionally high amyloid-beta concentrations. *Ann Neurol* 2000; **48:** 806–08.

89 Steiner H, Revesz T, Neumann M, et al. A pathogenic presenilin-1 deletion causes abberrant Abeta42 production in the absence of congophilic amyloid plaques. *J Biol Chem* 2001; **276:** 7233–39.

90 Ataka S, Tomiyama T, Takuma H, et al. A novel presenilin-1 mutation (Leu85Pro) in early-onset Alzheimer disease with spastic paraparesis. *Arch Neurol* 2004; **61:** 1773–76.

91 Rudzinski LA, Fletcher RM, Dickson DW, et al. Early onset familial Alzheimer disease with spastic paraparesis, dysarthria, and seizures and N135S mutation in PSEN1. *Alzheimer Dis Assoc Disord* 2008; **22:** 299–307.

92 Finckh U, Müller-Thomsen T, Mann U, et al. High prevalence of pathogenic mutations in patients with early onset dementia detected by sequence analyses of four different genes. *Am J Hum Genet* 2000; **66:** 110–17.

93 Hattori S, Sakuma K, Wakutani Y, et al. A novel presenilin 1 mutation (Y154N) in a patient with early onset Alzheimer's disease with spastic paraparesis. *Neurosci Lett* 2004; **368:** 319–22.

94 Moretti P, Lieberman AP, Wilde EA, et al. Novel insertional presenilin 1 mutation causing Alzheimer disease with spastic paraparesis. *Neurology* 2004; **62:** 1865–68.

95 Moehlmann T, Winkler E, Xia X, et al. Presenilin-1 mutations of leucine 166 equally affect the generation of the Notch and APP intracellular domains independent of their effect on AB42 production. *Proc Natl Acad Sci USA* 2002; **99:** 8025–30.

96 Sodeyama N, Iwata T, Ishikawa K, et al. Very early onset Alzheimer's disease with spastic paraparesis associated with a novel presenilin 1 mutation (Phe237Ile). *J Neurol Neurosurg Psychiatry* 2001; **71:** 556–57.

97 Judith M, Kevin T, Domizio S, et al. Two novel presenilin-1 mutations (Y256S and Q222H) are associated with early-onset Alzheimer's disease. *Neurobiol Aging* 2003; **24:** 655–62.

98 Caballero PEJ, Boguna CdD, Correa EM, Candela MS, Alonso CM. A novel presenilin 1 mutation (V261L) associated with presenile Alzheimer's disease and spastic paraparesis. *Eur J Neurol* 2008; **15:** 991–94.

99 Farlow M, Murrell J, Unverzagt F, et al. Familial Alzheimer's disease with spastic paraparesis associated with a mutation at codon 261 of the presenilin 1 gene. In: Iqbal K, Sisodia SS, Winblad B, eds. Alzheimer's disease: advances in etiology, pathogenesis and therapeutics. Chichester: John Wiley; 2001: 53–60.

100 Jacquemont ML, Campion D, Hahn V, et al. Spastic paraparesis and atypical dementia caused by PSEN1 mutation (P264L), responsible for Alzheimer's disease. *J Med Genet* 2002; **39:** e2.

101 Matsubara-Tsutsui M, Yasuda M, Yamagata H, et al. Molecular evidence of presenilin 1 mutation in familial early onset dementia. *Am J Med Genet* 2002; **114:** 292–98.

102 Assini A, Terreni L, Borghi R, et al. Pure spastic paraparesis associated with a novel presenilin 1 R278K mutation. *Neurology* 2003; **60:** 150.

103 Raman A, Lin X, Suri M, Hewitt M, Constantinescu CS, Phillips MF. A presenilin 1 mutation (Arg278Ser) associated with early onset Alzheimer's disease and spastic paraparesis. *J Neurol Sci* 2007; **260:** 78–82.

104 O'Riordan S, McMonagle P, Janssen JC, et al. Presenilin-1 mutation (E280G), spastic paraparesis, and cranial MRI white-matter abnormalities. *Neurology* 2002; **59:** 1108–10.

105 Rogaeva E, Bergeron C, Sato C, et al. PS1 Alzheimer's disease family with spastic paraplegia: the search for a gene modifier. *Neurology* 2003; **61:** 1005–07.

106 Tabira T, Chui D, Nakayama H, Kuroda S, Shibuya M. Alzheimer's disease with spastic paresis and cotton wool type plaques. *J Neurosci Res* 2002; **70:** 367–72.

107 Marrosu MG, Floris G, Costa G, et al. Dementia, pyramidal system involvement, and leukoencephalopathy with a presenilin 1 mutation. *Neurology* 2006; **66:** 108–11.

108 Brooks WS, Kwok JBJ, Kril JJ, et al. Alzheimer's disease with spastic paraparesis and 'cotton wool' plaques: two pedigrees with PS-1 exon 9 deletions. *Brain* 2003; **126:** 783–91.

109 Smith J, Kwok J, McLean C, et al. Variable phenotype of Alzheimer's disease with spastic paraparesis. *Ann Neurol* 2001; **49:** 125–29.

110 Mehrabian S, Traykov, L, Rademakers R, et al. A novel PSEN1 mutation in an EOAD family with spastic paraparesis and extrapyramidal signs. *Eur J Neurol* 2004; **11** (suppl 2)**:** 16 (abstr SC123).

111 Yasuda M, Maeda S, Kawamata T, et al. Novel presenilin-1 mutation with widespread cortical amyloid deposition but limited cerebral amyloid angiopathy. *J Neurol Neurosurg Psychiatry* 2000; **68:** 220–23.

112 Atsushi I, Yue-Shan P, Akinori M, et al. A mutant PSEN1 causes dementia with lewy bodies and variant Alzheimer's disease. *Ann Neurol* 2005; **57:** 429–34.

113 Larner A, Doran M. Clinical phenotypic heterogeneity of Alzheimer's disease associated with mutations of the presenilin-1 gene. *J Neurol* 2006; **253:** 139–58.

114 Verkkoniemi A, Kalimo H, Paetau A, et al. Variant Alzheimer disease with spastic paraparesis: neuropathological phenotype. *J Neuropathol Exp Neurol* 2001; **60:** 483–92.

115 Lazarov O, Morfini GA, Pigino G, et al. Impairments in fast axonal transport and motor neuron deficits in transgenic mice expressing familial Alzheimer's disease-linked mutant presenilin 1. *J Neurosci* 2007; **27:** 7011–20.

116 Pigino G, Morfini G, Pelsman A, Mattson MP, Brady ST, Busciglio J. Alzheimer's presenilin 1 mutations impair kinesin-based axonal transport. *J Neurosci* 2003; **23:** 4499–508.

117 Amtul Z, Lewis PA, Piper S, et al. A presenilin 1 mutation associated with familial frontotemporal dementia inhibits gamma-secretase cleavage of APP and notch. *Neurobiol Dis* 2002; **9:** 269–73.

118 Raux G, Gantier R, Thomas-Anterion C, et al. Dementia with prominent frontotemporal features associated with L113P presenilin 1 mutation. *Neurology* 2000; **55:** 1577–79.

119 Dermaut B, Kumar-Singh S, Engelborghs S, et al. A novel presenilin 1 mutation associated with Pick's disease but not beta-amyloid plaques. *Ann Neurol* 2004; **55:** 617–26.

120 Boeve BF, Baker M, Dickson DW, et al. Frontotemporal dementia and parkinsonism associated with the IVS1+1G->A mutation in progranulin: a clinicopathologic study. *Brain* 2006; **129:** 3103–14.

121 van der Zee J, Sleegers K, Broeckhoven CV. The Alzheimer disease-frontotemporal lobar degeneration spectrum. *Neurology* 2008; **71:** 1191–97.

122 Snider BJ, Norton J, Coats MA, et al. Novel presenilin 1 mutation (S170F) causing Alzheimer disease with Lewy bodies in the third decade of life. *Arch Neurol* 2005; **62:** 1821–30.

123 Piscopo P, Marcon G, Piras MR, et al. A novel PSEN2 mutation associated with a peculiar phenotype. *Neurology* 2008; **70:** 1549–54.

124 Dovey HF, John V, Anderson JP, et al. Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in brain. *J Neurochem* 2001; **76:** 173–81.

125 Cullion K, Draheim KM, Hermance N, et al. Targeting the Notch1 and mTOR pathways in a mouse T-ALL model. *Blood* 2009; **113:** 6172–81.

126 Engin F, Bertin T, Ma O, et al. Notch signaling contributes to the pathogenesis of human osteosarcomas. *Hum Mol Genet* 2009; **18:** 1464–70.

127 Meng RD, Shelton CC, Li Y-M, et al. Gamma-secretase inhibitors abrogate oxaliplatin-induced activation of the Notch-1 signaling pathway in colon cancer cells resulting in enhanced chemosensitivity. *Cancer Res* 2009; **69:** 573–82.

128 Arumugam TV, Chan SL, Jo D-G, et al. Gamma secretase-mediated Notch signaling worsens brain damage and functional outcome in ischemic stroke. *Nat Med* 2006; **12:** 621–23.

129 Jurynczyk M, Jurewicz A, Bielecki B, Raine C, Selmaj K. Inhibition of Notch signaling enhances tissue repair in an animal model of multiple sclerosis. *J Neuroimmunol* 2005; **170:** 3–10.

130 Milano J. Modulation of Notch processing by gamma-secretase inhibitors causes intestinal goblet cell metaplasia and induction of genes known to specify gut secretory lineage differentiation. *Toxicol Sci* 2004; **82:** 341–58.

131 Searfoss GH, Jordan WH, Calligaro DO, Galbreath EJ, Schirtzinger LM, Berridge BR. Adipsin, a biomarker of gastrointestinal toxicity mediated by a functional gamma-secretase inhibitor. *J Biol Chem* 2003; **278:** 46107–16.

132 Lewis HD. Apoptosis in T cell acute lymphoblastic leukemia cells after cell cycle arrest induced by pharmacological inhibition of notch signaling. *Chem Biol* 2007; **14:** 209–19.

133 Palomero T. CUTLL1, a novel human T-cell lymphoma cell line with t(7;9) rearrangement, aberrant NOTCH1 activation and high sensitivity to gamma-secretase inhibitors. *Leukemia* 2006; **20:** 1279–87.

134 Real PJ, Ferrando AA. NOTCH inhibition and glucocorticoid therapy in T-cell acute lymphoblastic leukemia. *Leukemia* 2009; **23:** 1374–47.

135 Effects of LY450139, on the progression of Alzheimer's disease as compared with placebo (IDENTITY-2). http://www.clinicaltrials.gov/ct2/show/NCT00762411?term=semagacestat&rank=2 (accessed November, 2009).

136 Siemers ER, Quinn JF, Kaye J, et al. Effects of a gamma-secretase inhibitor in a randomized study of patients with Alzheimer disease. *Neurology* 2006; **66:** 602–04.

137 Fleisher AS, Raman R, Siemers ER, et al. Phase 2 safety trial targeting amyloid-beta production with a gamma-secretase inhibitor in Alzheimer disease. *Arch Neurol* 2008; **65:** 1031–38.

138 Siemers E, Skinner M, Dean RA, et al. Safety, tolerability, and changes in amyloid beta concentrations after administration of a gamma-secretase inhibitor in volunteers. *Clin Neuropharmacol* 2005; **28:** 126–32.

139 Mayer SC, Kreft AF, Harrison B, et al. Discovery of begacestat, a Notch-1-sparing gamma-secretase inhibitor for the treatment of Alzheimer's disease. *J Med Chem* 2008; **51:** 7348–51.

140 Martone RL, Zhou H, Atchison K, et al. Begacestat (GSI-953): a novel, selective thiophene sulfonamide inhibitor of amyloid precursor protein gamma-secretase for the treatment of Alzheimer's disease. *J Pharmacol Exp Ther* 2009; **331:** 598–608.

141 Alzheimer Research Forum. Drugs in clinical trials. http://www.alzforum.org/drg/drc/default.asp?type=keyword&keyword=secretase+inhibitor (accessed Nov 7, 2009).

142 A phase II, multicenter, double blind, placebo-controlled safety, tolerability study of BMS-708163 in patients with mild to moderate Alzheimer's disease. http://www.clinicaltrials.gov/ct2/show/NCT00810147?term=BMS-708163&rank=2 (accessed Nov 7, 2009).

143 Pui C-H. T cell acute lymphoblastic leukemia: NOTCHing the way toward a better treatment outcome. *Cancer Cell* 2009; **15:** 85–87.

144 Real PJ, Tosello V, Palomero T, et al. Gamma-secretase inhibitors reverse glucocorticoid resistance in T cell acute lymphoblastic leukemia. *Nat Med* 2009; **15:** 50–58.

145 Real PJ, Tosello V, Palomero T, Castillo M, Hernando E, de Stanchina E. Gamma-secretase inhibitors reverse glucocorticoid resistance in T cell acute lymphoblastic leukemia. *Nat Med* 2009; **15:** 50–58.

146 Weggen S, Eriksen JL, Das P, et al. A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. *Nature* 2001; **414:** 212–16.

147 Fraering PC, Ye W, LaVoie MJ, Ostaszewski BL, Selkoe DJ, Wolfe MS. Gamma-secretase substrate selectivity can be modulated directly via interaction with a nucleotide binding site. *J Biol Chem* 2005; **280:** 41987–96.

148 Netzer WJ, Dou F, Cai D, et al. Gleevec inhibits beta-amyloid production but not Notch cleavage. *Proc Natl Acad Sci USA* 2003; **100:** 12444–49.

149 Lim GP, Yang F, Chu T, et al. Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. *J Neurosci* 2000; **20:** 5709–14.

150 Kukar TL, Ladd TB, Bann MA, et al. Substrate-targeting gamma-secretase modulators. *Nature* 2008; **453:** 925–29.

151 Tabet N, Feldman H. Indomethacin for Alzheimer's disease. *Cochr Database Syst Rev* 2002; **2:** CD003673.

152 Aisen PS, Schafer KA, Grundman M, et al. Effects of rofecoxib or naproxen vs placebo on Alzheimer disease progression: a randomized controlled trial. *JAMA* 2003; **289:** 2819–26.

153 Hüll M, Berger M, Heneka M. Disease-modifying therapies in Alzheimer's disease: how far have we come? *Drugs* 2006; **66:** 2075–93.

154 Eriksen JL, Sagi SA, Smith TE, et al. NSAIDs and enantiomers of flurbiprofen target gamma-secretase and lower Abeta42 in vivo. *J Clin Invest* 2003; **112:** 440–49.

155 Beher D, Clarke EE, Wrigley JDJ, et al. Selected non-steroidal anti-inflammatory drugs and their derivatives target gamma-secretase at a novel site: evidence for an allosteric mechanism. *J Biol Chem* 2004; **279:** 43419–26.

156 Morihara T, Chu T, Ubeda O, Beech W, Cole GM. Selective inhibition of Abeta42 production by NSAID R-enantiomers. *J Neurochem* 2002; **83:** 1009–12.

157 Ren Z, Schenk D, Basi GS, Shapiro IP. Amyloid-protein precursor juxtamembrane domain regulates specificity of gamma-secretase-dependent cleavages. *J Biol Chem* 2007; **282:** 35350–60.

158 Kukar T, Prescott S, Eriksen J, et al. Chronic administration of R-flurbiprofen attenuates learning impairments in transgenic amyloid precursor protein mice. *BMC Neuroscience* 2007; **8:** 54.

159 Wilcock GK, Black SE, Hendrix SB, Zavitz KH, Swabb EA, Laughlin MA. Efficacy and safety of tarenflurbil in mild to moderate Alzheimer's disease: a randomised phase II trial. *Lancet Neurol* 2008; **7:** 483–93.

160 Green RC, Schneider, LS, Amato DA, et al; for the Tarenflurbil Phase 3 Study Group. Effect of tarenflurbil on cognitive decline and activities of daily living in patients with mild Alzheimer disease: a randomized controlled trial. *JAMA* 2009; **302:** 2557–64.

161 Imbimbo BP, Hutter-Paier B, Villetti G, et al. CHF5074, a novel gamma-secretase modulator, attenuates brain beta-amyloid pathology and learning deficit in a mouse model of Alzheimer's disease. *Br J Pharmacol* 2009; **156:** 982–93.

162 Imbimbo BP, Del Giudice E, Colavito D, et al. 1-(3´,4´-Dichloro-2-fluoro[1,1'-biphenyl]-4-yl)-cyclopropanecarboxylic acid (CHF5074), a novel gamma-secretase modulator, reduces brain beta-amyloid pathology in a transgenic mouse model of Alzheimer's disease without causing peripheral toxicity. *J Pharmacol Exp Ther* 2007; **323:** 822–30.

163 Hébert SS, Serneels L, Dejaegere T, et al. Coordinated and widespread expression of gamma-secretase in vivo: evidence for size and molecular heterogeneity. *Neurobiol Dis* 2004; **17:** 260–72.

164 Shirotani K, Edbauer D, Prokop S, Haass C, Steiner H. Identification of distinct gamma-secretase complexes with different APH-1 variants. *J Biol Chem* 2004; **279:** 41340–45.

165 Schroeter EH. A presenilin dimer at the core of the gamma-secretase enzyme: insights from parallel analysis of Notch 1 and APP proteolysis. *Proc Natl Acad Sci USA* 2003; **100:** 13075–80.

166 Takasugi N, Tomita T, Hayashi I, et al. The role of presenilin cofactors in the gamma-secretase complex. *Nature* 2003; **422:** 438–41.

167 Serneels L, Dejaegere T, Craessaerts K, et al. Differential contribution of the three Aph1 genes to gamma-secretase activity in vivo. *Proc Natl Acad Sci USA* 2005; **102:** 1719–24.

168 Ma G, Li T, Price DL, Wong PC. APH-1a is the principal mammalian APH-1 isoform present in gamma-secretase complexes during embryonic development. *J Neurosci* 2005; **25:** 192–98.

169 Herreman A, Hartmann D, Annaert W, et al. Presenilin 2 deficiency causes a mild pulmonary phenotype and no changes in amyloid precursor protein processing but enhances the embryonic lethal phenotype of presenilin 1 deficiency. *Proc Natl Acad Sci USA* 1999; **96:** 11872–77.

170 Ni YX, Zhao XH, Bao GB, et al. Activation of beta-adrenergic receptor stimulates gamma-secretase activity and accelerates amyloid plaque formation. *Nat Med* 2006; **12:** 1390–96.

171 Thathiah A, Spittaels K, Hoffmann M, et al. The orphan G protein-coupled receptor 3 modulates amyloid-beta peptide generation in neurons. *Science* 2009; **323:** 946–51.