Understanding the roles of mutations in the amyloid precursor protein in Alzheimer Disease.

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Abstract

Many models of disease progression in Alzheimer's disease (AD) have been proposed to help guide experimental design and aid the interpretation of results. Models focussing on the genetic evidence include the amyloid cascade (ACH) and presenilin (PSH) hypotheses and the amyloid precursor protein (APP) matrix approach (AMA), of which the ACH has held a dominant position for over two decades. However, the ACH has never been fully accepted and has not yet delivered on its therapeutic promise. We review the ACH, PSH and AMA in relation to levels of APP proteolytic fragments reported from AD-associated mutations in *APP*. Different *APP* mutations have diverse effects on the levels of APP proteolytic fragments. This evidence is consistent with at least three disease pathways that can differ between familial and sporadic AD and two pathways associated with cerebral amyloid angiopathy. We cannot fully evaluate the ACH, PSH and AMA in relation to the effects of mutations in *APP* as the APP proteolytic system has not been investigated systematically. The confounding effects of sequence homology, complexity of competing cleavages and antibody cross reactivities all illustrate limitations in our understanding of the roles these fragments and the APP proteolytic system as a whole in normal aging and disease play. Current experimental design should be refined to generate clearer evidence, addressing both aging and complex disorders with standardised reporting formats. A more flexible theoretical framework capable of accommodating the complexity of the APP proteolytic system is required to integrate available evidence.

Word count = 244

Introduction

Alzheimer's disease (AD) is a clinicopathologically defined condition associated with aging and genetic causative or risk factors that leads to increasing cognitive impairment, difficulties in everyday living and neurodegeneration. There is no single accepted cause. In early-onset inherited forms of AD (FAD), accounting for <1% of dementia cases in populations [1], the presence of fully penetrant mutation in the amyloid precursor protein (*APP*), presenilin (PS) 1 (*PSEN1*) or more rarely PS 2 (*PSEN2*) confirms a diagnosis of AD. In late-onset sporadic AD (SAD), accounting for the majority of dementia syndrome, a clinical diagnosis can only be "probable" AD [2, 3] and is confirmed neuropathologically after death by deposits of amyloid beta protein (Aβ) and the presence of aggregated microtubule associated protein tau in neurofibrillary tangles (NFT) and neuritic plaques (NP) [4, 5]. Increasing use is being made of clinical imaging and standardised diagnostic criteria have been proposed [6] however, imaging and other biomarkers do not always correlate [7, 8]. AD-associated pathology may be present in those without cognitive impairment [9], does not correlate well with clinical dementia, and is associated additionally with ageing [10], raising questions around what the neuropathology represents. Dementia in the older population is rarely "pure" AD, and presents neuropathologically with mixed vascular and degenerative features [11, 12]. Thus, while the co-segregation of pathogenic and fully penetrant mutations within the same family permits diagnosis of monogenic FAD with a high degree of certainty, there is currently no unified clinical, neuropathological or molecular definition of SAD [13, 14].

Various experimental approaches have contributed to the body of evidence relating to AD. Clinical [2, 3, 15] and neuropathological features [4, 5] have been described. Blood and cerebrospinal fluid based biomarkers [16, 17], and MRI with markers such as Pittsburgh compound B [18] are being developed with the aim of following disease progression in humans. However, no marker reliably associates with clinical dementia [16, 18] in diagnosis or disease progression.

Various hypotheses have been proposed to guide investigations into disease pathways associated with AD, focussing on areas known to be perturbed in AD including the immune system [19-22], mitochondria and oxidative stress [23, 24], metabolism and diabetes [25-28], cholesterol regulation [29, 30], cell cycle [31, 32], neurotransmitters including acetylcholine in synaptic plasticity [33-37] and the role of tau deposition and tau oligomers [38-42]. However, none of these relate directly to interpreting the genetic evidence regarding the role of *APP* mutations in FAD. Hypotheses relating to the genetic evidence include the amyloid cascade hypothesis (ACH) [43-45], the presenilin hypothesis (PSH) [46] and the APP matrix approach (AMA) [47, 48] and of these, the ACH has maintained a dominant position guiding research for over two decades.

The ACH has not been universally accepted and periodic discussions have raised questions relating to the assertion that Aβ is causal in all forms of AD [14, 49-52] and instead highlight the complexity of the APP proteolytic system. Supporters of the ACH have referred to genetic evidence, where mutations associated with FAD lead to change in the expression of the various Aβ peptides, and Occam's razor, where clinical and neuropathological presentations of those with AD of both familial and sporadic forms share common features and so should be approached therapeutically as similar entities. Those that don't accept the ACH cite human studies where evidence is highly heterogenic [1, 12, 53, 54] and suggest that multiple pathways are possible [48]. The argument has two main perspectives, either Aβ is causal in AD and represents a unifying pathway to disease or complexity leads to multiple disease pathways.

Given that recent clinical trials guided by the ACH have not been as successful as hoped [55], it is important at this time to examine these hypotheses in greater detail with respect to accumulating evidence to see where failures in the translation of pre-clinical research to the human population might occur. Using mutations in *APP* as an illustrative example, we ask whether the research

community is well guided by the current hypotheses or whether a change in approach might bring new understanding.

The amyloid cascade hypothesis

The ACH interprets the genetic evidence from FAD to suggest that fully penetrant mutations in *APP* and *PSEN*s lead to changes in the levels of neurotoxic Aβ that initiate AD pathways [44, 45]. The original hypothesis, (Figure 1a), proposed that AD was caused by increased levels of Aβ however, this has been updated to include increased ratio of Aβ(1-42)/Aβ(1-40) [44, 45] or oligomers [56, 57]. All other features of AD, such as tau aggregation, inflammation, reduced metabolism, perturbed neural networks and cognitive impairments are proposed to follow on from causal events associated with increased Aβ [44, 45, 57]. The ACH assumes that all FAD mutations share molecular pathways associated with increases in neurotoxic forms of Aβ and in SAD, increased levels of Aβ, perhaps due to impaired degradation and clearance, contribute to disease [58, 59], therefore all FAD will respond to the therapeutic removal of Aβ. The ACH proposes that since SAD and FAD share common clinical and neuropathological features, then by Occam's razor, the simplest explanation suggests that FAD and SAD also share these disease mechanisms and therapeutic strategies developed for FAD should be applicable in SAD.

The presenilin hypothesis

~95% of FAD is caused by mutations in *PSEN1*. The PSH [46, 60], (Figure 1b), interprets the genetic data from *PSENs* mutations as showing loss of PS function, with several mutations showing almost complete abolition of γ-secretase activity with loss of physiologically relevant Aβ [60-63]. This contrasts with the over-production of Aβ or Aβ42 required by the ACH. However, some suggest that *PSENs* associated pathways may involve gain of function effects that are compatible with the ACH

such as increased Aβ42/43 [64-67]. Complex patterns of both gain and loss of PS functions that vary with each mutation [68] may better describe the contributions of *PSENs* mutations to variations seen in clinical features such as age of onset and seizures [69] and neuropathology [70].

The results from the randomised controlled trial of the γ-secretase inhibitor Semagacestat showing a worsening of dementia with increased risk of skin cancer [71] coupled with recent evidence of no clear associations between age of onset and Aβ levels or Aβ40/Aβ42 [62] support the PSH and suggest that enhancing γ-secretase could be a valuable therapeutic approach. Recent neuropathological evidence of increased size and number of cored amyloid plaques coupled with more severe cerebral amyloid angiopathy (CAA) and plaque distribution around vessels in those with *PSEN1* mutations after codon 200 compared to those with mutations before codon 200 suggest that *PSEN1* mutations may be associated with at least 2 disease pathways [72]. Whether these pathways relate to the dual carboxypeptide pathways associated with the production of Aβ [67] remains to be investigated.

Differences in levels of Aβ40 and Aβ42 [73] and differences in the APP β carboxy terminal fragment [74] between *PSENs* associated FAD and SAD, raise questions relating to the general applicability of the PSH. While studies have shown a rare coding variability in *PSEN1* may influence the susceptibility for apparently sporadic late-onset AD [75, 76], increases in Aβ production may not explain the majority of SAD cases. The PSH suggests that *APP* mutations around the α-, β'- and β-cleavage sites may act via conformational change to alter γ-cleavages, however, it is equally possible that this hypothesis may not be relevant to all FAD deriving from mutations in *APP* around the α-, β'- and β- cleavage sites. As with the ACH, the PSH focuses on Aβ as the outcome of interest however it could be usefully updated to include considerations of all products from γ-cleavage since loss or gain of function may affect all products equally [77]. The PSH allows for multiple pathways depending on the exact nature of the change in γ-cleavage arising from the different *PSENs* mutations [78]. The

complex mix of gain and loss of function for different *PSENs* mutations suggests that one therapeutic approach may not be adequate. A detailed investigation of the relationships between specific *PSENs* mutations and Alzheimer disease pathways is beyond the scope of this review however, a similar approach examining the proteolytic fragments for each *PSENs* mutation could usefully clarify our understanding of the contributions of *PSENs* mutations to AD pathways.

The amyloid precursor protein matrix approach

The AMA considers the effects of genetic mutations against the background of dynamic complexity of the APP proteolytic system as a whole. Mutations in *APP* may alter the balance between the different functional areas of this complex system with consequences for a wide variety of cellular processes, (Figure 1c). The functional consequences arising from APP proteolysis can be understood in terms of a dynamic balance between full length APP and fragments from the α- and β'- and β-cleavages as reflected in the ratios of sAPPα/sAPPβ/sAPPβ'/full length APP in functional module A, coupled with functions arising from the synergetic interactions of the P3-type/β'-type/Aβ-type fragments arising from γ-cleavage in functional module B. There are additional functional effects arising from the carboxy terminal membrane fragments (CTFs) following α-, β'- and β- cleavages, the various AICDs following γ- ε- and ζ- cleavages, and caspase cleavage [79]. The levels of sAPPβ/sAPPα may not mirror the levels of the Aβ- type/P3-type peptides as Aβ1-14/15/16 fragments generated either from the C99 membrane fragment [80] or as a product of Aβ catabolism [81, 82] have been reported. Additional η-cleavage has recently been reported, increasing the complexity of this proteolytic system [83, 84]. The expression level of *APP*, increased in Down syndrome (DS) and people with *APP* duplications, has been shown to be rate limiting in the production of Aβ [82], suggesting that α-, β- and other cleavages compete.

According to the AMA, the APP/PS proteolytic system is in dynamic balance around a homeostatic point that allows proper neuronal function. Shifts to either α- or β- pathways may be regulated by wide ranging factors from cholesterol to inflammation and synaptic activity and the system is able to feed forward iteratively via the ever changing ratios of proteolytic fragments that affect the same cell systems involved in its regulation [47, 48, 85]. Each mutation has the potential to alter the balance between the cleavage products and change the behaviour of the fragments to varying degrees depending on changes to hydrophobicity, electrostatic charge and aggregation properties. This can involve different gains or losses of function for each of the fragments and full length APP for each mutation. In effect the APP proteolytic system allows partial contributions to disease from various cellular systems via the complex regulation of all cleavage products in APP proteolysis, including Aβ. While genetic mutations in FAD potentially alter the balance in the APP proteolytic system, changes in the way that different feedback relationships from neuronal systems such as cholesterol homeostasis, immune signalling and synaptic plasticity also potentially alter this balance, leading to the possibility of multiple disease pathways.

In order to evaluate the hypotheses with respect to the relationships between mutations in *APP* and FAD, we examined the consequences of the various *APP* mutations on the levels of the peptide fragments resulting from the APP proteolytic system in studies describing human mutations, presented in Table 1 and Supplementary Table 1. *APP* duplications and triosomy of chromosome 21 found in DS have been included in Table 1 and Supplementary Table 1 for completeness however, given the limited space available, they are discussed only briefly. We considered the evidence from the different perspectives of the alternative hypotheses.

Experimental design, missing data and standardisation

No study in Supplementary Table 1 systematically measured all the APP proteolytic fragments and the roles of different APP proteolytic fragments have not been extensively investigated yet. While the Aβ-type fragments are well represented, other fragments are not, illustrating that our understanding of this complex proteolytic system is incomplete. Specifically, levels of APP, the large N-terminal sAPPα and sAPPβ, the membrane bound C-terminal fragments (CTF), the P3-type peptides and the various APP intracellular domains (AICD) are not well reported. Evidence relating to the APP proteolytic system as a synergistic whole is absent from the literature. From the perspective of the AMA, which focuses on the dynamic balance between all fragments in relation to the cellular environment, the distribution of evidence in Supplementary Table 1 shows that a full understanding of this system is not possible - too much evidence is missing. Although complexity in APP physiology and biochemistry has always been given as an alternative perspective [47-49, 52, 85, 86], this has not been considered in experimental design to date. The confounding complexity in the APP proteolytic system is highlighted in a recent investigation of anti Aβ antibody cross reactivities [87]. Cross reactivities of commonly used antibodies may undermine current interpretations of immunoreactivity and this is especially relevant to neuropathological investigations where only one antibody per feature may be used [72]. The antibody BC05, recognising Aβ C-terminals ending at amino acid 42 or 43, also recognises P3-42/43. BA07, recognising Aβ C-terminal ending at amino acid 40 also recognises P3-40, however very few experimental designs control for this cross reactivity and studies interpret immunoreactivities erroneously as representing Aβ. This confounding affects other antibodies raised against C-terminals [77]. Further cross reactivity may also derive from catabolic fragments of Aβ or Aβ' from cleavage by BACE2 [87]. From the perspective of the ACH, this may not be so important as P3 is not suggested to play a significant role in disease, from the perspective of the AMA, this is a fundamental confound between two or more cleavage pathways and the neuropathological evidence especially should be urgently clarified. Given the potential confounding of evidence relating to Aβ by P3- type and smaller catabolic peptides, current experimental design cannot support interpretations of Aβ as causal nor eliminate considerations of complexity from

disease pathways, raising profound implications for AD research strategies. Experimental designs should be adjusted to explicitly measure and report all proteolytic fragments where sequence homology predicts confounding. Mass spectrometry may be preferable to western blotting in conjunction with a panel of capture antibodies to identify each peptide [88].

Few studies have focussed on P3 type peptides, despite evidence that P3 is known to aggregate [89-92], has been associated with cotton wool type amyloid plaques [93, 94], is present in CAA [95], enhances the aggregation of Aβ1-40 [96], may have a signalling role in apoptosis via caspase activation[97], form Ca^{2+} channels [98] and may be affected similarly to Aβ by changes to γ-cleavage [77]. P3 peptides are not thought to contribute to disease progression in the ACH and their roles in disease and healthy ageing have largely been ignored. The AMA predicts modulatory relationships between P3-type and Aβ-type fragments in their predicted interactions as small binding proteins, (Figure 1c, functional module B), however, current experimental design is inadequate for investigations from this perspective as the AMA requires that each APP proteolytic fragment must be measured in any investigation. Neither the ACH nor PSH consider all fragments from the APP proteolytic system.

The use of cellular systems to investigate expression levels of Aβ is a useful approach to characterising these mutations and has been shown to reflect the amount deposited in the human brain [72]. In addition to different experimental procedures and the use of different cell models, (Supplementary Table 1), the reporting of the various expression levels of the proteolytic fragments is not standardised, making comparison between studies difficult beyond a qualitative measure of increase/decrease or no change. Some studies report concentrations as ng/ml^{-1} or Molar values [99-104], values normalised to full length APP levels [105-110] or total Aβ levels [111-113], relative to cell number [114, 115] or relative to WT/control [116-121]. Standardised reporting and experimental protocols would be useful in comparisons between studies. Given the different experimental

approaches, the qualitative changes in Table 1 appear generalizable and robust. However, given that evidence relating to Aβ is potentially confounded due to cross-reactivity of antibodies [87] we cannot be certain that these data are not confounded by P3.

Does evidence from FAD due to *APP* mutation describe one or many disease pathways?

Although the evidence for some mutations is sparse, the mutations can be grouped according to change in expression levels of the various Aβ fragments, (Table 1 and Figure 2). Group 1 shows increases in total [Aβ], Aβ40, Aβ42 and the Aβ42/Aβ40 ratio and is associated with mutations around the α-secretase site, (Table 1, Figure 2). Mutations in group 1, leading to increased Aβ expression, are compatible with the ACH. Group 2, including the protective *APP* p.A673T mutation [101] and mutations specific to *APP* at codon 693, shows reduced total [Aβ], Aβ40, Aβ42 and the Aβ42/Aβ40 ratio, (Table 1, Figure 2). Group 3 has reduced total [Aβ] and Aβ40 combined with increased Aβ42 and the Aβ42/Aβ40 ratio and these mutations are associated with the γ- secretase site, (Table 1, Figure 2). This third group also compares well with *PSENs* mutations showing similar reductions in total [Aβ] and Aβ40 combined with increased Aβ42 and the Aβ42/Aβ40 ratio [46]. Those that cannot be grouped due to a lack of data are in group x. Triosomy of chromosome 21 in DS and *APP* duplication and mutations in promoter regions that lead to increased levels of APP may show different changes in levels of Aβ species to other mutations and SAD [122, 123]. These genetic alterations may form a fourth group that represents an additional pathogenic pathway [122].

Genetic and molecular data suggest that there are at least three possible pathways to dysfunction and that these can be further modulated by features such as propensity of peptides to aggregate as oligomers and fibrils due to changes in electrostatic or hydrophobic natures of the substituted amino acids. Different molecular pathways associated with FAD have been proposed previously [124, 125] in relation to the phenotypic and neuropathological heterogeneity associated with *APP* mutations

[126] and *PSENs* mutations [53, 127]. In addition, *APP* duplication and triosomy of chromosome 21 appear to increase total tau and tau phosphorylation in a manner independent from Aβ while *PSENs* mutations may not [82]. This interpretation of the evidence contrasts with that of the ACH, which assumes that all forms of AD, inherited and sporadic, should share the same Aβ-related disease pathway.

Increases in Aβ42 seen in FAD with *PSENs* mutations and the *APP* mutations p.KM670/671NL and p.V717I have been found to precede dementia [103, 128], supporting the ACH where increasing Aβ, perhaps specifically Aβ42, is thought to cause AD. However, Scheuner et al also found that the average levels of Aβ42 in 71 individuals with SAD (29+/- 2pmol) were not significantly different to that measured in 75 controls (27 +/-3pmol) [103]. In this study, only 13% of those with SAD and 3% younger controls had elevated Aβ 1-42(3) levels similar to those found in individuals with FAD. This suggests that a minority of SAD may have similarities to *PSENs* associated FAD, supporting the multiple pathways perspective of the AMA and PSH. How imbalance between the all various peptides, including the shorter Aβ peptides [105, 111, 114, 128] contribute to AD disease processes is not clear. According to the ACH, mutations around the γ-cleavage site are associated with increased Aβ42 therefore removal of Aβ42 is a rational therapeutic approach. In contrast, both the PSH and AMA predict that *APP* and *PSENs* mutations associated with reductions in total Aβ may represent disease pathways associated with the loss of Aβ physiological functions [46, 48, 129] and removal of Aβ per se is unlikely to be beneficial; up-regulation of γ-cleavage or addition of physiologically relevant Aβ could be useful in humans.

It is interesting that the group 1 disease associated mutations involve the heparin binding domain, (Figure 2), and mutations N-terminal to this, such as *APP* p.T663M, are neutral. The AMA predicts that the group 1 mutations potentially also affect interactions of full length APP, sAPPα and sAPPβ,

with consequences for disease progression in addition to any affects due to changes in cleavages or behaviour of Aβ. This cannot be assessed with current evidence.

Is Aβ the only defining characteristic of the APP proteolytic system in AD?

The focus on Aβ proposed by the ACH in effect reduces description of the complexity of APP proteolytic system to Aβ levels. While *APP* mutations such as those in group 1 associated with the α-cleavage site lead to increased Aβ production, often with no change to the Aβ42/Aβ40 ratio, where measured, they also lead to a reduction in sAPPα [109, 114, 116]. Those mutations in group 3 showing reduced expression of total [Aβ] and Aβ40, where measured, lead to an increase in sAPPα [116]. Those studies that measure additional fragments [105, 114] independently suggest that it is not possible to assign absolute causality to any one fragment with certainty given the changes in expression or function of full length APP and other fragments.

Functions associated with sAPPα include promotion of long term potentiation (LTP) [130-132], neurite outgrowth [133] and various roles in neuroprotection [134-136]. Significant correlation between low levels of sAPPα and poor cognitive function was found in cases with the *APP* p.KM670/671NL double Swedish mutation while no association was found between the levels of Aβ and cognition [137] and low levels of sAPPα but not sAPPβ in cerebrospinal fluid (CSF) are associated with SAD [138]. The sAPPβ /sAPPα ratio has been found to be higher in those with amyloid neuropathological deposits than those without [139]. Both sAPPα and Aβ have important roles in regulating synaptic plasticity via LTP [130, 131] and long term depression (LTD) [140-142] respectively. Synaptic plasticity may be understood as a dynamic and coherent balance between both LTP and LTD and the AMA predicts that this will be associated with the ratios of sAPPα/sAPPβ coupled with P3/Aβ, (Figure 1c); neither LTP nor LTD alone can typify neurotoxicity or neuroprotection. In a recent study using animal models, immunotherapy targeting Aβ using two

different antibodies resulted in increased cortical hyperactivity and this was proposed to underlie the lack of cognitive improvement seen in human trials [143]. This hyperactivity is consistent with the AMA and PSH, where loss of physiologically relevant Aβ would be expected to reduce LTD and lead to increased hyperactivity via the actions of sAPPα and follow on failure of coherent synaptic plasticity but unexpected according to the ACH, where removal of Aβ would be expected to alleviate neurotoxicity. Taken together, the above evidence suggests that the role of sAPPα in disease progression may be more important than the ACH allows and experimental design should be refined to include sAPPα, sAPPβ and P3 when Aβ is reported with respect to synaptic plasticity.

How do different hypotheses relate to disease heterogeneity?

The mutations in *APP* and *PSENs* genes are only now being comprehensively described and summaries are available via the AD and FTD mutation database curated by Cruts et al [144] and the Alzforum database [145]. Rare mutations and those recently found e.g. *APP* p.D678H [146], *APP* p.K687N [109] and *APP* p.T719P [128], are not adequately described as too few individuals have come to autopsy.

Mutations affecting *APP* at codon 693, Group 2 in Table 1 and Figure 1, have diverse molecular and neuropathological effects. In *APP* Δ693, the charged acidic amino acid glutamic acid is deleted. This mutation is uniquely associated intraneuronal oligomerization with no fibrillization and with very low levels of amyloid [100, 147]. Both *APP* p.E693K, where glutamic acid is substituted by the larger charged basic side chain of lysine [148] and *APP* p.E693Q, where glutamic acid is replaced by the similarly sized, non- charged negatively polar side chain glutamine [107, 149-154], are associated with strokes, CAA and cognitive decline with no tau-related neurofibrillary changes. The *APP* p.E693G mutation, where glutamic acid is replaced by the small non-polar glycine, is associated with CAA, abundant plaques and typical tau related neurofibrillary pathology [102, 153-157]. The *APP*

p.E693K, *APP* p.E693Q and *APP* p.E693G mutations are also associated with increased deposition of Aβ38 not seen in DS, *PSEN1* mutations or sporadic disease [158] while *APP* p.E693G and *APP* p.E693Q are associated with reduced degradation by the insulin degrading enzyme [154]. It is not clear whether overall change in sequence (*APP* ΔE693), size (*APP* p.E693K and *APP* p.E693G), charge (all *APP* at codon 693 substitutions) or partial contributions from all these changes are responsible for the dramatic differences seen in aggregation, disease association and neuropathology for this codon.

The mutation *APP* p.A673V, as well as being associated with disease only in the homozygous state, is distinguished from all other *APP* mutations due to large plaque size and vessel associations [126] however it shares increased deposition of Aβ38 with *APP* mutations at codon 693 [158]. These mutations, associated with several different pathological presentations, perhaps represent different pathways that could be relevant to deposition of Aβ in various forms and tau-related neurofibrillary change. While group 2 may be generally defined by reduced levels of Aβ, individual mutations show unique neuropathological features that may derive from additional properties of any amino acid substitution. In this respect, each the effects of each mutation should be investigated not only with reference to levels of Aβ and other fragments but also the changed molecular properties arising from each mutation. It will be interesting to see if the *APP* p.L705V Italian mutation with CAA, increased deposition of Aβ38 [158] and few plaques is associated with reduced Aβ40, Aβ42 and Aβ42/Aβ40 in common with group 2 and how the change in charge from basic lysine to non-polar valine affects peptide interactions.

The genetic evidence is consistent with interpretations that these mutations lead to CAA affecting vessel walls and deposition of Aβ in brain parenchyma via different but not mutually exclusive disease pathways [148, 159, 160] and this may be usefully investigated in relation to neuropathologically defined CAA types [161, 162]. CAA may be a distinct pathological process from plaque formation, supported by evidence that Aβ(1-42) fragments are associated with diffuse

parenchymal deposits whereas Aβ(1-40) is associated with CAA vascular deposition [148]. Mutations resulting in changes to size and electrostatic charge may be associated with presence of CAA [163] that is independent from any interstitial fluid drainage effects [164, 165].

Mutations associated with FAD collectively offer an opportunity to describe in molecular detail a natural history of over and under expression for Aβ and other APP proteolytic fragments and also the associations with neuropathology and clinical features for each mutation. Following these individuals longitudinally will build a detailed understanding the different relationships between the APP proteolytic system, deposition of Aβ as plaques and CAA and how this proteolytic system relates to neurofibrillary change. A similar approach in populations to fully describe molecular and neuropathological change in ageing and disease will allow the identification of which pathways these mutations promote are most relevant to SAD.

All the hypotheses considered here, the AMA, the PSH and the ACH, allow changes in Aβ, whether due in concentration or structural features associated with substitution of amino acids, to modulate disease pathways. However, the ACH does not adequately explain the group 2 mutations, (Table 1, Figure 2), where levels of Aβ fragments are reduced. For *APP* mutations at codon 693, reduced Aβ is associated with disease, whereas for *APP* p.A673T reduced Aβ is not. A combination of the AMA and PSH for interpretation relating to *APP* mutations may be a better guide for experimental design.

From the perspective of the AMA and PSH, heterogeneity in clinical and neuropathological presentations in FAD and SAD suggests multiple pathways at the molecular level, where therapeutic strategies would be targeted. In contrast, the ACH suggests that all pathways are unified by Aβ and removal of Aβ is the best strategy. However, it is not clear whether what we currently understand as AD represents one or many disease subtypes or how SAD relates to FAD. A more detailed

characterisation of the range of amyloid and neurofibrillary deposits, both in terms of molecular composition and morphological appearance in the human population is urgently required.

Translating pre-clinical AD research to therapeutics

The translation of pre-clinical research to the human population presents significant challenges. Failure to replicate pre-clinical science has become a recent focus [166, 167] with various factors highlighted such as excess significance in animal research [168], poor use of statistics [169] and problems of inter-species generalizability [170, 171]. If we further consider the potential confounding due to anti Aβ antibody cross reactivities [87], we are uncertain as to what are relevant or irrelevant results that should be taken forward as therapeutic targets in AD.

While FAD can be readily identified and separated into subtypes by genetic characterisation, the lack of qualitative clinical markers in SAD is a significant impediment to the design of randomised controlled trials as there is no way to assign cases and controls with certainty. All AD clinical biomarkers lie on continua where thresholds are defined that best separate those with from those without dementia, however, no pre-defined threshold has been applied systematically between studies [16, 18, 172]. The relationship between biomarkers such as CSF or plasma levels of protein fragments, MRI markers of amyloid build up or atrophy and disease progression is not clear and different markers can lead to conflicting results [6, 7]. No biomarkers of AD have been systematically studied in population cohorts where most dementia syndrome occurs and where validity is best tested. Additionally, the relationship between neuropathology and disease progression is not yet fully understood so that it is not clear what the biomarkers or the neuropathology represent in relation to cognitive change. The lack of validated biomarkers underlies the difficulties involved in following human cohorts over time and is a serious limitation in the search for therapeutic treatments.

Within population studies, neuropathological (blind to clinical information) and clinical diagnoses of AD are not well matched and most report cases with dementia and no AD-related neuropathology and cases with significant neuropathological load and no dementia [11]. Case control studies often select cases and controls by combining clinical and neuropathological information with the effect of eliminating these two categories from the study, leading to selection bias and an over-estimate of associations. Population studies avoid this selection bias but because they do not separate out different disease types, this approach leads to under-estimates of associations. Both approaches reveal valuable information and should be used in combination. A population approach would be very useful in describing the clinical, neuropathological and molecular heterogeneity associated with different FAD mutations. This would give a better description of each and find specific differences and commonalities that would tease apart the possible disease pathways and allow the selection of cases and controls in randomised controlled trials with greater confidence.

Conclusions

Simplicity is one advantage of the ACH; it is easy to describe Aβ as neurotoxic and causal in AD. However, this simplicity is also its great weakness in that it does not allow the many roles and changing behaviours of Aβ to be placed in the wider context of the APP proteolytic system as a whole. Experimental design based on the ACH is focussed on Aβ and lacks the systematic approach demanded by the AMA that requires all fragments to be assessed in any investigation. The use of Occam's razor focuses attention solely on Aβ and in effect removes considerations of the complexity of APP physiology and biochemistry from experimental design, creating unnecessary division between the complexity of the APP proteolytic system and Aβ, one of its components. This has led to missing information and a poor understanding of the APP proteolytic system as a whole. We do

not yet have the evidence to say with certainty which model of disease progression is more representative of actual disease pathways in humans.

There are some research questions that the AMA will allow that the ACH does not, especially with respect to the loss of Aβ function and the dynamic balance between all the proteolytic fragments. Since the AMA includes other cellular systems as drivers in the regulation and control of APP proteolytic processing, the AMA throws a spotlight on other hypotheses ranging from those based on factors relating to wider cellular systems such as synaptic plasticity, cholesterol homeostasis, cell cycle, metabolism and oxidative stress, other cell signalling cascades, and ageing in a non-hierarchical manner. This may better represent multifactorial disease pathways recognised in SAD. An integrative approach should lead to a much better understanding of the relationships between all areas involved in AD research. We do not yet have the detailed evidence required to understand the role of the APP proteolytic system either in normal or disease states. We need refined theoretical disease models to generate better experimental designs both clinically and pre-clinically, in order to generate this evidence.

Supplementary information is available at *Molecular Psychiatry's* website

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Figure 1 Hypotheses of disease pathways in AD relevant to the interpretation of *APP* mutations

1a: Adapted from [44]; 1b: adapted from [46, 60]; 1c: Green α-cleavage; Red β-cleavage; Purple β'-cleavage; Blue γ-cleavage; Grey caspase cleavage. Thickness of arrows represents average percentage flow through the pathways as determined by ratios of P3:Aβ':Aβ as described in [111]. Functional block A arises due to the synergistic interactions of full length APP, sAPPα, sAPPβ and sAPPβ' and may involve examples of agonism and antagonism. Functional block B arises due to the synergistic interactions of the various fragment lengths following γ-cleavage with N- and C- terminal variations and may involve examples of agonism and antagonism. Other functions are associated with the AICDs following γ- and caspase cleavages and general catabolism of all fragments not represented here.

Figure 2 Disease associated *APP* Mutations by location

Adapted from [86] and [144]. Groups are defined by the qualitative changes in Aβ levels as described in Table 1.

Author contributions

SH wrote the paper in consultation and with contributions from CB

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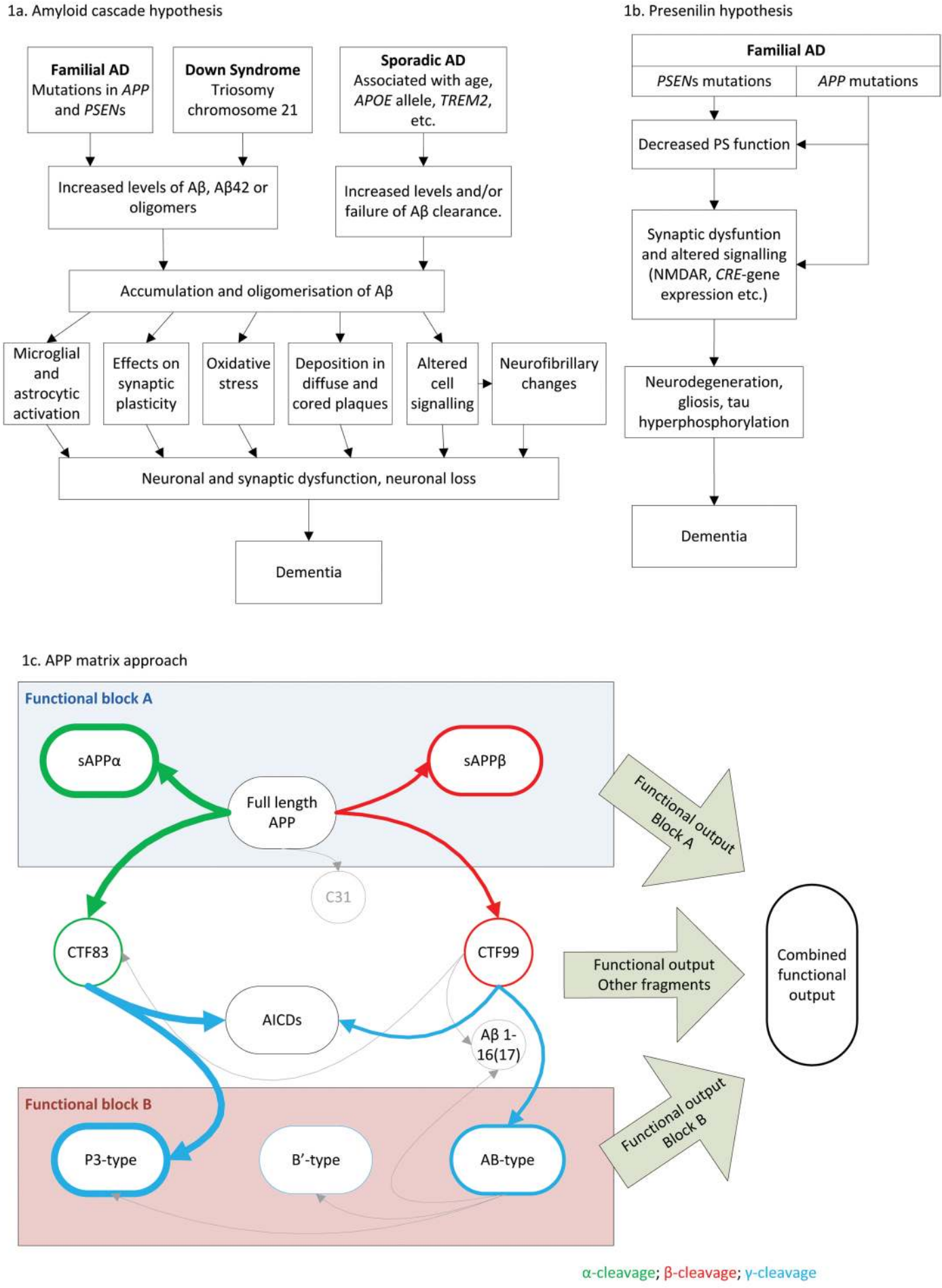
Competing interests

The authors declare that there are no competing interests


1a. Amyloid cascade hypothesis
Familial AD
Mutations in APP and PSENs
Down Syndrome
Triosomy chromosome 21
Sporadic AD
Associated with age, APOE allele, TREM2, etc.
Increased levels of Aβ, Aβ42 or oligomers
Increased levels and/or failure of Aβ clearance.
Accumulation and oligomerisation of Aβ
Microglial and astrocytic activation
Effects on synaptic plasticity
Oxidative stress
Deposition in diffuse and cored plaques
Altered cell signalling
Neurofibrillary changes
Neuronal and synaptic dysfunction, neuronal loss
Dementia
1b. Presenilin hypothesis
Familial AD
PSENs mutations
APP mutations
Decreased PS function
Synaptic dysfuntion and altered signalling (NMDAR, CRE-gene expression etc.)
Neurodegeneration, gliosis, tau hyperphosphorylation
Dementia
1c. APP matrix approach
Functional block A
sAPPα
sAPPβ
Full length APP
C31
CTF83
CTF99
AICDs
Aβ 1-16(17)
Functional block B
P3-type
B'-type
AB-type
Functional output Block A
Functional output Other fragments
Functional output Block B
Combined functional output
α-cleavage; β-cleavage; γ-cleavage

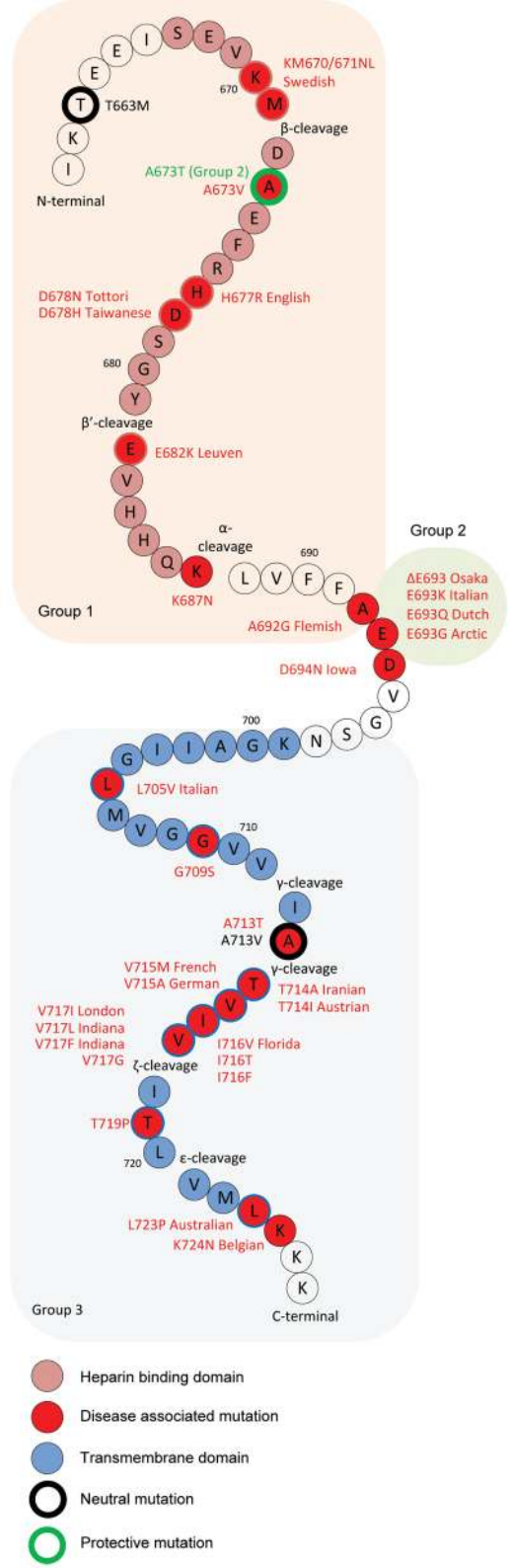

KM670/671NL
Swedish
T663M
β-cleavage
A673T (Group 2)
A673V
N-terminal
D678N Tottori
D678H Taiwanese
H677R English
β′-cleavage
E682K Leuven
α-
cleavage
Group 2
ΔE693 Osaka
E693K Italian
E693Q Dutch
E693G Arctic
K687N
Group 1
A692G Flemish
D694N Iowa
L705V Italian
G709S
γ-cleavage
A713T
A713V
γ-cleavage
V715M French
V715A German
T714A Iranian
T714I Austrian
V717I London
V717L Indiana
V717F Indiana
V717G
I716V Florida
I716T
I716F
ζ-cleavage
T719P
ε-cleavage
L723P Australian
K724N Belgian
C-terminal
Group 3
Heparin binding domain
Disease associated mutation
Transmembrane domain
Neutral mutation
Protective mutation

Table 1 Groupings of pathogenic *APP* mutations according to qualitative changes of Aβ fragments.

Mutation position	Group	Disease association/neuropathology	Fragments	refs
KM670/671NL Swedish	1	AD; numerous plaques and NFT, variable CAA; increases in expression of longer forms of APP but not APP695	↑[Aβ] ↑Aβ40 ↑ Aβ42 = / ↓ Aβ42/Aβ40	[103, 173, 174]
A673T	2	Protective mutation, reduced Aβ, Aβ40 and Aβ42, increased sAPPα	↓[Aβ] ↓ Aβ40 ↓ Aβ42	[101, 106]
A673V	1	AD only when homozygous; extensive Aβ deposition, CAA, increased Aβ40 (not Aβ42) fibrillization; amyloid plaques include both Aβ40 and Aβ42; few diffuse deposits; can be distinguished from other FAD or SAD by large plaque size and vessel associations	↑[Aβ] ↑Aβ40 ↑Aβ42 = Aβ42/Aβ40 ↑ Aβ 11-X	[106, 114, 126]
H677R English	x		=[Aβ] = Aβ42	[175]
D678H Taiwan	1	AD with CAA and micro-haemorrhages; changes in Aβ are extracellular; no change in intracellular levels; increased C99/C83 ratio; no change in BACE2 C89 product; mutation alters APP sorting	↑[Aβ] ↑Aβ40 ↑ Aβ42 ↑Aβ42/Aβ40	[117, 146, 175]
D678N Tottori	x	AD	=[Aβ] = Aβ42	[175, 176]

E682K Leuven	1	AD	↑[Aβ] ↑Aβ40 ↑ Aβ42 ↑Aβ42/Aβ40 ↓ Aβ 11-X	[105]
K687N	1	AD	↑[Aβ] ↑Aβ40 ↑ Aβ42	[109]
A692G Flemish	1	CAA, AD or both; large cored amyloid plaques centred on vessels; in contrast to other AD cored plaques are mostly Aβ40, diffuse Aβ42 deposits; severe neurofibrillary pathology	↑[Aβ] ↑Aβ40 ↑ Aβ42 ↑Aβ42/Aβ40	[102, 105, 107, 111, 153, 177-179]
ΔE693 Osaka	2	AD; very low levels of amyloid on PiB MRI; oligomerization with no fibrillization; uniquely increased intraneuronal Aβ oligomers	↓[Aβ] ↓ Aβ40 ↓ Aβ42 ↓ / =Aβ42/Aβ40	[100, 147]
E693K Italian	2	CAA, strokes and cognitive decline; no neurofibrillary changes; capillary CAA associated with Aβ42, vessels associated mostly with Aβ40; Aβ42 in diffuse deposits	↓ / =Aβ40 ↓ Aβ42 ↓ Aβ42/Aβ40	[148]

E693Q Dutch	2	CAA and cognitive decline; no neurofibrillary changes; mostly Aβ40 in vessels and Aβ42 in diffuse deposits; reduced Aβ proteolysis by IDE	↓ / =Aβ40 ↓ Aβ42 ↓ Aβ42/Aβ40	[107, 149-154]
E693G Arctic	2	CAA and AD, typical AD neurofibrillary pathology, abundant amyloid plaques reactive with both Aβ40 and Aβ42; many plaques ring-like and lacking cores; accelerated formation of oligomers and protofibrils by Aβ40; reduced Aβ proteolysis by IDE	↓ / =Aβ40 ↓ Aβ42 ↓ Aβ42/Aβ40	[102, 153-157]
D694N Iowa	x	CAA and AD; widespread NFT; increased Aβ40 in amyloid plaques		[180]
L705V Italian	x	CAA and cognitive decline; no amyloid plaques or NFT; vessels show both Aβ40 and Aβ42		[181]
G709S	x	AD; shifts Aβ profile from Aβ40 to Aβ39 Aβ37	↓ Aβ40 ↑ Aβ38 & Aβ39	[182]
A713T	x	CAA, stroke and AD; pathogenic in both heterozygous and homozygous states; later age of onset in heterozygotes	= Aβ42/Aβ40	[183-186]
T714A Iranian	x	AD; variable age at on-set; epilepsy	↓ Aβ42	[187, 188]
T714I Austrian	3	AD; variable CAA; epilepsy	↓ Aβ40 ↑ Aβ42 ↑ Aβ42/Aβ40	[94, 118, 189]

V715M French	3	AD	↓ [Aβ] ↓ Aβ40 = Aβ42 ↑ Aβ42/Aβ40	[116, 118, 190, 191]
V715A German	3	AD	↓ Aβ40 ↑ Aβ42 ↑ Aβ42/Aβ40	[118, 192, 193]
I716V Florida	3	AD	= or↑ Aβ40 ↑ Aβ42 ↑ Aβ42/Aβ40 ↑ Aβ38	[104, 113, 118]
I716F	3	AD with CAA; extensive neurofibrillary pathology; oligomeric N-truncated pyroglutamate Aβ deposition associated with clinical symptoms; Lewy bodies also present and associated with movement disorder	↓ [Aβ] ↓ Aβ40 ↑ Aβ42 ↑ Aβ42/Aβ40 ↑ Aβ38	[113, 119, 194, 195]
I716T	x	AD	↑ Aβ42/Aβ40 ↑ Aβ38	[113, 196]
V717I London	3	AD; numerous amyloid plaques and NFT, variable CAA	↓ [Aβ] ↓ Aβ40 ↑ Aβ42 ↑ Aβ42/Aβ40 ↑ Aβ38	[103, 104, 107, 108, 118, 119, 197]

V717L Indiana	3	AD	↓ Aβ40 ↑ Aβ42 ↑ Aβ42/Aβ40	[118]
V717F Indiana	3	AD	↓ Aβ40 ↑ Aβ42 ↑ Aβ42/Aβ40	[102]
V717G	3	AD; progressive amnesia	↓ Aβ40 ↑ Aβ42 ↑ Aβ42/Aβ40	[198, 199]
T719P	x	AD		[128]
L723P Australian	x	AD	↑ Aβ42 ↑ Aβ42/Aβ40	[121, 200]
K724N Belgian	3	AD	↓ Aβ40 ↑ Aβ42 ↑ Aβ42/Aβ40 ↑ Aβ38 & Aβ39	[201]
APP duplication	x	Duplication size varies and may include additional genes; duplications may not always be fully penetrant; those leading to increased APP levels share some features with DS		[202-206]
APP promoter	x	Promoter mutations leading to increased APP levels share some features with DS; may vary between specific mutations		[207, 208]

DS	x	Increased Aβ oligomers; complex changes in levels of Aβ species in plasma and CSF; levels of Aβ40 while initially higher in DS than normal controls are reduced with DS dementia; levels of Aβ42 and Aβ42/Aβ40 are initially lower but increase with DS dementia		[122, 123, 209-211]

-detailed descriptions are not available for recently discovered mutations as individuals have not yet come to autopsy. Further detail is available in Supplementary Table 1.