Catastrophe, chaos and Alzheimer's disease

The F E Williams Lecture

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The economic as well as the personal burden of dementia in the elderly is enormous. Cognitive impairment is the fate of at least one in five who live beyond 80 years, and by the age of 90 there is a one in two chance of being demented [1,2]. A cost of illness analysis in 1992 calculated UK annual health care costs to the NHS of £357m, which rose to £1,373m when community and non-NHS costs were included, far higher than any other disease [3].

Alzheimer's disease or inevitable ageing?

The very high prevalence of dementia, and more specifically Alzheimer's disease, in the elderly has led some to suggest that this is the inevitable fate of living too long. If ageing, in general, is considered to be a linear incremental process of accumulation of metabolic errors, then in the specific instance of Alzheimer's disease this is seen as the gradual appearance of plaques and tangles until a threshold is reached when the diagnosis of Alzheimer's disease is established. However, there are numerous well documented cases of long-lived individuals with well preserved cognitive skills. For example, Mme J C who celebrated her 120th birthday earlier this year, had been living independently until her 110th year and cognitive testing at 118 years showed a remarkably intact individual, allowing for her poor hearing and eyesight [4]. Do such individuals represent the tail of a normal distribution of function in the elderly or are they categorically different?

The concept of a gradual decline into cognitive failure occurring as the result of the accumulation of metabolic errors is a valuable model for diseases of old age but may not be the only interpretation for Alzheimer's disease. An alternative, which implies a categorical distinction between old age and Alzheimer's disease, proposes a more catastrophic onset for the disease; once the disease has been triggered the progress is non-linear, catastrophic and distinct from the linear accumulative effects of ageing. Some support for this model and the possible underlying mechanisms are beginning to emerge from the study of familial Alzheimer's disease.

Familial Alzheimer's disease

Alzheimer's original patient, a 51 year old lady, had no family history and indeed, the prevailing view for many years was that Alzheimer's disease represented a rare sporadic dementia of presenile onset. The histological features of senile plaques and neurofibrillary tangles had been described previously, but it was Alzheimer who associated both histological features with the clinical correlate of dementia. The realisation that the disease could occur on a genetic basis came only relatively recently, although some reports as early as the 1930s had been published [5]. The genetic basis for Alzheimer's disease is now well established, with a significant proportion, perhaps 5 to 10%, occurring on a clear autosomal dominant basis. However, the pattern of inheritance may be difficult to establish in the elderly in whom the family history may be censored by early death from other causes before the Alzheimer phenotype has been expressed.

In the search for the familial Alzheimer's disease (FAD) gene, many early genetic linkage studies focused on markers on chromosome 21, pursuing the clue provided by Down's syndrome. People with trisomy 21 Down's syndrome develop cognitive dysfunction associated with Alzheimer histopathological changes as they reach middle age [6]. Thus, if an extra copy of a gene on chromosome 21 can lead to Alzheimer's disease in Down's syndrome, then perhaps a mutation in the same gene could be responsible for FAD. This approach was rewarded in a study of four pedigrees by St George Hyslop et al who reported linkage to markers on chromosome 21 [7], although subsequent studies have revealed that FAD is, in fact, genetically heterogeneous [8,9].

The amyloid precursor protein

At the same time as the linkage to chromosome 21 markers in some FAD pedigrees was demonstrated, the gene for the amyloid precursor protein (APP) was identified [10]. This represented the culmination of many years' work attempting to characterise the abnormal proteins deposited in Alzheimer's disease and intimately associated with senile plaques and neurofibrillary tangles. The insoluble nature of these deposited proteins delayed their isolation and subsequent sequencing. The amyloid nature of the proteinaceous core of senile plaques had been recognised for many years on the basis of Congo red

staining and birefringence with polarised light, although the exact nature of the amyloid protein had not been identified.

Immunostaining established that the same protein was deposited in blood vessels and suggested that it was a novel amyloid protein distinct from those associated with systemic amyloidoses, although it shared with them the propensity to aggregate to form insoluble fibrils. In the early 1980s the amyloid from blood vessels was isolated and shown to contain a peptide of approximately 40 amino acids in length and named β-protein [11]. A peptide with a similar sequence was subsequently isolated from the plaque cores and termed A4 protein [12]. The reported sequences of the extracted amyloid suggested heterogeneity at the C-terminus of the peptide with molecules of varying lengths of 39–43 amino acids; vascular amyloid was predominantly 39 or 40 amino acids but plaque amyloid displayed greater heterogeneity of 40–43 amino acids [13]. There was initial doubt as to whether this was an artefact of the extraction procedure or a genuine reflection of peptide heterogeneity; this may now be viewed as a critical observation.

Once the primary amino acid sequence had been obtained it was possible by molecular cloning to show that the peptide was part of a much larger protein, the amyloid precursor protein (APP) [10,14,15]. The full length molecule is 770 amino acids long but at least six isoforms can be derived from alternatively spliced transcripts encoded by a single gene on chromosome 21. APP is a transmembrane protein and the 40 amino acid sequence of the β-amyloid peptide represents a small domain partially straddling the membrane region. There is a large extracellular component and a small cytoplasmic tail. The function of APP is not clearly established but there is a high degree of evolutionary conservation and it is widely expressed in non-neural as well as neural tissue [16]. The protein is transported down the axon by axoplasmic flow and can be found in abundance at the synapse where it is believed to be involved with synapse formation and maintenance; it may also have a role in repair mechanisms.

The molecular genetics of FAD

The identification of APP as the putative precursor of the amyloid in Alzheimer's disease brain and the location of the APP gene on chromosome 21, in the same region as that implicated by linkage studies, made it an obvious candidate for the FAD gene. Subsequently a variety of mutations has been identified in the APP gene associated with early onset FAD, albeit representing a very rare cause of the disease (Fig 1). The first mutation to be reported, a single point mutation resulting in an amino acid substitution of isoleucine for valine at APP 717 (APP 717 $_{val \rightarrow ile}$) was found in one UK and one US family [17]. Other families with this mutation have subsequently been reported from Canada, Italy and Japan but amounting to no more than 15 families worldwide [18,19,20]. Two other substitutions, either glycine or phenylalanine for valine (APP 717 $_{val \rightarrow gly}$; APP 717 $_{val \rightarrow phe}$) have also been reported in single pedigrees [21,22], and a double mutation at APP 670/671 has been reported in a single large Swedish pedigree (APP 670 $_{lys \rightarrow asn}$/671 $_{met \rightarrow leu}$) [23]. Two further mutations are associated predominantly with amyloid angiopathy (APP 693 $_{glu \rightarrow gln}$; APP 692 $_{ala \rightarrow gly}$) although the latter also exhibits cytoskeletal pathology [24,25].

Since APP mutations account for only a few FAD pedigrees, even those of early onset, there have to be other loci. Further genetic studies have established the nature of this heterogeneity (Table 1). A major determinant of early onset FAD, with an onset at around 40 years of age, is associated with a gene, as yet unidentified, on chromosome 14 [26]*. The reports of

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* (Note added in proof). A chromosome 14 FAD gene has now been identified. See Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, *et al.* Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* 1995;**375**:754–60.
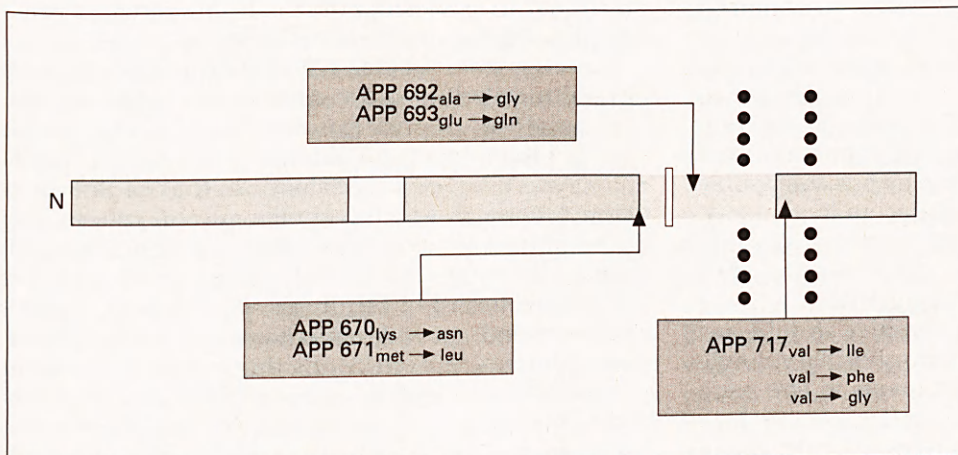

Fig 1. Amyloid precursor protein (APP) gene mutations associated with familial Alzheimer's disease and hereditary amyloid angiopathies

APP 692 $_{ala \rightarrow gly}$
APP 693 $_{glu \rightarrow gln}$

N

APP 670 $_{lys \rightarrow asn}$
APP 671 $_{met \rightarrow leu}$

APP 717 $_{val \rightarrow lle}$
val $\rightarrow$ phe
val $\rightarrow$ gly

Table 1. Genetic heterogeneity in Alzheimer's disease

Sporadic

Familial
Chromosome 21
    APP 717 $_{val \to ile}$
    APP 717 $_{val \to phe}$
    APP 717 $_{val \to gly}$
    APP 670/671 $_{lys \to asn/met \to leu}$
Chromosome 14
    Early onset
Chromosome 19
    apo ε 4 genotype
Late onset non-chromosome 19
Early onset non-chromosome 14, 19, 21
    (Volga German)

APP allelic variants
HCHWADAPP 693 $_{glu \to gin}$
Flemish mutation APP 692 $_{ala \to gly}$

linkage to chromosome 19 markers can now be attributed to inheritance of the apolipoprotein E4 genotype which is an important determinant of age at onset in FAD and a risk factor in sporadic disease [27,28]. In addition, some late onset FAD pedigrees appear not to be explicable by inheritance of the apo E4 genotype and the disease in Volga German pedigrees, families of German refugees from Russia who settled in the US in the early part of the century, appears to be associated with yet another genetic locus [29].

Disease mechanisms in APP mutation FAD

Clinically, the two main groups of early onset FAD, namely APP mutations and chromosome 14-linked, have relatively characteristic ages of onset at around 50 and 40 years respectively [30]. Clinical experience suggests that these patients are well, without obvious cognitive impairment, until the onset of disease in middle age. If this is correct, what is the underlying mechanism that permits normal brain function until middle life? Is there a mechanism which is compatible with a catastrophic onset as opposed to an incremental progression of disease? The APP mutation families with a defined molecular genetic defect are the ideal population in which to address this question.

The focus on APP mutation families should not ignore the involvement of the neurofibrillary tangle and constituent paired helical filaments in the pathology of Alzheimer's disease. A similar approach to that employed to study amyloid deposition has been taken towards the elucidation of the molecular pathology of neurofibrillary tangles with the isolation and sequencing of a protein from the central core of the paired helical filaments. Subsequent molecular cloning showed this to be the microtubule-associated protein tau [31] which, in paired helical filaments, is believed to be abnormally phosphorylated [32,33]. The relationship between the amyloid and tau pathology is unclear; they may be independent and it is conceivable, given the heterogeneity of Alzheimer's disease, that a subgroup may reflect primary tau pathology. However, in the specific case of APP mutation FAD, the primary abnormality must be in APP metabolism with subsequent neurofibrillary tangle formation. Moreover, the direct link between APP mutations and Alzheimer's disease has been recently confirmed by the report of transgenic mice bearing the APP 717 $_{val \to phe}$ mutation which have developed Alzheimer histopathological changes with neuritic plaques [34]. Some clues to the underlying mechanisms are now emerging and have relied upon the expanding knowledge of the normal physiological metabolism of APP.

The first metabolic pathway to be identified involves cleavage within the amyloid domain by an enzyme, still poorly characterised, which is now termed the α secretase [16,35,36]. Since such peptide cleavage precludes amyloid deposition, it was originally hypothesised that disruption of this normal pathway would underlie the pathophysiology of Alzheimer's disease. However, a further pathway was identified which involved internalisation of the molecule and sequential degradation within the endosomal/lysosomal pathway to produce a series of C-terminus products some of which included the entire amyloid sequence [16,37,38]. If this pathway were to predominate, then amyloid would be formed. However, it was subsequently discovered that the 40 amino acid peptide could be identified as a *soluble*, as opposed to *insoluble* fibrillary amyloid protein in neuronal cultures and in cerebral spinal fluid [39,40,41]. The production of this peptide, referred herein as Aβ involves putative α and β secretases (Fig 2a) but these remain poorly characterised. It is presumed to have some normal physiological role, although this has not yet been identified.

However, since soluble Aβ peptide is a product of normal metabolism this cannot itself readily explain the amyloid deposition but rather the post-translational structural change to β-amyloid fibrils emerges as a key event. Analogy can thus be drawn to prion disease in which a similar pivotal role in the pathogenesis is accorded to the post-translational structural change of the PrP cellular protein to the PrP scrapie form [42,43]. If this is a key event, it does not, as it stands, provide a mechanism of the toxicity of β amyloid and indeed conflicting results have been published on the toxicity of ß amyloid in neuronal cultures [44–47]. It remains a possibility that the disease is due to the loss of biological function of soluble Aβ
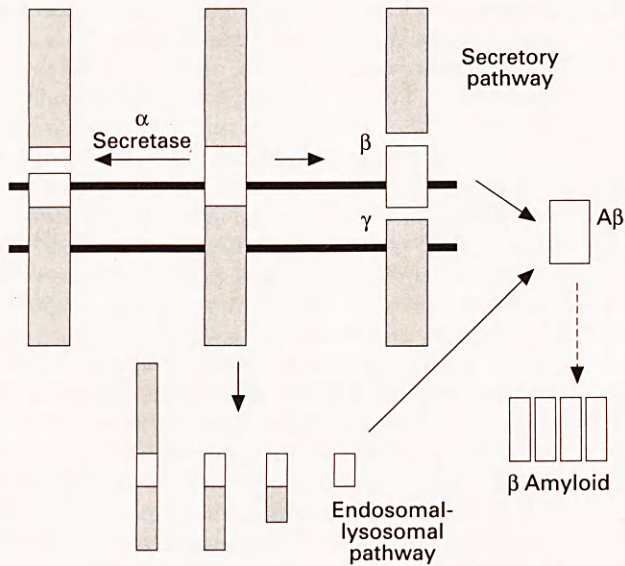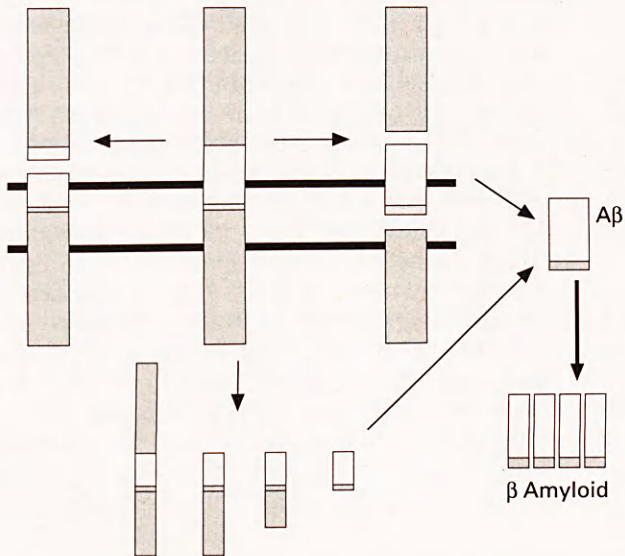
Fig 2a. Metabolism of APP

Fig 2b. Metabolism of APP with extension at the C terminus

consequent upon its modification to an abnormal β amyloid, rather than to direct toxicity.

If the change from soluble Aβ protein to insoluble β amyloid is critical, then how do APP disease mutations influence this? The first data came from studies of the Swedish double mutation. Transfection of neuronal cultures with constructs containing APP 670 lys→asn/671met→leu results in a six to eight fold increase in the production of soluble Aβ [48,49]. The increase is similar to, although quantitatively greater than, that found in Down's syndrome where it can be attributed to an increase in APP due to an increased gene dosage. If only the occasional molecule of Aβ undergoes a spontaneous change to β amyloid then an increase in Aβ will lead to a commensurate increase in

the rare event of β amyloid formation. Nevertheless, this *per se*, would still be an incremental increase in β amyloid formation. Clues to a more catastrophic event, at least at a molecular level, come from studies of the APP 717 mutations. Transfections of cell cultures with constructs bearing these mutations result not in an increase but in a subtle shift towards molecules that are extended by two or three amino acids at the C-terminus [50]. Thus the originally observed raggedness of the C-terminus on isolation of β amyloid from Alzheimer brain was a genuine observation, not an extraction artefact. What then is the effect of this shift in peptide length? Studies *in vitro* from Lansbury and colleagues have demonstrated that a subtle increase in the length of the molecule results

in a dramatic propensity to amyloid fibril formation in solution through a nucleation phenomenon [43,51]. Moreover, the precipitation of amyloid fibrils from the soluble 1–40 aminoacid Aβ peptide can be critically seeded by larger molecules of the 1–42 amino acid peptide. Thus a subtle shift in metabolism towards Aβ peptides which are extended at the C-terminus, can result in rapidly accelerating β amyloid formation. Once an event is triggered, and there may be many influences to raise the threshold of triggering, there is a catastrophic cascade of β amyloid formation (Fig 2b). To pursue the metaphor used by Lorenz in his 1979 lecture on predictability to the American Association for the Advancement of Science, 'Does the flap of a butterfly's wings in Brazil set off a tornado in Texas?', the C-terminus extension of Aβ is the butterfly equivalent which can precipitate the catastrophic events of Alzheimer's disease.

Many factors may interact with the proposed structural change (Fig 3). Thus, apolipoprotein E4 binds to β-amyloid more avidly than apolipoprotein E3 although differential binding to tau has also been observed [52, 53]. Free radicals and zinc also enhance fibril formation [54] and recently Pepys and colleagues demonstrated that the binding of serum amyloid P component (SAP) to amyloid fibrils inhibited their degradation [55]. All these observed interactions may have an effect on the age at onset and offer potential therapeutic targets.

Neuropathological and clinical correlates

These studies of the APP mutation cases provide a molecular mechanism for catastrophic change but can this be related to events at a tissue and clinical level? Immunohistochemical studies utilising antisera which can distinguish between Aβ peptides 1–40 and 1–42 suggest that deposition of Aβ1–42 is an early event associated with diffuse plaque formation. Neuritic plaques, by contrast, are associated with more β 1–40 immunostaining [56]. This can be interpreted as seeding by the deposition of a nucleus of β 1–42 followed by aggregation of β 1–40 as the disease progresses. This is consistent with the observed growth of plaques *in vitro* during which reversible deposition of labelled amyloid peptide can be demonstrated [57].

Can these observations be extended to the patterns of clinical onset and progression to demonstrate that in the FAD patients the change is sudden? The FAD pedigrees provide an opportunity to capture the onset and early progression of the disease since 50% of such individuals are predicted to develop the disease and there is a relatively characteristic age at onset. Thus a relatively small group of patients can be studied in great detail from a presymptomatic stage through to established disease. In general there has been no evidence that these at risk individuals are other than cognitively normal until they approach the age at onset. This contrasts, for example with the 144 base pair insert prion family, in whom behavioural abnormalities have been reported at an early age [58]. The few patients that have been followed do suggest that once the disease starts there is rapid progression. In such studies it is important to select neuropsychological tests of graded difficulty, in which the performance of a control population approaches a normal distribution, in order to avoid ceiling effects. Utilising such an approach, it has been possible to demonstrate a rapid decline over two years in one individual from a chromosome-14 linked pedigree [59]. Similarly, two patients from the APP 717 $_{val \rightarrow gly}$ mutation family declined rapidly by contrast with three as yet unaffected siblings. Regional magnetic resonance imaging (MRI) volumetry in these five family members also demonstrated a rapid decline in hippocampal volume, interpreted as tissue loss. Serial scanning over three years demonstrated no significant change in three cognitively stable individuals. By

Fig 3. Factors influencing the transition from Aβ to β amyloid
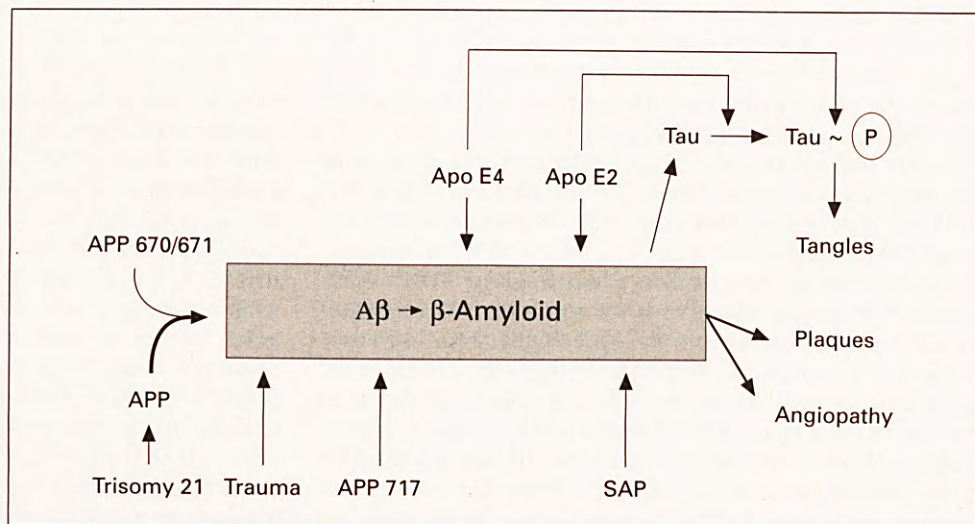
contrast, a loss of hippocampal volume of 5–10% per annum from an initial volume close to, although less than, the mean of the unaffected siblings was demonstrated in the two individuals with cognitive deterioration [60].

Summary

A model can be developed for familial APP mutation Alzheimer's disease to explain why a patient who is cognitively normal until middle age experiences a catastrophic amyloid deposition, which is to some extent mirrored in the clinical deterioration due to a subtle shift in Aβ metabolism. However, the analysis of young onset dementia hardly constitutes the study of 'the suffering and infirmities of old age' which F E Williams' bequest is intended to promote. It remains to be seen whether the models relevant to APP mutation FAD can be applied to Alzheimer's disease of old age, or indeed other degenerative diseases of later life. Such models, however, do provide an alternative to the view that Alzheimer's disease is an incremental process virtually indistinguishable from old age itself. With an incremental linear process, treatment is akin to a war of attrition. By contrast, with a catastrophic process the difference between a normal elderly person and a patient with incipient Alzheimer's disease at the start may be minimal, perhaps only a few molecules of extended Aβ peptide, but they diverge very rapidly. If treatment can be directed at the metabolic events at the onset then there is a real opportunity for optimism. If ultimately successful, prevention rather than delay becomes a realistic goal, echoing Duc de La Rochefoucauld's desire 'to die as young as possible as late as possible'.

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