The presence of numerous senile plaques (SP) and neurofibrillary tangles (NFT) within association areas of the neocortex and within the hippocampus and amygdala, is generally regarded as providing the histopathological hallmarks of Alzheimer's disease (AD) [1]. It is now becoming more widely accepted that these pathological changes play more than just a diagnostic role in the disorder, but are of fundamental importance within the pathogenic process [1-3].

The NFT represents an accumulation within the perikaryon and nerve terminals of proteinaceous filamentous material and this accumulation has been associated with a progressive reduction in capacity for protein synthesis [4] and interruption of axoplasmic flow [5]. While it has yet to be directly shown that NFT are, in themselves, neurotoxic, their excessive presence within the nerve cell and its processes are likely to impair function and make communication between the cell body and its terminals more difficult. Because NFT are found in a wide variety of neurological disorders other than AD [6, 7], it is possible that the formation of this material reflects a response of the neuron to a particular kind of damage rather than to a single specific agent. The site of the damage may occur at the nerve terminals and within the confines of the other pathological characteristic of AD, namely the SP [1, 3].

Similar pathological changes to those of AD are seen in the brains of nearly all persons with Down's syndrome (DS) who live beyond 40 years of age; such features are only rare in those who live beyond 40 years of age; such features are only rare before 20 years of age [8]. Because of this similarity between DS, with obvious implications as to the changes of NFT in DS, the brains of nearly all persons with Down's syndrome (DS) provide a useful model for the pathological process of AD.

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The staining was even in distribution with no tendency towards the formation of an amyloid core. In this same patient, numerous patchy areas showing an enhanced density of staining combined with an increased granularity were demonstrated with Con A but similar positive staining was not detected with the other three lectins. None of the immunochemical markers for NFT revealed any NFT in these two patients.

In nine of the 11 patients aged between 35 and 50 years, using silver staining, a moderate number of SP were seen within the molecular grey matter of the dentate gyrus and sometimes also within areas CA1, CA4/5 and subiculum. Occasional SP were also present within the adjacent entorhinal and temporal neocortex of all except two of these patients (aged 38 and 42 years). With anti-A4 staining, a moderate number of SP were identified in the hippocampus and neocortex of all nine patients. Although in most instances the distribution of anti-A4 reactivity was again even, an occasional SP within the temporal neocortex showed a typical well-defined plaque core with a surrounding halo of staining [14]. With Con A, all nine patients showed a strong staining of SP both within the aforementioned regions of hippocampus and in the adjacent entorhinal and temporal neocortex. The pattern of staining was either finely granular and evenly distributed (as in dentate gyrus) or was aggregated into large coarse clumps of material admixed with other unstained areas. In these patients SP were (in apparent instances) similarly stained with both PSA and WGA, although the intensity of the reaction was much less, with WGA staining less than PSA. With ePHA, SP in the hippocampus of all nine patients were only weakly stained, whereas in temporal neocortex only five patients showed a weak staining of SP. In no patient did SP within entorhinal cortex stain with ePHA.

In eight of these same nine patients (except a 42-year-old patient) numerous NFT were demonstrated (using either silver, anti-PHF or anti-t methods) within the large stellate neurons of layer II of the entorhinal cortex. NFT were less commonly seen within CA1 and CA4/5 areas of hippocampus and occasionally within the subiculum and adjacent neocortex. Each staining method demonstrated NFT in approximately similar numbers (Table 1).

In the other two patients under 50 years of age (aged 43 and 48 years) and in all 11 patients aged over 53 years, numerous SP and NFT were seen, in silver-stained sections, throughout the hippocampus, entorhinal and temporal neocortex and amygdala, which is in agreement with previous findings [20].

Table 1. NFT distribution in patient groups

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Silver</th>
<th>Anti-PHF</th>
<th>Anti-t</th>
</tr>
</thead>
<tbody>
<tr>
<td>No NFT (n = 3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Few NFT (n = 8)</td>
<td>49 ± 72</td>
<td>49 ± 77</td>
<td>57 ± 90</td>
</tr>
<tr>
<td>Numerous NFT (n = 13)</td>
<td>557 ± 128</td>
<td>563 ± 124</td>
<td>442 ± 182</td>
</tr>
</tbody>
</table>

Results are means ± s.e.m. Statistical significance (Wilcoxon's matched pairs test): *P < 0.05 compared with silver.
cortex. In these 13 patients using the anti-A4 method, most SP were strongly stained, showing a well-defined core and halo of staining[14]. Many less intensely and more uniformly staining SP were also widely present, scattered among the more intensely staining SP. While in most instances these SP were still strongly stained with Con A, some SP showed only a weak staining of fewer areas of the SP. However, in these patients the degree of staining with PSA, WGA and ePHA was now increased; indeed the intensity of staining was usually equivalent to, or sometimes greater than, that with Con A. In all 13 patients in this group intracellular NFT were as strongly stained with anti-\( \tau \) or anti-PHF antisera as with silver staining techniques not bound by a limiting nerve cell membrane (extracellular or 'ghost' tangles) anti-PHF staining was reduced and anti-\( \tau \) weak, or absent, in many instances. The number of cells staining with anti-\( \tau \) was thus significantly less than those staining with silver or anti-PHF (Table 1).

Staining of amyloid within cerebral vessels was patchy and patient specific. In a few of the patients under 50 years of age only an occasional artery (in either hippocampus or neo-cortex) showed weak staining. In all except one of the patients over 50 years of age, several, and sometimes many, vessels overlaying the temporal cortex, and some intraparenchymal arteries, showed strong anti-A4 staining. In some of these patients occasional vessels in the hippocampus were also, but to a lesser extent, affected.

The results of this study imply therefore that the earliest morphological changes that characterize the formation of the SP, as detectable by those immunocytochemical and lectin histochemical methods used here, involve a deposition of amyloid (A4) protein and an accumulation of an oligosaccharide recognized by the lectin from Con A. At this stage such 'pre-plaque' areas are mostly unrecognizable using conventional staining techniques.

The amyloid deposits within SP (and cerebral vessels also) are derived from aggregations of polypeptide subunits each of which comprise 42-44 amino acid residues (A4 protein)[15, 16]. Such a polypeptide represents the transmembrane domain of a much larger precursor protein(s) with a C-terminal intracellular part and a bulky N-terminal extracellular part, the whole molecule containing a basic 695 amino acid residues [10] with variants containing a 57 amino acid[17] or a 75 amino acid[18] insert at residue 289, to give a 751[17] or a 770[18] amino acid structure, respectively. It has been proposed[10] that after damage to (nerve) cell membranes, this precursor protein is cleaved to yield the A4 subunit, although the fate of the remaining intracellular and extracellular domains is not yet known. The extracellular domain of the precursor protein is glycosylated at positions 467-469 and 496-498[10] and it is possible that one or both of these glycosylation sites contains, in vivo, the oligosaccharide sequence that is recognized by Con A. At present it is not possible to determine whether Con A binding reflects the presence of the intact precursor, the cleaved extracellular domain or a processed glycoprotein portion. However, if the latter is the case, the finely granular nature of the early Con A binding, like that of the A4 deposits, suggests that membrane damage occurs evenly and locally over a large region of tissue. As such changes progress with time the conventional SP morphology becomes clearly recognizable: A4 protein subunits aggregate, become \( \beta \)-pleated and concentrate into the familiar plaque core and halo[14] (they then become recognizable by thioflavin and Congo Red). PHF-containing neurites within SP become discernible by silver methods and the Con A-positive material concentrates into larger aggregates and undergoes a series of biochemical elaborations.

The precise anatomical localization of the lectin binding is not known, although the later clumped nature of the staining resembles the dystrophic neurites seen under the electron microscope and raises the possibility that the staining may be localized to the membrane-bound lamellar bodies numerous within such altered terminals[19]. The oligosaccharides recognized by Con A, PSA, WGA and ePHA are all of the mannose-containing type[20]. Con A recognizes simple mannose-containing sequences and there is a progression in the complexity of saccharides recognized by WGA and PSA through to ePHA. Hence the pattern of build up of staining intensity with time from initial high Con A binding to late high ePHA binding would be consistent with a series of morphological changes that might begin with the intracellular uptake of part of the cleaved precursor and culminate with its metabolic modification into membrane-bound lamellar bodies.

The appearance of anti-\( \tau \) immunoreactivity within NFT at a similar time (and in similar numbers) to that seen with silver and anti-PHF methods is consistent with the view that \( \tau \) is a major antigenic determinant of NFT[5]. However, because in the youngest patients, no greater numbers of \( \tau \)-positive neurones, than silver or anti-PHF, or that \( \tau \)-positive cells were seen in the absence of silver or anti-PHF-staining neurones, suggests that it is possible that NFT are only associated with \( \tau \) and not necessarily derived from it.

Since in all except the youngest patient (in whom no Anti-A4 staining was seen), areas of A4 protein deposition were present within brain parenchyma (plaques), although often in the absence of significant amyloidosis within adjacent cerebral vessels, it is possible that the deposition of amyloid (A4) protein within cerebral vessel walls to give a congophilic angiopathy is an event that follows the deposition of A4 within SP. Such findings would imply, as argued by Masters & Beyreuther[21], that the amyloid deposits in AD are indeed of a neuronal or at least a parenchymal origin, and assuming that they are both of the same material, A4 protein becomes eventually embedded in vessel walls. An origin of A4 protein from a serum precursor[3, 16] would seem inconsistent with such a pattern of changes. It is, however, still possible that the two (similar) \( \beta \)-proteins are produced and accumulated locally (and independently) by separate cells of origin with the SP amyloid being neuronally derived and the vascular amyloid perhaps from endothelial cells (these latter cells have been shown[22] to genetically express the A4 amyloid protein precursor).


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Molecular genetics of Alzheimer’s disease

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It is not clear what proportion of Alzheimer’s disease cases are genetic in origin because of the late age of onset and because of the difficulties in absolute clinical diagnosis. Despite this uncertainty, it is clear that pedigrees in which Alzheimer’s disease shows autosomal dominant inheritance (e.g. Nee et al., 1983) offer an opportunity to investigate the pathogenesis of the disease in more detail. In such pedigrees, the technique of linkage analysis offers a route to chromosomal localization, isolation and, finally, characterization of the defective gene and its product. Largely through the Alzheimer’s Disease Society, we have identified more than a hundred families, multiply affected by Alzheimer’s disease, and have collected blood samples and/or established cell lines from about 20 of these in which the pedigree structure is such that genetic linkage analysis is possible.

Linkage analysis relies upon the fact that alleles of DNA sequences close together on a chromosome will be co-inherited more often than the 50% predicted for unlinked markers. The closer they are together, the more frequently alleles will co-segregate. Molecular genetics allows the segregation of such sequence alleles (restriction fragment length polymorphisms) to be followed through families. Since the chromosomal positions of a large number of restriction fragment length polymorphisms are now known, the inheritance of these can be followed through families to see whether any of the loci are close (i.e. genetically linked) to the disease locus.

The observation that infants with Down’s Syndrome who survive into mid-life develop histopathological features similar to those found in Alzheimer’s disease suggested that abnormalities of the expression of a gene or set of genes on chromosome 21 can lead to the development of the disease. This implied that linkage analysis using probes to loci on this chromosome might be a fruitful strategy. This has recently been proved to be so and linkage of two polymorphic loci (D21S1 and D21S16) to a locus causing early-onset Alzheimer’s disease has been reported. Since these loci are on the proximal segment of the long arm of chromosome 21 (21q1-2; St. George-Hyslop et al., 1987a) it follows that a familial Alzheimer’s disease locus should, in principle, be in this region.

This localization of the Alzheimer gene is interesting for two reasons. First, although people had predicted that the Alzheimer gene might be on chromosome 21, they had thought that it would be at the end of the long arm, since individuals with just this segment triplicated (by translocation) develop phenotypic Down’s syndrome. Secondly, at the same time that the Alzheimer’s gene was localized, the gene coding for β-amyloid (a protein component of the senile plaques and cerebrovascular amyloid; Glenner & Wong, 1984) was cloned and localized to the same chromosomal region (Kang et al., 1987; Goldgaber et al., 1987, Tanzi et al., 1987). The β-amyloid gene was an excellent candidate for the Alzheimer gene and its localization to the correct chromosomal region naturally prompted speculation that the genetic locus leading to Alzheimer’s disease was within the β-amyloid gene. Expectations were further raised when it was reported that the β-amyloid gene was duplicated in sporadic Alzheimer’s disease (Delabar et al., 1987). This suggested a unitary hypothesis for Alzheimer’s disease with four tenets:

(1) Aβ-amyloid deposition is central to the aetiology of Alzheimer’s disease.
(2) Down’s syndrome cases develop Alzheimer’s disease because they have three copies of the A4-amyloid gene.
(3) Sporadic Alzheimer’s disease develops in people who have duplicated their A4-amyloid gene, i.e. they too have three copies.
(4) Familial Alzheimer’s disease is caused by over-expression, or expression of an abnormal variant of the A4-amyloid gene.

The attraction of this hypothesis is that tenets (3) and (4) are testable. Unfortunately, they do not stand up to experimental scrutiny. Further genetic analysis of families with Alzheimer’s disease, using polymorphic markers of the A4-amyloid gene, clearly showed that there were recombinants between the two loci in several families. This strongly suggests the two loci are distinct (Van Broeckhoven et al., 1987; Tanzi et al., 1987b). Furthermore, other groups have not been able to replicate the localization of the amyloid gene locus in other affected individuals (Van Broeckhoven et al., 1987, St. George-Hyslop et al., 1987b; Tanzi et al., 1987c; Podlisny et al., 1988). It therefore seems most likely that the location of the β-amyloid gene on chromosome 21 is coincidental.

The identification of a genetic linkage of Alzheimer’s disease to the loci S1/S11 and S16 should, in principle, make it a relatively straightforward task to determine whether other families, multiply affected by Alzheimer’s disease, share the same disease locus. Clearly, linkage analysis of a number of new families should confirm the linkage between the chromosome 21 probes and the disease if there is only one disease locus. A multi-centre collaboration has been established to determine the generality of the linkage to the chromosome 21 probes. So far, approximately 40 families, including 10 families collected by our group, have been analysed (St. George-Hyslop et al., 1988). The mean ages of onset in these families ranges from 30 to 80 years of age. The null hypothesis being tested in this collaboration is that all familial cases of Alzheimer’s disease are caused by the locus close to the probes D21S1/D21S11. The results analysed so