

Is there a spatial association between senile plaques and neurofibrillary tangles in Alzheimer's disease?

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Abstract

Objective: To test the hypothesis that the clusters of senile plaques (SP) and neurofibrillary tangles (NFT) in patients with Alzheimer's disease (AD) are spatially associated as predicted by the 'Amyloid Cascade Hypothesis'.

Methods: The spatial association between the SP and NFT was studied in the cerebral cortex and hippocampus in six cases of sporadic Alzheimer's disease (AD) using contingency tables. The coefficient C_7 was used as an index of spatial association while chi-square with correction for continuity was used as a test of significance.

Results: In the brain regions analysed, values of C_7 were in the range -0.31 to +0.32 but a statistically significant spatial association between SP and NFT was present in only 8/39 (21%) regions. The degree of spatial association between the SP and NFT was similar in different brain regions and did not vary with apolipoprotein ϵ genotype of the patient. However, the magnitude of C_7 in a region was positively correlated with the density of the NFT and with the total density of SP and NFT but not with the density of SP alone.

Conclusion: There was little evidence that SP and NFT were spatially associated except in brain areas with high densities of lesions. The data support the hypothesis that SP and NFT are distributed relatively independently in the cerebral cortex and hippocampus and therefore, could be distinct phenomena in AD.

Key words: Alzheimer's disease, senile plaques, neurofibrillary tangles, contingency tables, coefficient of association (C_7)

Introduction

Alzheimer's disease (AD) is characterised by the presence of two hallmark lesions in the brain, viz., senile plaques (SP) and neurofibrillary tangles (NFT). The most important constituent of the SP is the protein amyloid- β ($A\beta$) [15]. $A\beta$ is an approximately 4-kDa peptide arising by cleavage of a larger trans-membrane amyloid precursor protein (APP) present in most brain cells. The $A\beta$ peptide exists in

alternative spliced forms, the most common being $A\beta_{42/43}$ found predominantly in SP and the more soluble $A\beta_{40}$ found in association with blood vessels [23]. Deposition of $A\beta$ in familial [10] and sporadic AD [30] is probably the result of processes that increase the proportion of the highly amyloidogenic peptide $A\beta_{42/43}$ [16]. By contrast, NFT are intracellular inclusions composed of paired helical filaments (PHF), the most important molecular constituent of

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which is the microtubule protein tau. There is a single gene for tau and different isoforms result from alternative splicing and post-transcriptional changes [13]. In AD, all six isoforms of tau are abnormally phosphorylated and aggregated into PHF [9].

The 'Amyloid Cascade Hypothesis (ACH)' [17] is the most important theory to date to explain the pathology of AD. This hypothesis proposes that there is a direct causal relationship between the pathogenesis of the SP and NFT, viz., the deposition of A β is the initial pathological event in AD leading to the formation of NFT, neuronal loss, and the development of dementia [17]. Despite attempts to relate the development of abnormal tau to A β [31], little evidence has been found that directly connects the two pathologies [6] suggesting that the processes leading to the formation of SP and NFT could be distinct phenomena [11]. Previous studies [6,7] have concluded that in the cerebral cortex in AD, SP and NFT are distributed in clusters that often exhibit a regular distribution parallel to the pia mater. If A β deposition leads to tau pathology as proposed by the ACH, then the clusters of SP and NFT should not be randomly distributed with reference to each other but exhibit a degree of spatial association. To test this hypothesis, the degree of spatial correlation between the SP and NFT was measured in various brain regions in cases of sporadic AD using a statistical method based on contingency tables.

Materials and methods

Cases

Six cases of sporadic AD, without evidence of a family history of the disease, were obtained from

the Brain Bank, Dept. of Neuropathology, Institute of Psychiatry, King's College, London, UK. Informed consent was given for the removal of all brain tissue and followed the principles embodied in the 1964 Helsinki declaration. Patients (details in Table I) were clinically assessed and all fulfilled the 'National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association' (NINCDS/ADRDA) criteria for probable AD [32]. The histological diagnosis of AD was confirmed by the presence of widespread neocortical SP consistent with the 'Consortium to Establish a Registry of Alzheimer Disease' (CERAD) criteria [24]. All cases also had numerous NFT in the cerebral cortex and hippocampus. Apolipoprotein ϵ (apo ϵ) genotype is a significant risk factor for sporadic AD [28], individuals with allele ϵ 4 having an increased risk of developing the disease. In the present study, four of the cases had an apo ϵ genotype of 3/4, one case was 3/3, and the remaining case 2/3.

Histological methods

Tissue blocks of the frontal cortex (B8) at the level of the genu of the corpus callosum, parietal cortex (B7) at the level of the splenium of the corpus callosum, occipital cortex including the calcarine sulcus (B17), superior temporal gyrus (B38), lateral occipitotemporal gyrus (B36), parahippocampal gyrus (B28), and hippocampus were taken from each case. Tissue was fixed in 10% phosphate buffered formal saline, embedded in paraffin wax, and 7 μ m coronal sections were cut and stained with the Gallyas silver impregnation method [14] to reveal the SP and NFT.

Table I. Details of Alzheimer's disease cases used in the study

Case	Sex	Age (yrs)	Onset (yrs)	PM delay (months)	Apo ϵ	Cause of death
A	F	70	64	24	3/4	Brochopneumonia
B	F	66	59	–	3/4	Brochopneumonia
C	M	73	66	24	2/3	Brochopneumonia
D	F	88	–	16	3/3	Brochopneumonia
E	M	82	78	11	3/4	Brochopneumonia
F	F	87	82	27	3/4	Myocardial infarction

Abbreviations: M = male, F = female, PM = Postmortem, Apo ϵ = Apolipoprotein ϵ genotype, – data not available

Morphometric methods

In the cerebral cortex, a sample of between 64 and 128, 500x500 µm microscopic fields were located at random throughout the cortical ribbon in each brain area studied. Sample fields must not be so small that they are incapable of containing at least two individuals of the histological features under study. Conversely, fields should not be too large so that individuals of the two features occur in every field. Consideration of the densities, distribution and range of sizes of lesions suggested that a 500x500 µm field would be optimal for sampling the SP and NFT. The presence or absence of SP and NFT was recorded within each sample field. A lesion was recorded in a sample field when at least 50% of its area was present within the field. In the hippocampus, sample fields were located at random in the pyramidal cell layer and included sectors CA1 to CA4.

Statistical methods

In each brain region, the frequencies of the SP and NFT in all sample fields were first summarised as a 2x2 contingency table (Table II). From these frequencies, a 'coefficient of association' (C_7) can be calculated which varies from +1, when the maximum possible co-occurrence is present, to -1 the minimum possible co-occurrence [18]. Values close to zero indicate that the frequencies of the two features are close to those that would be expected to occur by chance. The calculation of C_7 depends on the relationships between the numerical values in the contingency table and is dependent on first, whether the product of the joint presences and joint absences (ad) is greater or less than the product of samples which contain one feature alone (cb) and second, on the relative magnitude of 'c' and 'b' and 'a' and 'd' (see Table II) [27]. In addition to C_7 , a value of chi-square (χ^2) can be calculated from the frequencies in the contingency table. Chi-square is a test of the null hypothesis that the two histological features are distributed independently. Since the χ^2 distribution is continuous and is being used to approximate a discrete distribution, it is necessary to make a 'correction for continuity' [26] and this statistic is given the symbol X^2 . Differences in the degree of association between brain regions were tested using a one-way analysis of variance (ANOVA). Differences between cases with apo ε genotype 3/4 and those of genotype 2/3 and 3/3 were tested using Student's 't'

Table II. The 2x2 contingency table method for measuring the degree of spatial association between senile plaques (SP) and neurofibrillary tangles (NFT) in Alzheimer's disease (AD) showing the calculation of the index of association C_7 and chi-square with correction for continuity (χ^2)

		SP	
		present	absent
NFT	present	a	b
	absent	c	d
When $ad \geq bc$ and $c < b$		$C_7 = ad - bc / (a+b)(b+d)$	
When $ad \geq bc$ and $c \geq b$		$C_7 = ad - bc / (a+c)(c+d)$	
When $ad < bc$ and $a \geq d$		$C_7 = ad - bc / (a+b)(a+c)$	
When $ad < bc$ and $a > d$		$C_7 = ad - bc / (b+d)(c+d)$	
$\chi^2 = [ad - bc - N/2]^2 N / (a+b)(c+d)(a+c)(b+d)$			

test. The relationship between C_7 and the density of lesions in a brain region was tested using correlation and regression methods.

Results

The frequencies of SP and NFT in 104 randomly located sample fields in a single brain region (Case A, Superior temporal gyrus) are shown in Table III. In 43/104 fields, either both SP and NFT occur in the same field (35/104) or both lesions were absent (8/104). By contrast, there were 61/104 fields in which either SP or NFT were recorded alone. From these data, a value of C_7 of -0.27 was obtained indicating a weak but negative spatial association between the SP and NFT, i.e., there was a greater tendency for the SP and NFT to occur alone rather than together in a sample field. The value of χ^2 ($\chi^2=1.51$) calculated from these data was not significant suggesting that there was no statistically significant degree of association between the SP and NFT, i.e., essentially the SP and NFT are distributed independently in this region.

The values of C_7 and χ^2 obtained from all brain regions and cases investigated are summarised in Table IV. The value of C_7 varied from -0.31 to +0.32 suggesting weak spatial associations but significant values of χ^2 were observed in only 8/39 (21%) of the

Table III. Frequencies of senile plaques (SP) and neurofibrillary tangles (NFT) in 104 randomly located sample fields in the superior temporal gyrus of a case of Alzheimer's disease (Case A)

		SP		total
		present	absent	
NFT	present	35	49	84
	absent	12	8	20
	total	47	57	
$C_7 = ad - bc / (b+d)$ ($c+d$) = -0.27; $\chi^2 = 1.51$ (1DF, $P > 0.05$)				

brain areas studied. In all regions where there was a significant degree of association between the SP and NFT, the two lesions were positively associated. There was no significant difference in the magnitude of C_7 between brain regions ($F=1.06$, $P > 0.05$) or between patients with different apo ϵ genotypes ($t=0.54$, $P > 0.05$). However, the value of C_7 was positively correlated with the overall density of NFT ($r=0.36$, $P < 0.05$) and with the total density of lesions (SP+NFT) ($r=0.44$, $P < 0.01$) (Fig. 1) but not with SP alone ($r=0.27$, $P > 0.05$).

Discussion

A significant spatial association between the SP and NFT was found in approximately 20% of the

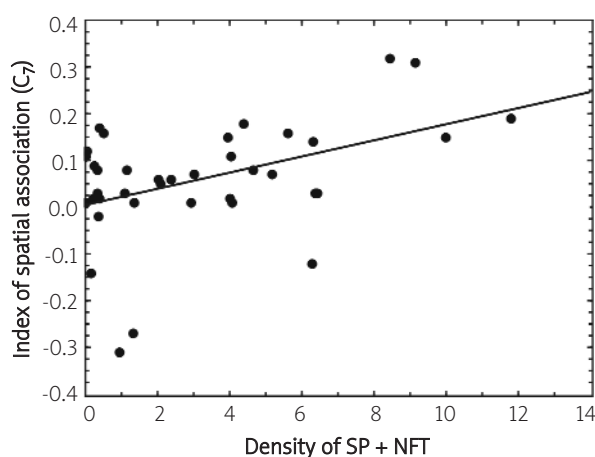


Fig. 1. Correlation between the degree of association (C_7) and the total density of senile plaques (SP) and neurofibrillary tangles (NFT) in a brain area ($r=0.44$, $P < 0.01$)

brain areas studied consistent with previous studies [6,19]. A number of hypotheses could explain the lack of spatial association in the majority of brain areas. First, the degree of association is dependent on field size [26] and the sample field may have been too small to have a significant chance of including both lesions. However, considering the densities, sizes, and distribution of lesions, the field size used was large enough to sample both the SP and NFT adequately. Second, the degree of association between SP and NFT could depend on the stage of the disease. For example, early in the disease process, the SP and NFT may be present in small co-localised clusters [6,7] but later, the correlation may disappear if one of the lesions becomes more abundant and widely distributed than the other. In the present study, however, both lesions become widely distributed and the degree of association between the SP and NFT becomes greater as their density increases. Third, the Gallyas stain may preferentially stain the primitive and classic subtypes of SP but not the diffuse SP [2,3]. Hence, a more significant association between the SP and NFT may be detected using methods that stain all the SP in the tissue [5]. The Gallyas-stained SP, however, contain tau positive PHF and are likely to be the most closely related population of plaques to the cellular NFT [8]. Fourth, SP and NFT may not be spatially associated within the same area of the cortex but there could be a more complex relationship between the two lesions, e.g., SP may develop on the axon terminals of NFT containing neurons [8,12,22,25,29].

The degree of association between SP and NFT (C_7) in a region was positively correlated with NFT density and with the total density of both lesions but not with the density of SP alone. These results suggest that the probability of observing a spatial association between the SP and NFT is more likely in areas with larger numbers of lesions. Two histological features may appear to be positively associated if large numbers of lesions are present if there are some areas of the section that are 'unsuitable' for the development of either feature. In such a circumstance, the number of empty plots ('d' in a 2x2 table) may be large leading to a spurious positive association. This could arise in the present study during random sampling across the cerebral cortex as SP and NFT may occur with greater abundance in laminae II/III and V/VI respectively and are not randomly distributed across the section [4]. In

Table IV. The degree of spatial association between the senile plaques (SP) and neurofibrillary tangles (NFT) in various brain regions (FC = frontal cortex, PC = parietal cortex, OC = occipital cortex, STG = superior temporal gyrus, LOT = lateral occipitotemporal gyrus, PHG = parahippocampal gyrus, HC = hippocampus sectors CA1 to CA4) in six cases of Alzheimer's disease (C7 = degree of association, χ^2 = chi-square with correction for continuity, – insufficient densities of SP and NFT to test association, * $P < 0.05$, ** $P < 0.01$)

Case	Spatial Association	Brain region						
		FC	PC	OC	STG	LOT	PHG	HC
A	C ₇	0.06	0.06	0.01	-0.27	0.32	0.14	0.19
	χ^2	0.35	0.32	0.08	1.51	2.90	7.72**	8.96**
B	C ₇	–	0.02	0.16	0.11	0.01	0.15	0.16
	χ^2	–	0.03	5.89*	2.90	0.09	0.01	5.89*
C	C ₇	0.05	0.01	0.18	–	0.02	-0.02	0.08
	χ^2	0.19	0.06	6.43*	–	0.01	0.28	0.27
D	C ₇	0.09	0.11	0.12	0.01	-0.31	-0.12	0.31
	χ^2	0.53	2.06	0.76	0.07	1.80	0.28	8.70**
E	C ₇	0.02	0.02	0.17	–	0.07	0.08	0.15
	χ^2	1.68	0.08	5.44	–	1.51	3.10	8.45**
F	C ₇	0.02	0.02	-0.14	0.08	-0.02	0.03	0.03
	χ^2	0.05	0.19	0.16	0.03	0.30	0.23	1.81

Differences in the degree of association between SP and NFT: 1) Between brain regions, analysis of variance $F=1.06$ ($P > 0.05$), 2) Between cases with apo ϵ genotype 3/4 and the remaining cases $t=0.54$ ($P > 0.05$).

addition, a positive association can arise if both histological features studied are common and distributed in patches throughout the entire area. If the patches are large relative to the total area sampled (a 'coarse grained' pattern) [1], then there may be a considerable area of overlap between the two features that is largely due to chance. In such areas, it may be possible to record sufficient sample fields with both SP and NFT to achieve a significant χ^2 . Consistent with this hypothesis, large scale clustering of SP [6] and NFT [2] is often observed in the cerebral cortex in AD. A similar hypothesis has been proposed to explain the positive spatial associations observed between SP and blood vessels in AD [20,21], viz., when SP are especially abundant, there is a high probability that a sufficient proportion of them will contact a blood vessel profile by chance.

In conclusion, the data suggest that clusters of SP and NFT are not spatially associated in the majority of

areas of the cerebral cortex and hippocampus studied in cases of sporadic AD. Where positive associations are present, they are likely to be attributable to the abundance and wide distribution of the lesions in the tissue rather than reflect a causal relationship between them. Hence, it is unlikely that the formation of SP and NFT occurs in relation to either the same or closely related cell bodies as predicted by the 'amyloid cascade hypothesis'. The data therefore support the hypothesis that A β and tau pathology are discreet phenomena in AD [6,11].

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