PAPER

Is there a relation between *APOE* expression and brain amyloid load in Alzheimer's disease?

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Background: It has been proposed that, independent of the $\epsilon 4$ allele, *APOE* promoter polymorphisms (-491 A/T and -219 G/T) may be risks factor for Alzheimer's disease by modulating *APOE* expression.
Objective: To measure the level of *APOE* expression in Alzheimer's disease.
Methods: Brains were obtained at necropsy from 114 patients with early and late onset sporadic Alzheimer's disease in Greater Manchester (UK) during years 1986 to 2001. Total RNA was extracted from 84 brains. Purified lymphocytes were obtained from fresh blood from 16 probable Alzheimer cases from Lille (France). *APOE* and β-actin gene expression was determined by reverse transcriptase polymerase chain reaction in brain and lymphocytes.
Results: An inverse correlation between *APOE* expression level and Aβ loads was observed. As previously described and extended to 114 cases here, an association between the -219 TT genotype and a higher level of parenchymal Aβ deposition was found, irrespective of *APOE* $\epsilon 4$ allele status. This effect was more pronounced in older individuals, whereas higher Aβ load appeared more closely related to $\epsilon 4$ in the younger age group (cut off point at the median age at death (72.5 years)). The -219 TT genotype was associated with a decrease in *APOE* expression. There was a 60% decrease in *APOE* expression in lymphocytes from probable Alzheimer cases v controls (p = 0.01).
Conclusions: In the oldest individuals, reduced *APOE* expression, modulated in part by -219 G/T polymorphism, may influence risk and constitute a determinant Aβ load in Alzheimer's disease.

Epidemiological and molecular studies suggest that multiple genes and environmental factors underlie the aetiology of Alzheimer's disease. To date, four genes have been implicated. The apolipoprotein E (*APOE*) gene is recognised as a major risk factor for sporadic forms of Alzheimer's disease with non-Mendelian patterns of inheritance,[1] while pathogenic mutations in the amyloid precursor protein (*APP*), presenilin-1 (*PS1*), and presenilin-2 (*PS2*) genes are responsible for some rare early onset autosomal dominant forms.[2]

In vitro and in vivo studies show that pathogenic mutations in *APP, PS1*, and *PS2* favour Aβ peptide production, particularly A$\beta_{n-42(43)}$, the species suspected of initiating the formation of amyloid plaques. These latter observations support the amyloid cascade hypothesis, namely that of overproduction of or failure to degrade the Aβ peptide, which leads to amyloid deposition and perhaps provokes neurofibrillary degeneration and finally neuronal death.[2]

The exact role of apolipoprotein E protein (apoE) in the development of Alzheimer's disease is still not understood. It has been suggested that apoE, and in particular the E4 isoform, may influence or provoke impaired dendritic outgrowth and arborisation,[3] or enhance oxidative neuronal damage.[4] Most interest has been focused on the amyloid cascade hypothesis whereby apoE may induce cholesterol dependent modifications of APP metabolism,[5] enhanced Aβ aggregation and toxicity,[6] or reduced Aβ clearance.[7]

Recent work suggests that, besides exerting a qualitative effect on the occurrence of Alzheimer's disease through variations in *APOE* $\epsilon 2/\epsilon 3/\epsilon 4$ coding polymorphisms, the quantitative expression of *APOE* may also be a key disease determinant, for the following reasons:

- although contradictory, numerous studies have reported a potential disruption of apoE levels in the brain,[8–13] CSF, or plasma of Alzheimer cases compared with controls[14–24];

- Aβ load in transgenic murine brain seems to be dependent on the extent of *APOE* expression. In these transgenic models, mouse apoE appears to increase amyloid deposition whereas human apoE decreases it[25 26];

- four polymorphisms have been discovered in potential regulatory regions of the *APOE* gene.[27–30] Two of them, which lie within the promoter, have received much attention (-491 A/T and -219 G/T). Both may be functional—that is, they appear to modify gene expression in vivo and in vitro[29–36] and have been associated with an increased risk of developing Alzheimer's disease[30 31 35–37] and modulation of Aβ load.[38–41]

However, not all studies have found an association between these promoter polymorphisms and Alzheimer's disease,[42–44] so a better characterisation of their association with Alzheimer pathology and in particular with the hallmarks of Alzheimer's disease will contribute to our understanding of their true role.

To examine further the possible role of apoE in the pathology of Alzheimer's disease, we measured *APOE* mRNA levels in Alzheimer brains and assessed the association between *APOE* expression and Aβ loads. We also studied the relation between *APOE* promoter polymorphisms and both *APOE* expression and Aβ load. Finally, we measured the *APOE* mRNA levels in lymphocytes from probable Alzheimer cases and controls and related these to genotype.

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Abbreviations: apoE, apolipoprotein E protein; APP, amyloid precursor protein; CAA, cerebral amyloid angiopathy; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; DSM-III-R, *Diagnostic and Statistical Manual of Mental Disorders*, third edition, revised; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association
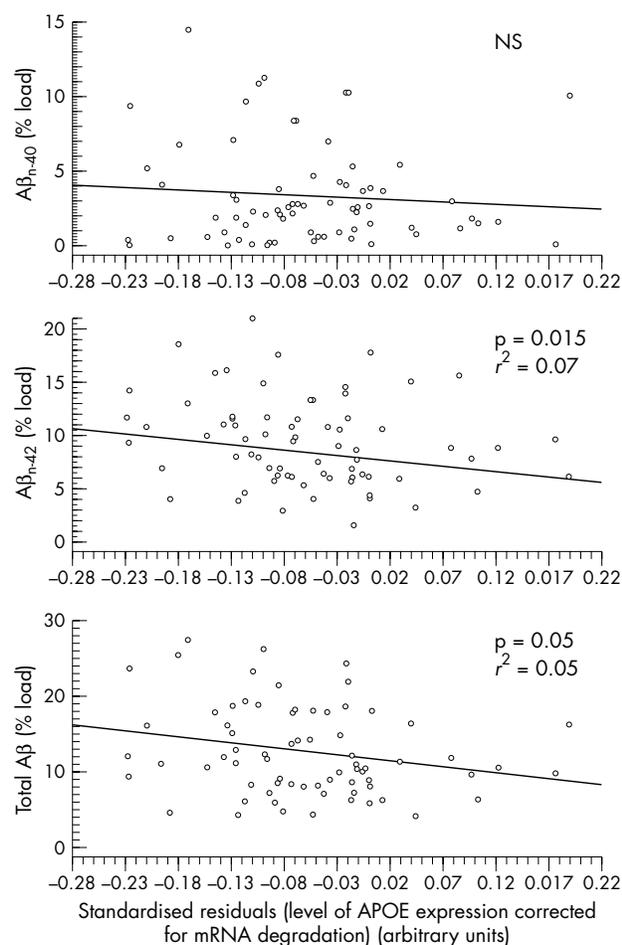
Figure 1 Correlation between Aβ load and standardised residuals of the amount of *APOE* mRNA (that is, the level of *APOE* expression adjusted according to the level of total RNA degradation). The level of significance was estimated using the non-parametric Spearman test.

METHODS
Brain samples

Alzheimer's disease brains were obtained at necropsy from 114 patients with early and late onset sporadic Alzheimer's disease accessioned from the Greater Manchester region of the United Kingdom during years 1986 to 2001 (mean (SD) age at death, 73.1 (9.1) years; mean age at onset, 65.9 (10.3) years; 51% male). All patients were white.

Pathological diagnoses were made in accordance with CERAD neuropathological criteria for Alzheimer's disease.[45] All patients were at Braak stages 5 or 6 at time of death.[46] The amount of plaque associated $A\beta_{n-40}$, $A\beta_{n-42(43)}$, and total Aβ ($A\beta_{n-40}+A\beta_{n-42(43)}$) was quantified by image analysis in the frontal cortex (Brodmann areas 8 and 9) as previously described.[47] The extent of cerebral amyloid angiopathy (CAA) in leptomeningeal and intraparenchymal arteries was rated semiquantitatively in 91 of the 114 patients (80%).[48] The severity of CAA was assessed semiquantitatively on a five point scale (grades 0 to 4). Briefly, CAA was rated as follows:

- 0, no blood vessels (small arteries, arterioles, and capillaries) are stained;
- 1, a few leptomeningeal vessels only are involved;
- 2, a few leptomeningeal vessels only are affected; mild intracortical vascular involvement;
- 3, many leptomeningeal and intracortical vessels affected;

- 4, many leptomeningeal and intracortical vessels affected, with dyshoric angiopathy associated with intracortical vessels.

Tau load in the frontal cortex was determined by image analysis in 86 of the patients after immunostaining for phosphorylated tau, using a standard procedure employing monoclonal antibody AT8 (Innogenetics, Gent, Belgium) as primary antibody.[49]

Lymphocyte samples

Purified lymphocytes from fresh blood of 16 probable cases of Alzheimer's disease (mean (SD) age, 81.8 (5.8) years, 25% male) and 36 controls (80.3 (6.5) years, 29.3% male) were cultured for 72 hours in the presence of 0.1% phytohaemagglutinin. These individuals were recruited from the same geographical area (Greater Lille) and all were also white. The diagnosis of probable Alzheimer's disease was established according to the DSM-III-R and NINCDS-ADRDA criteria. The white controls were defined as subjects without DMS-III-R dementia criteria and with full integrity of their cognitive functions. Each individual or next of kin gave informed consent.

Genotyping

DNA was extracted from frozen brain tissue and used to genotype *APOE* coding and promoter polymorphisms.[36]

Real time reverse transcriptase polymerase chain reaction

Total RNA from brains of 84 of the 114 patients with Alzheimer's disease (74%) was extracted from frozen brain tissue using a phenol/chloroform protocol (TRIzol® reagent, Invitrogen, San Diego, California, USA). Following harvesting, total RNA from lymphocytes was extracted using the RNeasy mini kit (Qiagen, Stanford, California, USA) and DNase treatment. The quality of total RNA was assessed using an Agilent 2100 bionalyser, and the ratio of ribosomal RNA 28S/18S systematically estimated employing Agilent 2100 biosizing software (range from 0.0 to 1.8 in brains or more than 1.7 in lymphocytes).[50–52]

Reverse transcription was done using 50 ng of total RNA, and expression was measured by real time polymerase chain reaction (PCR), using Taqman technology to coamplify cDNA from the *APOE* and β-actin genes, as described by the supplier (Applied Biosystems, Foster City, California, USA).

Statistical analyses

Statistical analysis was carried out using SAS statistical software, v.7.0 (SAS Institute Inc, Cary, North Carolina, USA). As the level of total RNA degradation (estimated by rRNA 28S/18S ratio) was identified as a potential confounder, this degradation level was subsequently included in a multivariate analysis of covariance, using a general linear model for comparison of the amount of mRNA between Alzheimer's disease and *APOE* promoter genotypes. Standardised residuals of linear regression analysis between degradation and APOE/β-actin ratio were withheld for further correlations between Aβ loads and mRNA amount. The relations between Aβ loads and standardised residuals were tested using the non-parametric Spearman test.

Aβ load data were compared between −219 TT and GG+GT genotypes because homozygosity for the T allele has been associated with an increased risk of developing Alzheimer's disease. Aβ load data were log transformed to normalise distributions. The effect of the coding and promoter polymorphisms on plaque associated Aβ load or the degree of CAA was tested with a multivariate analysis of covariance using a general linear model, or with Wilcoxon non-parametric test according to the number of individuals in each category.

Table 1 Effect of the −219 G/T and coding *APOE* polymorphisms on amyloid loads and cerebral amyloid angiopathy in the frontal cortex of 114 patients with Alzheimer's disease

Polymorphism	Genotype		p Value*	p Value†
−219 G/T	**GG+GT**	**TT**		
n	78	36		
Plaque $A\beta_{n-40}$	1.4 (0.3 to 7.3)	3.1 (1.2 to 8.4)	0.007	0.02
Plaque $A\beta_{n-42}$	8.5 (5.1 to 14.1)	10.4 (6.5 to 16.5)	NS	NS
Total plaque $A\beta$	11.5 (6.9 to 19.3)	14.4 (8.7 to 23.9)	0.032	0.045
n	60	31		
Frontal CAA level (mean (SD))	1.9 (0.8)	1.7 (0.8)	NS	NS
−491 A/T	**TT+AT**	**AA**		
n	20	94		
Plaque $A\beta_{n-40}$	1.6 (0.4 to 6.8)	1.8 (0.4 to 8.7)	NS	NS
Plaque $A\beta_{n-42}$	10.0 (6.6 to 15.1)	9.3 (5.5 to 15.6)	NS	NS
Total plaque $A\beta$	12.3 (7.8 to 19.5)	12.5 (7.3 to 21.2)	NS	NS
n	15	76		
Frontal CAA level (mean (SD))	1.8 (0.6)	1.7±0.5	NS	NS
APOE	**ε4⁻**	**ε4⁺**		
n	40	74		
Plaque $A\beta_{n-40}$	0.9 (0.2 to 4.3)	2.3 (0.5 to 11.4)	0.014	NS
Plaque $A\beta_{n-42}$	9.8 (6.1 to 15.8)	9.1 (5.4 to 15.2)	NS	NS
Total plaque $A\beta$	11.8 (7.5 to 18.7)	12.7 (7.3 to 22.2)	NS	NS
n	32	59		
Frontal CAA level (mean (SD))	1.7 (1.9)	1.9 (0.8)	NS	NS

Values are mean per cent amyloid load and (range) except where specified.
*p Values non-adjusted.
†p Values adjusted for impact of *APOE* −491 A/T, −219 G/T, and ε4 polymorphism on the general linear model of covariance.
CAA, cerebral amyloid angiopathy.

RESULTS

RNA is notoriously labile and highly sensitive to RNAse. As a result, postmortem delay can strongly affect the results when studying mRNA expression. However, to use only postmortem delay as a potential confounder does not allow one to correct for storage, transportation, or extraction, which may have lead to further autolysis. In order to take these biases into account, the quality of total RNA and its degradation were assessed using the 28S/18S ratio of ribosomal RNA, measured by the Agilent 2100 bionalyser and biosizing software, a new technology allowing estimation of RNA degradation (and particularly RNAse action).[50–52] In Alzheimer's disease and control brains, the level of total RNA degradation was inversely correlated with the APOE/β-actin ratio by linear regression (p<0.0001, $r^2 = 0.164$). Adjustments for this variable were thus made systematically.

We measured the level of *APOE* mRNA in the brain of 84 of the Alzheimer cases (fig 1) and observed that the *APOE* mRNA level correlated inversely with the level of $A\beta_{n-42}$ (p = 0.015) and with total Aβ load (p = 0.05) using the non-parametric Spearman test.

We next studied the association of the −491 A/T and −219 G/T polymorphisms in the *APOE* promoter that have been
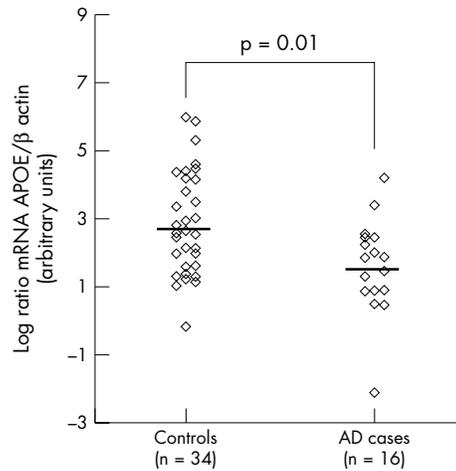
Figure 2 Comparison of the level of *APOE* expression in Alzheimer's disease and control lymphocytes by real time reverse transcriptase polymerase chain reaction. Bold lines indicate the mean values. AD, Alzheimer's disease.

Table 2 Effect of the −219 G/T polymorphism on amyloid load according to age in the brains of 114 patients with Alzheimer's disease

Genotype	Age less than median*			Age more than median*		
	GG+GT	TT	p Value	GG+GT	TT	p Value
n	42	16		36	20	
Plaque $A\beta_{n-40}$	2.0 (0.3 to 12.3)	3.0 (1.1 to 8.35)	NS	0.9 (0.2 to 5.5)	3.2 (1.2 to 8.9)	0.007
Plaque $A\beta_{n-42}$	9.3 (5.8 to 15.0)	9.7 (6.1 to 15.4)	NS	8.4 (5.2 to 13.6)	11.0 (6.9 to 17.5)	0.035
Total plaque $A\beta$	12.4 (7.5 to 20.6)	13.5 (8.1 to 22.5)	NS	10.5 (6.4 to 17.4)	15.2 (9.1 to 25.2)	0.016

Values are mean per cent amyloid load with (range).
*Age classes were defined by median age at death (72.5 years).

associated with levels of *APOE* expression. As the TT genotype of the −219 G/T polymorphism and the AA genotype of the −491 A/T polymorphism were reported potentially to increase the risk of developing Alzheimer's disease in a study of 1732 Alzheimer cases and 1926 controls from six independent populations,[36] we assessed whether these genotypes were associated with the neuropathological hallmarks and level of *APOE* expression in Alzheimer brains. The distributions of the *APOE* coding and −491 A/T and −219 G/T polymorphisms were similar to those previously reported in other Alzheimer populations.[36] Our initial study on just 74 Alzheimer cases was increased here by 40 brains to 114. In this extended population, we still observed a significant effect of the −219 G/T polymorphism on Aβ loads.[38] Aβ$_{n-40}$ and total Aβ load were increased by 124% and 35%, respectively, in individuals bearing the −219 TT genotype, compared with GT and GG (table 1; p = 0.007 and p = 0.032, respectively) while a non-significant borderline increase of 25% was observed for Aβ$_{n-42}$ deposition (p = 0.08). The amount of Aβ$_{n-40}$ was also significantly increased (p = 0.014) in Alzheimer patients carrying at least one ε4 allele (table 1). However, this latter increase was no longer significant when adjusted for the effect of the −219 G/T polymorphism (table 1). Finally, adjustment for a putative effect of the ε2 allele did not modify the association of the −219 G/T polymorphism with Aβ burden.

The effect of the *APOE* −219 G/T polymorphism may be specific to amyloid deposition in the form of plaques, as no effect of this polymorphism on the severity of CAA was detected, neither was ε4 detected, even though Aβ$_{n-40}$ is the major Aβ peptide species associated with this particular pathology (table 1). We may hypothesise we were not able to detect an effect of the *APOE* polymorphisms on CAA because the frontal region was not the area most affected by such lesions. However, the *APOE* polymorphisms were not associated with the CAA severity even in the occipital region which is the most affected (data not shown).[53] Furthermore, no effect of the *APOE* promoter polymorphisms on tau load was detected (data not shown). No effect of the −491 A/T polymorphism on Aβ deposition was observed (table 1) and neither of the *APOE* promoter polymorphisms was associated with differential disease duration (data not shown).

Previous reports have described an increased risk of developing Alzheimer's disease associated with the *APOE* −219 G/T polymorphism, but only in the oldest individuals.[36 37] We divided our population into two subgroups, using median age at death (72.5 years) as the cut off point. In the older subgroup, Aβ$_{n-40}$, Aβ$_{n-42}$, and total Aβ loads were significantly increased, by 257% (p = 0.007), 31% (p = 0.035), and 45% (p = 0.016), respectively, in individuals bearing the *APOE* −219 TT genotype (table 2). This increase of Aβ$_{n-40}$, Aβ$_{n-42}$, and total Aβ loads may be independent of the coding *APOE* polymorphisms (+204%, p = 0.07; +35%, p = 0.21; and +54%, p = 0.05, respectively, in ε3/ε3 bearers; +98%, p = 0.05; +45%, p = 0.06; and +55%, p = 0.04 in ε3/ε4 bearers). Conversely, the *APOE* ε4 allele was only associated with an increase in Aβ$_{n-40}$ load in the younger subgroup (+238%, p = 0.006).

It is important to note that when the population was divided using other ages at death as the cut off, determined by tertile or quartile design (oldest age class above 76 years or above 79 years, respectively), the results were clearer in term of Aβ loads . For instance, in the highest quartile class of age (≥79 years), the Aβ$_{n-40}$, Aβ$_{n-42}$, and total Aβ loads were increased by, respectively, +274% (p = 0.05), +41% (p = 0.05), and +53% (p = 0.02) in individuals bearing the *APOE* −219 TT genotype.

We did not observe any significant differences in the amount of *APOE* mRNA between any of the polymorphic variants for −219 G/T, −491 A/T or coding polymorphisms in Alzheimer brains (data not shown). However, as the association of the −219 G/T polymorphism on Aβ load was strongest in the oldest individuals, we assessed whether the amount of *APOE* mRNA was dependent on age according to the *APOE* −219 G/T polymorphism. We observed that the −219 TT genotype was associated with an age dependent decrease in the amount of *APOE* mRNA (p = 0.02, non-parametric Spearman test). This correlation was not observed for cases bearing at least one −219 G allele (p = 0.77, non-parametric Spearman test). In the oldest Alzheimer subgroup (cut off at 72.5 years), the level of *APOE* mRNA was decreased by 65% in individuals bearing the −219 TT genotype compared with younger individuals of the same genotype (respectively, 0.23 (0.11) and 0.15 (0.06); p = 0.02). Such a decrease was not detectable in the oldest Alzheimer subjects bearing at least a G allele when compared with younger individuals (respectively, 0.20 (0.11) and 0.20 (0.11); NS).

Finally, we assessed whether the level of *APOE* expression was different in lymphocytes between Alzheimer cases and controls. We found a 60% decrease in APOE mRNA in the Alzheimer cases compared with the controls (p<0.01; fig 2). This may indicate that a decrease in *APOE* expression is a marker of Alzheimer's disease. No significant effect of the *APOE* promoter or coding polymorphisms was detected (data not shown).

DISCUSSION

In this study we found that a decrease of *APOE* mRNA in human Alzheimer's disease brains was correlated with an increase in Aβ loads. In the oldest cases, the *APOE* −219 TT genotype was associated with a decrease in *APOE* expression and an increase in Aβ loads. These data may indicate that variations in *APOE* expression are important for the development of amyloid plaques in the brain in Alzheimer's disease, independent of the *APOE* genotype. This observation is of interest for the following reasons. First, although the finding is controversial, a majority of studies reported decreases in apoE levels in the CSF of Alzheimer patients[14–20] while other found an increase[21 22] or no change.[23 24] Second, in brain tissues, both decreases[8–11] and increases[12 13] of *APOE* mRNA and proteins have been reported, but despite these discrepancies, overall it has been proposed that levels of apoE are reduced in Alzheimer's disease.[54] Our observations in human lymphocytes support this, even though they are peripheral cells.

Some points need further discussion. For instance, we previously described a specific ε4 allele overexpression in Alzheimer's disease brains carrying the ε3/ε4 genotype compared with controls of the same genotype.[55] At that time, we did not measure the global level of expression of the *APOE* gene, and our previous data cannot be compared directly with the results reported here. However, even though our previous observation may in part have resulted from a technical bias, restricting their significance,[56] this observation raises an important issue—that there may be a contradiction between the consequences of a decrease in *APOE* expression and the deleterious effect of the apoE4 isoform (generally assumed to be an increase in negative function compared with the apoE3 isoform).[57] Specific age dependent mechanisms may nevertheless help us to gain a better understanding of this potential contradiction. For instance, it is well documented that the apoE4 isoform facilitates Aβ$_{n-40}$ deposition[47] and this relation may underlie the pathogenesis of Alzheimer's disease in younger persons. It is well established that the risk of Alzheimer's disease associated with the ε4 allele decreases in patients who develop the disease after 65 to 70 years.[1 58] Our present findings may indicate that possession of the *APOE*

−219 TT genotype is associated with a reduced *APOE* expression and a greater Aβ accumulation in such older people, consistent with the increased risk for Alzheimer's disease conferred by this genotype in older subjects.[36 37] We may thus hypothesise that it is an insidious overall lack of brain apoE, rather than any particular isoform or allele specific process that promotes Aβ formation and the development of Alzheimer's disease in such older people.

In respect of our results, there are some further caveats that need to be considered. First, there may be systematic bias in the measurement of *APOE* expression resulting from, for example, postmortem autolysis, storage, and transportation. As a result, we systematically corrected our results for degradation of extracted total RNA by the use of the Agilent technology, which, to date, is likely to be the most powerful way of estimating total RNA quality.[50–52] However, we are aware that this correction may be insufficient for highly degraded RNA samples, and replication of our observations in other large sets of brain tissue is necessary. Furthermore, measurement of apoE protein level is pertinent, as this may be less susceptible to postmortem autolysis, and APOE protein synthesis is highly dependent on regulation at post-transcriptional levels. Second, we are also aware that quantification of low abundance mRNAs is more sensitive to problems derived from mRNA degradation, although *APOE* is highly expressed in brain.[59] Finally, multiple stratifications may lead to false positive results, and corrections for multiple testing could be applicable. However, it is important to note that the polymorphisms are not completely independent, nor are the tested variables, and the results need to be coherent at both epidemiological and biological level.

Our present data substantiate our initial preliminary report on the effects of −219 G/T polymorphism on Aβ load.[18] However, in this study we were not able to detect any effect of the −491 A/T polymorphism on the pathological variables studied. Our findings do not confirm our initial report of an association of the −491 AA genotype on Aβ$_{n-40}$ load in a subset of non-ε4 Alzheimer's disease bearers[22]; in the present study, we observed a non-significant ($p = 0.27$) increase of 78% in Aβ$_{n-40}$ load (+78%) in non-ε4 bearing Alzheimer cases with the −491 AA genotype.

It is not clear why these two polymorphisms—both capable of modulating *APOE* expression[12–18]—do not apparently act in a similar way in the aetiology of Alzheimer's disease. However, several observations may explain this apparent discrepancy. As the decrease in *APOE* expression only appears in Alzheimer's disease carriers of the −219 TT genotype in an age dependent manner, we may hypothesise that the cellular mechanism leading to this decrease causes the development of Alzheimer's disease only in the oldest individuals, thereby explaining why the association of the −219 G/T and −491 A/T polymorphisms with dementia differs according to age.[36 37] Consequently, it is likely that transcriptional factors able to bind the sequences containing the −491 A/T and −219 G/T polymorphisms are different. For example, we have observed that the −219 G/T, but not the −491 A/T polymorphism, modulates the binding of oestrogen receptors.[60] Similarly, Campillos *et al* described a specific interaction of the heterogeneous nuclear ribonucleoprotein A1 protein with the −219T allele, leading to the modulation of the *APOE* promoter activity.[61] Furthermore, there may be a tissue dependent effect of the *APOE* promoter polymorphisms on expression, leading to a local modulation of this expression in the brain. Regulation of the transcription of *APOE* is likely to be highly complex and is influenced not only by functional proximal polymorphisms (−491 A/T and −219 G/T) but also by distal regulatory regions. Environmental factors are also likely to influence this complex genetic regulatory mechanism.

In conclusion, numerous coherent observations indicate that a decrease in *APOE* expression may be important for the development of Alzheimer's disease. Indeed, in mice a decrease in amyloid deposition is inversely associated with the copy number of the human *APOE* gene, this effect being the stronger when the human clusterin (apoJ) is present.[62] Likewise, in our study, the highest level of *APOE* mRNA found correlated with lowest amyloid depositions. We also showed that the −219 TT genotype is associated with both an increased risk of developing Alzheimer's disease and an increased amount of amyloid deposition in brains in the oldest subjects. These associations might be explained by the fact that the −219 T allele is correlated with a decrease in gene reporter activity in vitro,[29] with low *APOE* mRNA levels in the brain of old cases as well as with a low plasma level in young people.[32] Finally, several reports have described an increased risk of myocardial infarction associated with the −219 TT genotype.[32 63 64] This is of particular interest as it has been suggested that apoE may participate at a local level in the degradation of arteriosclerotic plaques.[32] We hypothesise that apoE participates in the degradation of amyloid deposits, as suggested by our data and by recent transgenic mouse results.[65]

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REFERENCES

1 **Farrer LA**, Cupples LA, Haines JL, *et al*. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* 1997;**278**:1349–56.
2 **Hardy J**. Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci* 1997;**20**:154–9.
3 **Nathan BP**, Bellosta S, Sanan DA, *et al*. Differential effects of apolipoproteins E3 and E4 on neuronal growth in vitro. *Science* 1994;**264**:850–2.
4 **Miyata M**, Smith JD. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. *Nat Genet* 1996;**14**:55–61.
5 **Koudinov AR**, Berezov TT, Koudinova NV. Alzheimer's amyloid beta and lipid metabolism: a missing link? *FASEB J* 1998;**12**:1097–9.
6 **Holtzman DM**. Role of apoe/Abeta interactions in the pathogenesis of Alzheimer's disease and cerebral amyloid angiopathy. *J Mol Neurosci* 2001;**17**:147–55.
7 **Yang DS**, Small DH, Seydel U, *et al*. Apolipoprotein E promotes the binding and uptake of beta-amyloid into Chinese hamster ovary cells in an isoform-specific manner. *Neuroscience* 1999;**90**:1217–26.
8 **Bertrand P**, Poirier J, Oda T, *et al*. Association of apolipoprotein E genotype with brain levels of apolipoprotein E and apolipoprotein J (clusterin) in Alzheimer disease. *Brain Res Mol Brain Res* 1995;**33**:174–8.
9 **Beffert U**, Cohn JS, Petit-Turcotte C, *et al*. Apolipoprotein E and beta-amyloid levels in the hippocampus and frontal cortex of Alzheimer's disease subjects are disease-related and apolipoprotein E genotype dependent. *Brain Res* 1999;**843**:87–94.
10 **Ramassamy C**, Averill D, Beffert U, *et al*. Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain. *Neurobiol Dis* 2000;**7**:23–37.
11 **Hesse C**, Bogdanovic N, Davidsson P, *et al*. A quantitative and immunohistochemical study on apolipoprotein E in brain tissue in Alzheimer's disease. *Dement Geriatr Cogn Disord* 1999;**10**:452–9.
12 **Diedrich JF**, Minnigan H, Carp RI, *et al*. Neuropathological changes in scrapie and Alzheimer's disease are associated with increased expression of apolipoprotein E and cathepsin D in astrocytes. *J Virol* 1991;**65**:4759–68.

13 **Yamagata K**, Urakami K, Ikeda K, *et al.* High expression of apolipoprotein E mRNA in the brains with sporadic Alzheimer's disease. *Dement Geriatr Cogn Disord* 2001;**12**:57–62.

14 **Blennow K**, Hesse C, Fredman P. Cerebrospinal fluid apolipoprotein E is reduced in Alzheimer's disease. *Neuroreport* 1994;**5**:2534–6.

15 **Hesse C**, Larsson H, Fredman P, *et al.* Measurement of apolipoprotein E (apoE) in cerebrospinal fluid. *Neurochem Res* 2000;**25**:511–17.

16 **Kunicki S**, Richardson J, Mehta PD, *et al.* The effects of age, apolipoprotein E phenotype and gender on the concentration of amyloid-beta (A beta) 40, A beta 4242, apolipoprotein E and transthyretin in human cerebrospinal fluid. *Clin Biochem* 1998;**31**:409–15.

17 **Landen M**, Hesse C, Fredman P, *et al.* Apolipoprotein E in cerebrospinal fluid from patients with Alzheimer's disease and other forms of dementia is reduced but without any correlation to the apoE4 isoform. *Dementia* 1996;**7**:273–8.

18 **Lehtimaki T**, Pirttila T, Mehta PD, *et al.* Apolipoprotein E (apoE) polymorphism and its influence on apoE concentrations in the cerebrospinal fluid in Finnish patients with Alzheimer's disease. *Hum Genet* 1995;**95**:39–42.

19 **Skoog I**, Hesse C, Fredman P, *et al.* Apolipoprotein E in cerebrospinal fluid in 85-year-old subjects. Relation to dementia, apolipoprotein E polymorphism, cerebral atrophy, and white matter lesions. *Arch Neurol* 1997;**54**:267–72.

20 **Song H**, Saito K, Seishima M, *et al.* Cerebrospinal fluid apo E and apo A-I concentrations in early- and late-onset Alzheimer's disease. *Neurosci Lett* 1997;**231**:175–8.

21 **Carlsson J**, Armstrong VW, Reiber H, *et al.* Clinical relevance of the quantification of apolipoprotein E in cerebrospinal fluid. *Clin Chim Acta* 1991;**196**:167–76.

22 **Fukuyama R**, Mizuno T, Mori S, *et al.* Age-dependent decline in the apolipoprotein E level in cerebrospinal fluid from control subjects and its increase in cerebrospinal fluid from patients with Alzheimer's disease. *Eur Neurol* 2000;**43**:161–9.

23 **Lefranc D**, Vermersch P, Dallongeville J, *et al.* Relevance of the quantification of apolipoprotein E in the cerebrospinal fluid in Alzheimer's disease. *Neurosci Lett* 1996;**212**:91–4.

24 **Rosler N**, Wichart I, Bancher C, *et al.* Tau protein and apolipoprotein E in CSF diagnostics of Alzheimer's disease: impact on non Alzheimer's dementia? *J Neural Transm Suppl* 1996;**47**:259–66.

25 **Bales KR**, Verina T, Dodel RC, *et al.* Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat Genet* 1997;**17**:263–4.

26 **Holtzman DM**, Bales KR, Wu S, *et al.* Expression of human apolipoprotein E reduces amyloid-beta deposition in a mouse model of Alzheimer's disease. *J Clin Invest* 1999;**103**:R15–21.

27 **Mui S**, Briggs M, Chung H, *et al.* A newly identified polymorphism in the apolipoprotein E enhancer gene region is associated with Alzheimer's disease and strongly with the epsilon 4 allele. *Neurology* 1996;**47**:196–201.

28 **Lambert JC**, Pasquier F, Cottel D, *et al.* A new polymorphism in the APOE promoter associated with risk of developing Alzheimer's disease. *Hum Mol Genet* 1998;**7**:533–40.

29 **Artiga MJ**, Bullido MJ, Frank A, *et al.* Allelic polymorphisms in the transcriptional regulatory region of apolipoprotein E gene. *FEBS Lett* 1998;**421**:105–8.

30 **Bullido MJ**, Artiga MJ, Recuero M, *et al.* A polymorphism in the regulatory region of APOE associated with risk for Alzheimer's dementia. *Nat Genet* 1998;**18**:69–71.

31 **Lambert JC**, Berr C, Pasquier F, *et al.* Pronounced impact of Th1/E47cs mutation compared with −491 AT mutation on neural APOE gene expression and risk of developing Alzheimer's disease. *Hum Mol Genet* 1998;**7**:1511–16.

32 **Lambert JC**, Brousseau T, Defosse V, *et al.* Independent association of an APOE gene promoter polymorphism with increased risk of myocardial infarction and decreased APOE plasma concentrations – the ECTIM study. *Hum Mol Genet* 2000;**9**:57–61.

33 **Roks G**, Cruts M, Houwing-Duistermaat JJ, *et al.* Effect of the APOE-491A/T promoter polymorphism on apolipoprotein E levels and risk of Alzheimer disease: the Rotterdam study. *Am J Med Genet* 2002;**114**:570–3.

34 **Laws SM**, Taddei K, Martins G, *et al.* The −491AA polymorphism in the APOE gene is associated with increased plasma apoE levels in Alzheimer's disease. *Neuroreport* 1999;**10**:879–82.

35 **Laws SM**, Taddei K, Martins G, *et al.* Variation at the APOE −491 promoter locus is associated with altered brain levels of apolipoprotein E. *Mol Psychiatry* 2002;**7**:886–90.

36 **Lambert JC**, Araria-Goumidi L, Myllykangas L, *et al.* Contribution of APOE promoter polymorphisms to Alzheimer's disease risk. *Neurology* 2002;**59**:59–66.

37 **Heijmans BT**, Slagboom PE, Gussekloo J, *et al.* Association of APOE epsilon2/epsilon3/epsilon4 and promoter gene variants with dementia but not cardiovascular mortality in old age. *Am J Med Genet* 2002;**107**:201–8.

38 **Lambert JC**, Mann D, Goumidi L, *et al.* Effect of the APOE promoter polymorphisms on cerebral amyloid peptide deposition in Alzheimer's disease. *Lancet* 2001;**357**:608–9.

39 **Berr C**, Lambert JC, Sazdovitch V, *et al.* Neuropathological epidemiology of cerebral aging: a study of two genetic polymorphisms. *Neurobiol Aging* 2001;**22**:227–35.

40 **Myllykangas L**, Polvikoski T, Reunanen K, *et al.* ApoE epsilon3-haplotype modulates Alzheimer beta-amyloid deposition in the brain. *Am J Med Genet* 2002;**114**:288–91.

41 **Pahnke J**, Walker LC, Schroeder E, *et al.* Cerebral beta-amyloid deposition is augmented by the −491AA promoter polymorphism in non-demented elderly individuals bearing the apolipoprotein E epsilon4 allele. *Acta Neuropathol* 2003;**105**:25–9.

42 **Rebeck GW**, Cheung BS, Growdon WB, *et al.* Lack of independent associations of apolipoprotein E promoter and intron 1 polymorphisms with Alzheimer's disease. *Neurosci Lett* 1999;**272**:155–8.

43 **Perry RT**, Collins JS, Harrell LE, *et al.* Investigation of association of 13 polymorphisms in eight genes in southeastern African American Alzheimer disease patients as compared to age-matched controls. *Am J Med Genet* 2001;**105**:332–42.

44 **Zurutuza L**, Verpillat P, Raux G, *et al.* APOE promoter polymorphisms do not confer independent risk for Alzheimer's disease in a French population. *Eur J Hum Genet* 2000;**8**:713–16.

45 **Mirra SS**, Heyman A, McKeel D, *et al.* The consortium to establish a registry for Alzheimer's disease. Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 1991;**41**:479–86.

46 **Braak H**, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol* 1991;**82**:239–59.

47 **Mann DM**, Iwatsubo T, Pickering-Brown SM, *et al.* Preferential deposition of amyloid beta protein (Abeta) in the form Abeta40 in Alzheimer's disease is associated with a gene dosage effect of the apolipoprotein E E4 allele. *Neurosci Lett* 1997;**221**:81–4.

48 **Tian J**, Shi J, Bailey K, *et al.* Relationships between arteriosclerosis, cerebral amyloid angiopathy and myelin loss from cerebral cortical white matter in Alzheimer's disease. *Neuropathol Appl Neurobiol* 2004;**30**:46–56.

49 **Thaker U**, McDonagh AM, Iwatsubo T, *et al.* Tau load is associated with apolipoprotein E genotype and the amount of amyloid β protein, Aβ$_{40}$, in sporadic and familial Alzheimer's disease. *Neuropathol Appl Neurobiol* 2003;**29**:35–44.

50 **Kuschel M**, Muller O, Buhlmann C. Lab-on-a-chip technology. *Innovations in Pharmaceutical Technology* 2002;(Oct 9):38–45.

51 **Ricicova M**, Palkova Z. Comparative analyses of Saccharomyces cerevisiae RNAs using Agilent RNA 6000 nano assay and agarose gel electrophoresis. *FEMS Yeast Res* 2003;**4**:119–22.

52 **Gomes LI**, Silva RL, Stolf BS, *et al.* Comparative analysis of amplified and non-amplified RNA for hybridization in cDNA microarray. *Anal Biochem*, 2003 15, **321**:244–51.

53 **Tian J**, Shi J, Bailey K, *et al.* Relationships between arteriosclerosis, cerebral amyloid angiopathy and myelin loss from cerebral cortical white matter in Alzheimer's disease. *Neuropathol Appl Neurobiol* 2004;**30**:46–56.

54 **Champagne D**, Pearson D, Dea D, *et al.* The cholesterol-lowering drug Probucol increases apolipoprotein E production in the hippocampus of aged rats: implications for Alzheimer's disease. *Neuroscience* 2003;**121**:99–110.

55 **Lambert JC**, Perez-Tur J, Dupire MJ, *et al.* Distortion of allelic expression of apolipoprotein E in Alzheimer's disease. *Hum Mol Genet* 1997;**6**:2151–4.

56 **Growdon WB**, Cheung BS, Hyman BT, *et al.* Lack of allelic imbalance in APOE epsilon3/4 brain mRNA expression in Alzheimer's disease. *Neurosci Lett* 1999;**272**:83–6.

57 **Teter B**. ApoE-dependent plasticity in Alzheimer's disease. *J Mol Neurosci* 2004;**23**:167–79.

58 **Meyer MR**, Tschanz JT, Norton MC, *et al.* APOE genotype predicts when – not whether – one is predisposed to develop Alzheimer disease. *Nat Genet* 1998;**19**:321–2.

59 **Mahley RW**. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988;**240**:622–30.

60 **Lambert JC**, Coyle N, Lendon C. The allelic modulation of apolipoprotein E expression by estrogen. *J Med Genet* 2004;**41**:104–12.

61 **Campillos M**, Lamas JR, Garcia MA, *et al.* Specific interaction of heterogeneous nuclear ribonucleoprotein A1 with the −219T allelic form modulates APOE promoter activity. *Nucleic Acids Res* 2003;**31**:3063–70.

62 **DeMattos RB**, Cirrito JR, Parsadanian M, *et al.* apoE and Clusterin Cooperatively Suppress Abeta Levels and Deposition. Evidence that apoE Regulates Extracellular Abeta Metabolism In Vivo. *Neuron*, 2004 Jan 22, **41**:193–202.

63 **Viitanen L**, Pihlajamaki J, Miettinen R, *et al.* Apolipoprotein E gene promoter (−219G/T) polymorphism is associated with premature coronary heart disease. *J Mol Med* 2001;**79**:732–7.

64 **Ye S**, Dunleavey L, Bannister W, *et al.* Independent effects of the −219 G>T and epsilon 2/ epsilon 3/ epsilon 4 polymorphisms in the apolipoprotein E gene on coronary artery disease: the Southampton Atherosclerosis Study. *Eur J Hum Genet* 2003;**11**:437–43.

65 **Koistinaho M**, Lin S, Wu X, *et al.* Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-beta peptides. *Nat Med* 2004;**10**:719–26.