

The ACE gene and Alzheimer's disease susceptibility

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Abstract

A recent study suggested that the insertion (I) allele in intron 16 of the angiotensin converting enzyme gene (ACE) is associated with Alzheimer's disease (AD) risk. In our series of 239 necropsy confirmed late onset AD cases and 342 elderly non-demented controls aged >73 years, we found significantly different ACE genotype distributions in the case and control groups (p=0.007). Homozygotes for both the I and D alleles were associated with a higher risk compared to DI heterozygotes. While the APOE ε4 allele was strongly associated with AD risk in our series, we found no evidence for an interaction between the APOE and ACE loci. In addition, no interactions were observed between ACE and gender or age at death of the AD cases. A meta-analysis of all published reports (12 case-control series in total) suggested that both the II and ID ACE genotypes are associated with increased AD risk (odds ratio (OR) for II v DD 1.36, 95% confidence interval (CI)=1.13-1.63, OR for DI v DD 1.33, 95% CI=1.14-1.53, p=0.0002).

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Alzheimer's disease (AD) is characterised clinically by dementia and neuropathologically by the presence of β-amyloid plaques and neurofibrillary tangles. Although the pathological changes are similar at all ages at onset, AD is often divided into early and late onset disease using an arbitrary age at onset of 65 years. Most AD cases are late onset. Genetic factors modulate AD susceptibility and the risk in the first degree relative of an AD proband is 3.5-5. The only locus unequivocally associated with late onset AD risk is APOE. However, this locus accounts for less than half of the genetic variance.^{1 2}

Kehoe *et al*³ recently reported an association between AD and the insertion allele of an insertion/deletion (I/D) polymorphism in intron 16 of the DCP1/ACE gene, which encodes angiotensin converting enzyme (ACE). ACE forms angiotensin II by cleaving the two carboxy-terminal amino acids from angiotensin I. Kehoe *et al*³ reported that the effect of the I allele was dominant, as both the II and ID genotypes were over-represented in cases. The overall odds ratio (OR) for AD in carriers of one or two I alleles was 2.22 (95% CI=1.6-

3.1). This effect was independent of APOE. Although the diagnostic criteria for the cases were not clearly described, it appears that many, if not most, of the cases were defined using clinical criteria. In this study, we have tried to replicate these findings in a series of cases with necropsy confirmed AD and have performed a meta-analysis of all published association studies of ACE and AD.

Methods

Anonymised cases (n=239) with necropsy confirmed AD with an onset after 65 years (using CERAD criteria) were obtained from Brain Banks in Cambridge, Oxford, and London. Cases were of white English origin and comprised 86 males and 153 females; mean age at death was 81.2 years (SD 7.8). Our white, English, non-demented controls aged 73 years and older with MiniMental State Examination (MMSE) scores of 24 or more were collected around Oxford and Cambridge (n=342) as part of ongoing community based studies, the MRC Multicentre Study of Cognitive Function and Ageing and the Cambridge City Study.^{4 5} Controls comprised 140 males and 202 females and had a mean age of 82.1 years (SD 3.8). There was no significant difference in the age at death of the cases compared to the age of examination of the controls and the sex distributions in the two groups. Power calculations show that a sample of 162 cases and 162 controls would detect an effect size of 2 with the genotype frequencies observed in the normal population at 5% significance and 80% power. Thus our larger sample size should be able to detect an effect of the size reported by Kehoe *et al*.³

ACE genotypes were determined using the "triple primer" method described by Evans *et al*.⁶ APOE genotypes were performed as described previously.⁷ Calculation of odds ratios with 95% confidence intervals was determined using Stata 6.0 software. Meta-analysis was performed using unconditional logistic regression, using S-Plus software.

Results

ACE genotype frequencies differed significantly in our case and control groups (p=0.007) (table 1). This was because of an over-representation of both II and DD homozygotes compared to the ID heterozygotes in the cases v the controls. Allele frequencies were not significantly different in cases v controls (table 1).

Table 1 Allele and genotype frequencies at the ACE locus in necropsy confirmed AD cases and non-demented elderly controls

	Controls	%	Cases	%	Odds ratios with 95% CI
Allele D	361	53	247	52	1
Allele I	323	47	231	48	1.05 (0.83–1.32)
Total	684	100	478	100	
Genotype DD	79	23	68	28	1
Genotype DI	203	59	111	46	0.64 (0.43–0.95)
Genotype II	60	18	60	25	1.16 (0.72–1.89)
Total	342	100	239	100	

Allele I v D: $\chi^2_1=0.14$; $p=0.71$.

Genotypes: $\chi^2_2=9.81$; $p=0.007$.

Table 2 Allele and genotype frequencies at the APOE locus in necropsy confirmed AD cases and non-demented elderly controls. APOE alleles were differently distributed in cases and controls ($\chi^2_2=78$, $p<0.001$). APOE $\epsilon 4$ carriers had an odds ratio for disease of 4.87 (95% CI 3.38–7.01, $\chi^2_1=77$, $p<0.001$) compared to non-carriers. Genotype frequencies were significantly different in cases v controls ($\chi^2_5=94$, $p<0.001$)

	Controls	%	Cases	%	Odds ratios with 95% CI
Allele $\epsilon 2$	70	10	15	3	0.36 (0.20–0.64)
Allele $\epsilon 3$	537	79	318	67	1
Allele $\epsilon 4$	77	11	145	30	3.18 (2.33–4.33)
Total	684	100	478	100	
Genotype $\epsilon 2/2$	1	0.3	1	0.4	2.20 (0.13–35.47)
Genotype $\epsilon 2/3$	56	16	6	3	0.24 (0.10–0.56)
Genotype $\epsilon 2/4$	12	4	7	3	1.28 (0.50–3.35)
Genotype $\epsilon 3/3$	213	62	97	41	1
Genotype $\epsilon 3/4$	55	16	118	49	4.71 (3.16–7.03)
Genotype $\epsilon 4/4$	5	1	10	4	4.39 (1.46–13.19)
Total	342	100	239	100	

The presence of the APOE $\epsilon 4$ allele was strongly associated with AD risk in our sample (table 2). In keeping with the trends summarised in recent meta-analyses,^{1,2} APOE $\epsilon 3/4$ was associated with increased AD risk (OR compared to $\epsilon 3/3$ 4.71, 95% CI=3.16–7.03) and APOE $\epsilon 2/3$ was associated with decreased risk (OR compared to $\epsilon 3/3$ 0.24, 95% CI=0.10–0.56). The wide confidence intervals associated with APOE $\epsilon 2/2$ and APOE $\epsilon 4/4$ were probably a function of the relative paucity of these genotypes. We observed no significant interactions between the ACE locus and APOE, gender, or age at death (for the AD cases) in our sample ($p>0.05$ in all analyses; interactions between ACE and presence or absence of APOE $\epsilon 4$ allele and ACE and gender tested by Mantel-Haenszel test; one-way ANOVA used to test for difference in age at death of AD cases with the three different ACE genotypes).

We performed a meta-analysis of our data with those reported previously for AD before 7 April 2000. The demographic data and odds ratios for ACE alleles and genotypes for each series are shown in table 3. In the meta-analysis

of these 12 case-control series, the I allele is associated with significantly increased AD risk (OR=1.18, 95% CI=1.07–1.28, $\chi^2_1=12.78$, $p<0.001$, heterogeneity $\chi^2_{11}=13.46$, $p=0.26$) and both the II and ID genotypes are associated with significantly increased risks, compared to DD homozygotes (OR (II v DD)=1.36, 95% CI=1.13–1.63, OR (ID v DD)=1.33, 95% CI=1.14–1.53, $\chi^2_2=16.74$, $p=0.0002$, heterogeneity $\chi^2_{22}=45.83$, $p=0.002$).

Discussion

Our data comparing late onset necropsy confirmed AD cases to non-demented controls were consistent with those of Kehoe *et al*,³ in that the II genotype was associated with increased risk. In a meta-analysis of all published studies with our own data, both the ID and II genotypes were associated with significantly increased AD risk, confirming the suggestion that the effect of the I allele may be dominant.³ The modest odds ratios associated with both the II and ID genotypes may explain why the effect was not observed in a number of the published studies. Since there is significant heterogeneity between the studies, this effect may be more pronounced in particular populations. Our data are consistent with those of Kehoe *et al*,³ who also did not find any ACE interactions with APOE, age, or gender.

The increased AD risk associated with the I allele is consistent with the increased longevity associated with the D allele.^{15,16} However, this longevity association could potentially confound the interpretation of our results if the effect is mainly because of the D allele being a marker of healthy ageing. Although ACE genotypes were not associated with different ages at death in our AD cases, this caveat will need to be addressed in large scale epidemiologically based cohort studies. Although these associations of ACE and AD/longevity are not easily reconciled with previous studies implicating the D allele in a number of cardiovascular diseases, like myocardial infarction and left ventricular hypertrophy, the latter findings have not been consistently replicated.³

The potential role of ACE in AD pathogenesis is not clear. AD risk may be related to blood pressure regulation. Blood pressure is partly regulated by angiotensin II, which is a potent arterial vasoconstrictor. Angiotensin II also increases aldosterone synthesis from the

Table 3 Demographic details and odds ratios for AD for ACE alleles and genotypes for specific case control series used for meta-analysis

Reference region	Source	Cases			Controls		Odds ratio (95% confidence interval)		
		No	Age (SD)	Diagnostic criteria ¹⁷	No	Age (SD)	I v D	DI v DD	II v DD
Scacchi <i>et al</i> ⁸	Italy	80	83.5 (5.9)	NINCDS-ADRDA	153	78.3 (7.0)	1.05 (0.71–1.56)	1.26 (0.70–2.27)	0.96 (0.39–2.39)
Chapman <i>et al</i> ⁹	Israel	49	74.8 (9.7)	NINCDS-ADRDA	40	72.7 (7.8)	1.00 (0.53–1.86)	1.32 (0.55–3.21)	0.72 (0.17–3.10)
Kehoe <i>et al</i> ³	Cardiff	198	70.3 (9.4)	NINCDS-ADRDA	77	73.5 (6.2)	1.56 (1.07–2.28)	2.39 (1.29–4.43)	2.56 (1.14–5.78)
Kehoe <i>et al</i> ³	London	135	82.3 (6.7)	NINCDS-ADRDA	111	80.8 (4.5)	1.39 (0.97–1.99)	3.13 (1.69–5.79)	1.79 (0.83–3.86)
Kehoe <i>et al</i> ³	Belfast	209	76.6 (6.3)	NINCDS-ADRDA	198	77.1 (6.4)	1.27 (0.96–1.67)	1.86 (1.10–3.13)	1.75 (0.99–3.11)
Palumbo <i>et al</i> ¹⁰	Perugia	64	73 (5)	NINCDS-ADRDA	40	71 (4)	1.07 (0.60–1.90)	1.01 (0.41–2.47)	1.13 (0.39–3.22)
Alvarez <i>et al</i> ¹¹	Spain	350	72 (9.0)	NINCDS-ADRDA	400	21–65 (range)	1.28 (1.04–1.58)	1.26 (0.92–1.73)	1.61 (1.06–2.44)
Hu <i>et al</i> ¹²	Japan	132	73.7 (11.0)	NINCDS-ADRDA	148	75.5 (9.9)	1.59 (1.11–2.26)	1.85 (0.81–4.23)	2.70 (1.87–6.13)
Crawford <i>et al</i> ¹³	Florida	171	72.2 (6.7)	NINCDS-ADRDA	175	73.6 (6.7)	1.23 (0.91–1.67)	1.81 (1.12–2.90)	1.27 (0.69–2.33)
Farrer <i>et al</i> ¹⁴	Moscow	151	64.6 (8.8)	NINCDS-ADRDA	206	64.9 (11.4)	0.90 (0.67–1.22)	1.27 (0.77–2.07)	0.77 (0.42–1.42)
Farrer <i>et al</i> ¹⁴	Toronto	236	75.2 (7.6)	NINCDS-ADRDA	169	70.2 (10.2)	0.96 (0.72–1.28)	1.17 (0.76–1.80)	0.84 (0.47–1.48)
Narain <i>et al</i>	England	239	81.2 (7.8)	CERAD	342	82.1 (3.8)	1.05 (0.83–1.32)	0.64 (0.43–0.95)	1.16 (0.72–1.88)

adrenal cortex and stimulates catecholamine synthesis and release from peripheral noradrenergic neurones. Angiotensin II is formed after cleavage of two carboxy-terminal amino acids from angiotensin I by ACE. While the D allele has been associated with higher circulating ACE levels in man, this locus is not clearly associated with essential hypertension in humans. Kehoe *et al*⁶ suggested that it may be important to consider other functions of ACE, since it may play a role in inflammation in AD. While the role of ACE in AD pathogenesis is not clear, this meta-analysis suggests that both II and ID genotypes are associated with a modest increase in risk.

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