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Original Article

ACE variants and association with brain Aβ levels in Alzheimer's disease

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Abstract: *ACE* is a candidate gene for Alzheimer's disease (AD) and associations have been reported between *ACE* variants and plasma ACE levels, AD risk, AD age at onset of disease and cerebrospinal fluid levels of Aβ. Despite evidence that ACE can degrade Aβ, the relationships between *ACE* variants and the levels of different types of Aβ in the brain are not known. We have investigated the relationship between AD-associated *ACE* variants, for which the associations with brain activity of ACE were previously analysed, and brain homogenate levels of soluble, insoluble and oligomeric Aβ. Reported AD risk variants in the *ACE* indel (rs1799752) and its 'proxy' rs4343 were significantly associated with soluble Aβ level in AD only (p=0.001), as was rs1800764 but less so (p=0.014). In contrast, insoluble Aβ was associated with *ACE* indel and rs4343 variants in controls only ($p < 0.01$). No associations were found for oligomeric Aβ. These data indicate a complex relationship between *ACE* and Aβ that differs between AD and control brains.

Keywords: Alzheimer's disease, angiotensin, ACE, amyloid, neuropathology, soluble Aβ, insoluble Aβ, SNP, association, Aβ degrading enzyme, cerebral blood flow, hypertension

Introduction

Angiotensin converting enzyme (ACE) plays a key role in the renin-angiotensin system (RAS) pathway. ACE catalyses the formation of the vasoconstrictor angiotensin II (Ang II) from angiotensin I and is also responsible for the cleavage and inactivation of the vasodilator bradykinin, resulting in vasopression. The actions of the RAS have been extensively studied in the periphery, particularly the role of Ang II in hypertension. However, it is now recognised that nearly all organs of the body have their own local paracrine-like RAS, with organ-specific actions [1]. The actions of Ang II within the central nervous system are of increasing interest in the context of Alzheimer's disease (AD). Ang II inhibits the release of acetylcholine (ACh) and has a pro-inflammatory effect (for review see [2]).

The level and activity of ACE within the cerebral cortex are generally elevated in AD [3-6]. This would be expected to increase the production of Ang II, the proinflammatory, anticholinergic and vasopressor actions of which could all exacerbate cognitive dysfunction in AD. That elevated ACE activity may be deleterious in AD is supported by recent population-based observational studies. Here RAS-acting antihypertensives such as ACE inhibitors (ACE-Is) and angiotensin receptor blockers (ARBs), which inhibit Ang II signalling, were associated with reductions in the incidence and rate of cognitive decline in MCI and AD [7-15].

The ACE gene (*ACE*) has featured for a considerably long period as one of the top susceptibility genes for AD [16-18], according to the ongoing meta-analysis database of AD candidate genes listed on Alzgene (www.Alzgene.org). *ACE* indel and related haplotypes associated with AD risk have reduced plasma ACE [19-22] whereas protective genotypes have elevated ACE [16]. We have recently observed reduced ACE activity

in CSF from AD patients [23] in agreement with previous work [24-25].

Several studies have shown ACE to be capable of degrading Aβ in vitro [26-30]. Variations in *ACE* were associated with differences in CSF Aβ level [16, 31] and some animal data suggested that Aβ level was increased by administration of ACE inhibitors [32-33]. It is not currently known whether ACE inhibitor use in humans influences Aβ accumulation. Few studies have tested whether AD-associated 'risk' *ACE* variants are associated with Aβ level in the brain [34-35] which may also shed further light on possible in vivo interactions and, to our knowledge, none has examined this association in relation to the different forms of Aβ.

Prompted by our previous observation of associations between *ACE* variants and ACE protein level and activity in CSF and human brain tissue [23], we have now explored the relationship between *ACE* variants and different forms of Aβ in greater detail, in mid-frontal cortex from AD and control brains.

Materials and methods

General materials

For this study, we analysed data that we had obtained in recent studies of Aβ species in the context of AD [36-38]. Measurements of total soluble and insoluble Aβ and of oligomeric $Aβ_{1-42}$ had been made on samples of mid-frontal cortex (Brodmann area 6). In samples from this region we had also previously measured ACE levels and activity. The tissue was from the South West Dementia Brain Bank, University of Bristol, and the studies had Research Ethics Committee approval. The AD cases (N=72, 64% female, 79.58 ± 9.0 years at death, 45.01 ± 24.0 hours post-mortem delay) had 'probable' or 'definite' AD according to the criteria of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) [39]. Controls (N=33, 49% female, 79.21 ± 10.9 years at death, 45.42 ± 42.7 hours post-mortem delay) were defined by the absence of AD or other neuropathological abnormalities. The age, gender and post-mortem delay were matched as closely as possible between the AD and control groups (see above). As previously described for this cohort, information on prior use of ACE-inhibitors (ACE-Is) was available for only a small number of subject, and ACE level and activity were not related to the past history of ACE-I use in these cases [23].

ACE genotype data and the genotypic and allelic frequencies of *ACE* frequencies in this cohort were previously reported [23]. The four SNPs analysed were examined in several other studies in the context of hypertension and ACE plasma level [19, 40] and AD [16, 31, 41-43]. The most common *ACE* variant , the intron 16 indel (rs1799752) has close linkage disequilibrium (LD) with rs4343 (in exon 17 of *ACE*) (r2 =0.91) [19], whilst rs4291 and rs1800764 are located in the *ACE* promoter and 5' untranslated region (UTR) of the gene and were previously shown to be associated with AD.

Measurement of total soluble and insoluble Aβ and oligomeric $Aβ_{1-42}$

The measurement of total soluble and insoluble Aβ in the cases in this study was reported previously [38], as was the measurement of oligomeric $Aβ_{1-42}$. Soluble and guanidine-HCl extracted (insoluble) fractions were analysed by sandwich ELISA in which monoclonal anti-Aβ (4G8 clone, raised against amino acids 18-22; Millipore, Watford, UK) was used for the capture step and biotinylated anti-human β-amyloid monoclonal antibody (10H3 clone) (Thermo Fisher Scientific, Northumberland, UK) for the detection step. Measurement of oligomeric $Aβ_{1-42}$ in the soluble fraction was by sandwich ELISA using a rabbit polyclonal $Aβ_{1-42}$ antibody (Millipore, Watford, UK) for the capture step and monoclonal mouse anti-oligomeric Aβ antibody (clone 7A1a, New England Rare Reagents, ME, USA) for the detection step [38, 44].

Statistical analysis

Statistical Package for Social Science software (12.0.1) was used to examine the relationship between *ACE* genotype and Aβ load by analysis of variance (ANOVA), adjusting for multiple testing by the Bonferroni method. Values of $p < 0.05$ were considered significant.

Results

Soluble Aβ

The level of total soluble Aβ was significantly influenced by *ACE* indel polymorphism in the

Table 1. Analysis of genetic association between *ACE* indel polymorphism and Aβ levels

Indel rs	DD (mean±SEM)	ID (mean±SEM)	II (mean±SEM)	*ANOVA*	*Post-Hoc*
Total Soluble Aβ*					
Control	1.854 ± 0.5	1.452 ± 0.2	1.056 ± 0.51	*NS*	*NS*
AD	1.571 ± 0.3	2.444 ± 0.6	5.231 ± 0.9	*P=0.001*	*DDvII p=0.009, DDvII p=0.002*
Combined	1.699 ± 0.3	2.2 ± 0.4	4.361 ± 0.8	*p=0.002*	*DD v II p=0.002, ID v II p=0.011*
Total Insoluble Aβ*					
Control	12.285 ± 6.3	55.441 ± 13.7	10.818 ± 6.9	*P=0.009*	*DDvID p= 0.060, IDvII p= 0.016*
AD	146.204 ± 22.9	133.444 ± 16.0	158.854 ± 20.2	*NS*	*NS*
Combined	91.800 ± 18.1	110.209 ± 12.9	124.692 ± 19.9	*NS*	*NS*
Oligomeric 42 Aβ**					
Control	2.869 ± 0.4	2.609 ± 0.6	3.390 ± 0.9	*NS*	*NS*
AD	2.689 ± 1.0	4.800 ± 1.1	3.219 ± 1.0	*NS*	*NS*
Combined	2.774 ± 0.5	4.151 ± 0.8	3.266 ± 0.8	*NS*	*NS*

Legend: * refers to units of measurement in nM range while ** corresponds to measurement units of micro(greek letter m)g/ml

combined AD and control cohorts (ANOVA p = 0.002) and in AD cases alone (ANOVA p = 0.001) but not in controls alone (**Table 1**). Post-hoc Bonferroni testing showed significantly higher soluble Aβ in individuals with *ACE* II genotype than with ID or DD genotypes in the combined and AD cohorts (**Table 1**). No significant difference in soluble Aβ was observed between ID and II genotypes, although there was a suggestion of a gene-dose effect. Soluble Aβ level was significantly associated with *ACE* SNPs rs4343 and rs1800764 in the combined and AD cohorts but not in controls alone and not with rs4291 although similar patterns were apparent (**Table 2**). There was no association between *ACE* genotype and oligomeric $A\beta_{1\text{-}42}$ level in any of the cohorts (**Table 1** and **2**).

Insoluble Aβ

Total insoluble (guanidine-extracted) Aβ level did not differ according to the *ACE* indel (**Table 1**) or any of the other *ACE* SNPs (**Table 2**) in the AD or combined AD and control cohorts. In contrast, in the control cohort alone, insoluble Aβ load differed significantly according to *ACE* indel (ANOVA p = 0.009) but not in the allele dose dependent manner observed for soluble Aβ in AD. Instead, in controls insoluble Aβ was elevated solely in cases heterozygous (ID) for the *ACE* indel compared to both homozygous groups (**Table 1**). For *ACE* SNP rs4343, a proxy SNP of the indel because of its close linkage disequilibrium (LD) [16], insoluble Aβ was similarly significantly different (ANOVA p = 0.006, post-hoc AG>GG p=0.020; AG>AA p=0.080) following the same pattern of association as for the indel and consistent with the allelic distributions expected by their LD (**Table 2**).

Discussion

These data reveal a complex relationship between *ACE* variants and the levels of different species of Aβ in AD. Individuals carrying the *ACE* II 'risk' genotype for AD [17] have lower plasma [21] and CSF [23] ACE protein compared to samples homozygous for the 'protective' D allele and heterozygotes with reportedly intermediate risk [17]. These *ACE* genotypes have also previously been associated with variation in the CSF level of $A\beta_{1\text{-}42}$ in AD [16, 31].

The current data show that the AD risk II geno-

Table 2. Analysis of genetic association between common *ACE* polymorphisms and Ab levels

A. rs4343	GG (Mean ±SEM)	AG (Mean ±SEM)	AA (Mean ±SEM)	*ANOVA*	*Bonferroni post-hoc*
Total soluble Aβ*					
Controls	1.887 ± 0.7	1.524 ±0.3	1.056 ± 0.5	*NS*	*NS*
AD	1.507 ± 0.3	2.301 ± 0.6	5.614 ± 1.1	*P = 0.001*	*GG V AA p=0.002, AG v AA p=0.005*
Combined	1.676 ± 0.3	2.081 ± 0.5	4.529 ± 0.9	*P = 0.003*	*GG v AA p=0.004, AA v AG p=0.010*
Total Insoluble Aβ*					
Controls	9.735 ± 6.4	60.919 ± 13.9	10.818 ± 7.0	*P = 0.006*	*GG v AG p=0.011, AA v AG p=0.033*
AD	151.624 ± 26.2	130.708 ± 16.8	166.395 ± 22.9	*NS*	*NS*
Combined	93.817 ± 20.7	109.106 ± 13.3	125.810 ± 22.3	*NS*	*NS*
Oligomeric Aβ42					
Controls	2.300 ± 0.4	2.559 ± 0.5	3.781 ± 1.0	*NS*	*NS*
AD	2.902 ± 1.1	3.911 ± 0.9	3.297 ± 1.1	*NS*	*NS*
Combined	2.653 ± 0.6	3.451 ± 0.6	3.459 ± 0.8	*NS*	*NS*
B. rs1800764	CC (Mean ±SEM)	TC (Mean ±SEM)	TT (Mean ±SEM)	*ANOVA*	*Bonferroni post-hoc*
Total soluble Aβ*					
Controls	2.017 ± 0.7	1.331 ± 0.3	.787 ± 0.3	*NS*	*NS*
AD	2.609 ± 1.3	1.975 ± 0.3	4.911 ± 0.9	*P = 0.014*	*TC V TT p= 0.013*
Combined	2.351 ± 0.8	1.736 ± 0.2	4.374 ± 0.9	*P = 0.003*	*CC v TT p=0.082, TC v TT p=0.002*
Total insoluble Aβ*					
Controls	11.113 ± 5.6	33.404 ± 10.0	13.440 ± 10.7	*NS*	*NS*
AD	122.170 ± 25.3	155.666 ± 18.4	148.014 ± 20.1	*NS*	*NS*
Combined	76.738 ± 19.0	108.842 ± 14.8	126.483 ± 19.6	*NS*	*NS*
Oligomeric Aβ42					
Controls	3.091 ± 0.8	2.933 ± 0.4	3.033 ± 1.2	*NS*	*NS*
AD	3.245 ± 1.2	3.338 ± 0.9	3.666 ± 0.5	*NS*	*NS*
Combined	3.182 ± 0.8	3.164 ± 0.5	3.562 ± 0.9	*NS*	*NS*
C. rs4291	AA (Mean ±SEM)	AT (Mean ±SEM)	TT (Mean ±SEM)	*ANOVA*	*Bonferroni post-hoc*
Total soluble Aβ*					
Controls	2.418 ± 0.8	1.307 ± 0.3	.924 ± 0.5	*NS*	*NS*
AD	2.905 ± 1.6	2.290 ± 0.4	3.740 ± 0.8	*NS*	*NS*
Combined	2.674 ± 0.9	1.886 ± 0.3	3.314 ± 0.7	*NS*	*NS*
Total insoluble Aβ					
Controls	26.107 ± 12.6	42.626 ± 14.0	17.683 ± 8.4	*NS*	*NS*
AD	126.597 ± 31.1	138.365 ± 18.6	154.437 ± 19.2	*NS*	*NS*
Combined	76.352 ± 19.9	101.133 ± 14.7	130.993 ± 18.2	*NS*	*NS*
Oligomeric Aβ42**					
Controls	2.498 ± 0.6	2.434 ± 0.5	3.368 ±0.7	*NS*	*NS*
AD	2.589 ± 1.2	2.904 ± 1.0	3.800 ± 0.9	*NS*	*NS*
Combined	2.548 ± 0.7	2.713 ± 0.6	3.675 ± 0.7	*NS*	*NS*

Legend: * refers to units of measurement in nM range while ** corresponds to measurement units of micro(greek letter m)g/ml

type and related alleles in nearby SNPs are also associated with a significantly higher concentration of total soluble Aβ in AD. This may reflect more efficient cleavage by ACE of soluble than insoluble forms of Aβ and is consistent with the hypothesis that higher-risk *ACE* variants increase the risk of AD through reduced ACE-mediated degradation of insoluble Aβ. However, the relationship between *ACE* genotype and ACE activity is complicated and differs for plasma [21] and CSF [23] from that in brain tissue. ACE activity and, to a lesser extent, ACE protein concentration, are elevated not reduced in AD [3-6], and we previously found that *ACE* variation has no major influence on ACE activity in brain tissue [23].

Increases in ACE protein level and activity have been demonstrated in human SH-SY5Y neuroblastoma cells following their exposure to oligomeric and fibrillar $A\beta_{1-42}$ [23], in keeping with observations in brain tissue of a positive association between parenchymal Aβ and ACE activity [6]. These findings suggest that ACE activity may increase as a protective response to accumulation of Aβ, possibly irrespective of the underlying genetic background, but again with differing levels of efficiency in the degradation of soluble and insoluble Aβ. The biological implications of this increase in terms of the influence on Aβ level are unclear. Studies in mouse models of AD have also yielded inconclusive data: in some administration of ACE-inhibitors did not result in an increase in Aβ load [45], in others [32-33] involving longer periods of treatment, increased Aβ accumulation was observed. Further studies are needed to clarify the long-term effects of ACE-inhibitors on Aβ (see [1] for review).

Epidemiological data indicate that elevated blood pressure in mid-life is associated with increased risk of dementia later in life (reviewed in [1]), although some people with prior hypertension are no longer hypertensive by the time dementia develops [46]. Elevated ACE activity in AD, possibly in response to Aβ deposition, could have an influence on the risk of developing AD or its exacerbation over this sort of timescale, perhaps mediated via localised central hypertensive actions of Ang II. Hypertension has already been shown to alter blood-brain barrier (BBB) permeability and increase Aβ deposition in mouse models [47-48]. A partly self-perpetuating cycle could therefore be envisaged in which a slow increase in the amount of Aβ upregulates brain ACE as a means of attenuating Aβ accumulation but also causes increased production of Ang II, which could exacerbate hypertension and with that affect cerebral blood flow, increase inflammation and inhibit ACh release. We have proposed a similar model to account for the upregulation of another putative Aβ-degrading enzyme in AD, endothelin converting enzyme 2 (ECE-2) [49]. ECE-2 is responsible for the production of another vasoconstrictor peptide ET-1 (i.e. in addition to Ang II). A further example of this sort of feedback loop is provided by the vasoactive Aβ-degrading enzyme, neprilysin, the production of which was also shown to be increased in neuronal, microglial and cerebrovascular cells by exposure to Aβ and which can produce a vasoconstrictor effect through its degradation of the vasodilator bradykinin [49] and in human APP transgenic mice [50].

The observed increases to the activity of brain ACE in response to Aβ may also influence the recently reported association between recurrent ICH and *ACE* [51]. Recurrent ICH is often a manifestation of Aβ-related cerebral amyloid angiopathy [52]. Elevated risk of recurrent ICH was associated with the AD-protective indel D allele. Healthy controls possessing the TT genotype of another *ACE* variant (rs4311) associated with recurrent ICH, had significantly higher peripheral (serum) levels of ACE [51]. It is possible that elevation of ACE in response to escalating Aβ levels (in this case perhaps related to cerebral amyloid angiopathy) may increase the likelihood of ICH through hypertensive effects [53].

The present data provide further evidence implicating ACE in the pathogenesis of AD. We suggest that this association may relate to the divergent roles of ACE in degradation of Aβ mediating a short-term neuroprotective action and the production of Ang II which may lead to longer term more wide-ranging deleterious consequences (i.e. hypertension, damage to the blood-brain barrier, reduced CBF, Aβ deposition, inflammation and reduced cholinergic activity).

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