Association of Polymorphisms in the Angiotensin-Converting Enzyme Gene with Alzheimer Disease in an Israeli Arab Community

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Several lines of evidence support for a role of angiotensin converting enzyme (ACE) in Alzheimer disease (AD). Most genetic studies have focused on an *Alu* insertion/deletion (*I/D*) polymorphism in the ACE gene (*DCP1*) and have yielded conflicting results. We evaluated the association between 15 single-nucleotide polymorphisms (SNPs) in *DCP1*, including the *I/D* variant, and AD in a sample of 92 patients with AD and 166 nondemented controls from an inbred Israeli Arab community. Although there was no evidence for association between AD and *I/D*, we observed significant association with SNPs *rs4343* ($P = .00001$) and *rs4351* ($P = .01$). Haplotype analysis revealed remarkably significant evidence of association with the SNP combination *rs4343* and *rs4351* (global $P = 7.5 \times 10^{-7}$). Individuals possessing the haplotype "GA" (frequency 0.21 in cases and 0.01 in controls) derived from these SNPs had a 45-fold increased risk of developing AD (95% CI 6.0–343.2) compared with those possessing any of the other three haplotypes. Longer range haplotypes including *I/D* were even more significant (lowest global $P = 1.1 \times 10^{-12}$), but the only consistently associated alleles were in *rs4343* and *rs4351*. These results suggest that a variant in close proximity to *rs4343* and *rs4351* modulates susceptibility to AD in this community.

Alzheimer disease (AD [MIM #104300]) is a progressive, neurodegenerative disease characterized clinically by gradual loss of memory and pathologically by neurofibrillary tangles and amyloid plaques in the brain. Currently, the apolipoprotein E (APOE [MIM +107741]) $\varepsilon 4$ allele is the only broadly recognized genetic risk factor for late-onset AD (LOAD) in most populations.[1] Much attention has been focused on the connection between angiotensin I converting enzyme (ACE [MIM +106180]) and AD. ACE is a dipeptidyl carboxypeptidase that plays an important role in regulation of blood pressure by converting angiotensin I to biologically active angiotensin II. Several studies show that an *Alu* insertion/deletion (*I/D*) polymorphism in the ACE gene (symbol DCP1) is associated with plasma level of ACE,[2,3] although the genetic regulation of ACE levels in the brain is poorly understood. Studies showing association between the *I/D* polymorphism and cardiovascular disease risk[4–8] and evidence suggesting cardiovascular risk factors promote AD[9–11] are consistent with the idea that ACE might play a role in AD via a cardiovascular mechanism. However, the observation that ACE degrades Aβ, the pathological hallmark of AD, in vitro[12–14] suggests that variation in *DCP1* may directly modulate susceptibility to AD.

Nearly all investigations of the association between *DCP1* and AD have examined the *I/D* polymorphism. Of the published reports on 41 independent samples,[15–17] 11 showed significant association with the *I* allele, 1 showed significant association with the *D* allele, and 29 found no association with this marker. These conflicting results prompted four meta-analyses of ACE studies, which considered 39 samples published before September 2004.[17–20] Two SNPs in the *DCP1* promoter region (*rs4291* and *rs1800764*) and one synonymous coding SNP (*rs4343*) proximate to *I/D* have been associated with AD in a combined sample of four case-control samples from Sweden and the United Kingdom.[18] The

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rs4343 A allele was associated with both risk and age at onset of AD.[21]

In this study, we evaluated the association between *DCP1* and AD in Wadi Ara, an Israeli Arab community with a high prevalence of AD.[22] A total of 92 individuals meeting DSM-IV criteria for AD[23] and 166 nondemented controls aged 60 and older were included in this study. Although most members of this community of >81,500 people trace their ancestors to ~14 founder families, investigation of family history by use of multiple informants (since genealogical records do not exist) revealed that most subjects belonged to distinct multigenerational pedigrees. Thus, for analytical purposes, this group was treated as a case-control sample. A detailed description of subject ascertainment and evaluation is provided elsewhere.[24] We profiled the sample for the *I/D* polymorphism and 21 SNPs spanning a 575.53-kb region including the 5′ and 3′ portions of *DCP1* which were selected from public databases, primarily dbSNP. Four nonsynonymous coding SNPs (*rs4317, rs4318, rs4364,* and *rs4976*) were almost completely monomorphic. Assays for SNPs *rs4342* and *rs13030* were unsuccessful. SNPs available for the statistical analyses include 11 within the coding region, 2 within the promoter region, and 2 in the flanking regions (see table 1). Genotyping was perfomed either by the MassARRAY Homogenous MassEXTEND (hME) Assay (Sequenom) or by TaqMan SNP Genotyping Assays (Applied Biosystems).

Analyses of individual SNPs were performed using SAS software, release 9.1. A χ^2 test was used to determine whether the genotype distribution in control subjects conformed to Hardy-Weinberg equilibrium. Genotype and allele frequencies were compared between cases and controls by χ^2 analysis. Fisher's exact test was used when the expected frequency of one or more cells was too small for the χ^2 test. Differences were considered significant if the *P* value was ≤.05. A Bonferroni correction was applied to results of analyses with individual SNPs. The linkage disequilibrium (LD) structure among SNPs was examined with the program Haploview.[25] Haplotype blocks were defined using an algorithm which created 95% confidence boundaries on *D′* to define SNP pairs in strong LD. Haplotype analysis was carried out using Haplo.stats v1.1.1.[26] Haplotypes were considered significant if the empirical global *P* value was below .05.

Of the 15 polymorphic *DCP1* markers, only the genotypes for *rs4353* were not in Hardy-Weinberg equilibrium in controls and therefore excluded from further analysis. The remaining panel of 14 markers displayed variable and often weak intermarker linkage disequilibrium (LD) (fig. 1). SNPs *rs1518772* and *rs894407* are respectively located in the genes immediately proximal (*TANC2*) and distal (*FTJS3*) to *DCP1,* and are not in LD with any of the *DCP1* intragenic markers. The LD block structure derived from this sample of AD cases and controls from Wadi Ara is nearly identical to the structure obtained for the Caucasian sample in the HapMap project[27] although not all SNPs in this study are included in HapMap.

Analysis of individual markers revealed significant disease association with SNPs *rs4343* and *rs4351* (table 1). The allele frequency difference between cases and controls for *rs4343* remained significant after correcting for multiple testing (adjusted $P = .0002$). To assess whether a particular marker profile including *rs4343* accounts for or clarifies the association with AD, we first performed haplotype analysis of all marker pair combinations including *rs4343*. The most significant haplotypes

Table 1

Characteristics of *DCP1* Markers and Their Association with AD

Marker Name	Map Position	Location or Type	Minor Allele	Minor Allele Frequency		*P*
				Cases	Controls	
rs1518772	58,679,848	Upstream of 5′ UTR	T	.45	.40	.36
rs1800764	58,904,261	Promoter SNP	A	.37	.39	.57
rs4291	58,907,926	Promoter SNP	T	.36	.33	.56
rs4295	58,910,030	Intron	C	.42	.39	.61
rs4311	58,914,495	Intron	C	.47	.53	.26
rs4329	58,917,190	Intron	G	.27	.33	.15
rs4335	58,918,757	Intron	A	.43	.45	.75
Alu I/D	58,919,636	Intron	I	.27	.31	.44
rs4343	58,919,763	T776T	G	.42	.18	**.00001**
rs4351	58,923,464	Intron	A	.44	.32	**.01**
rs4353	58,924,154	Intron	G	.38	.51	NT[a]
rs4362	58,927,493	F1129F	C	.49	.40	.06
rs4575595	58,930,947	Intron	A	.37	.30	.18
rs4267385	58,937,488	Intron	C	.30	.31	.73
rs894407	59,255,373	Downstream of 3′ UTR	A	.35	.41	.30

[a] NT = not tested (see text).

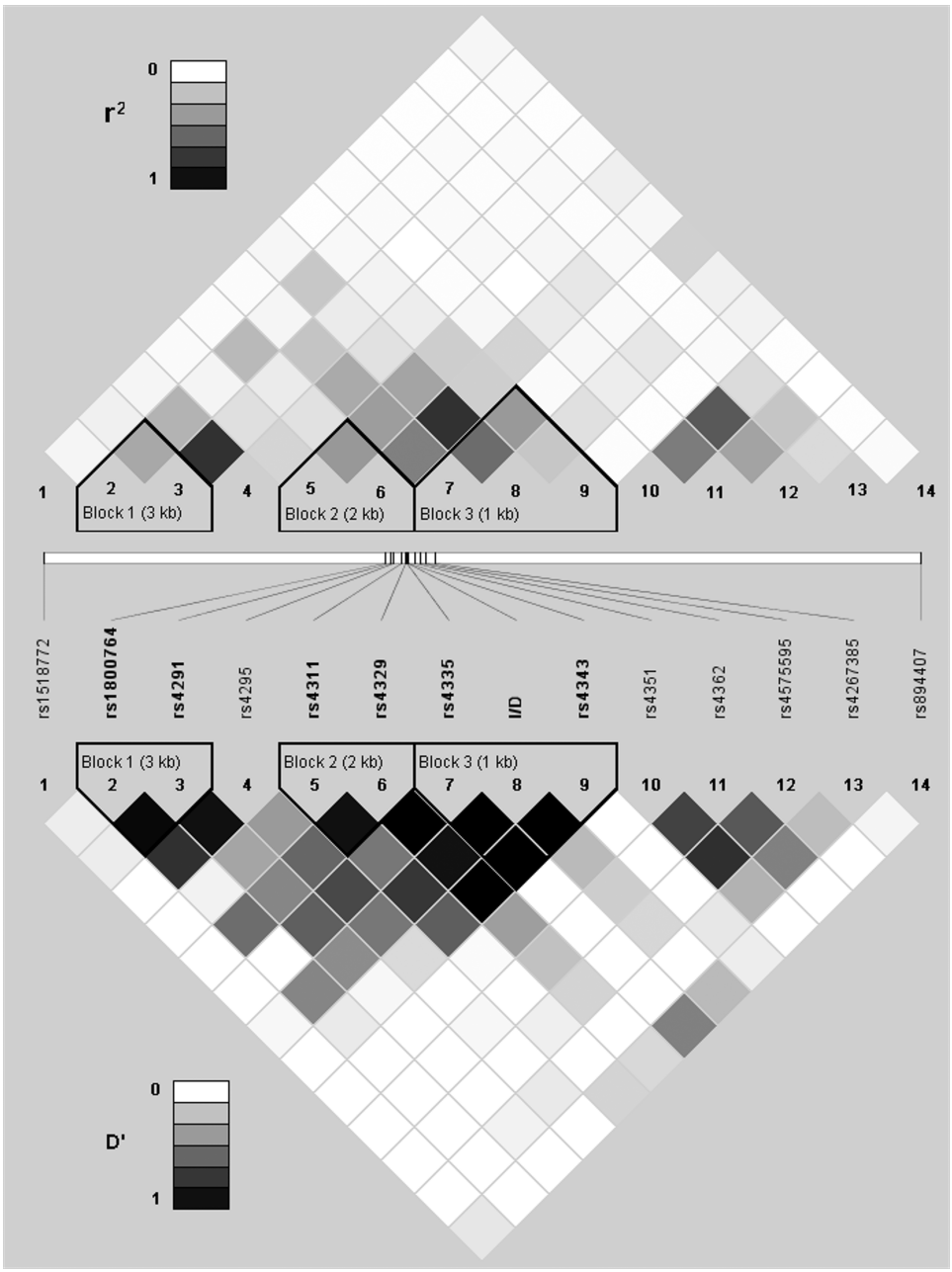

Figure 1 LD block structure in the *DCP1* region. The upper triangle shows LD calculated using the r^2 measure, and the lower triangle shows LD calculated using the D' measure.

included *rs4343* and *rs4351* (data not shown). Next, to assess whether adding more markers would significantly modify the effect, we evaluated 3–5 marker haplotypes, including these two SNPs using a sliding-window approach. Compared to the haplotype containing only *rs4343* and *rs4351* (global $P = 7.5 \times 10^{-7}$), noticeable improvement was obtained by extending the haplotypes in the 5′ direction (most significant global $P = 1.1 \times 10^{-12}$), however, the same alleles in these proximal SNPs (including the *I/D* polymorphism) were present in both the risk and protective haplotypes suggesting that the source of the effect on AD risk is more proximate to *rs4343* and *rs4351* than to the *I/D* site (table 2). Close examination of the haplotype containing *rs4343* and *rs4351* showed a significant enrichment of the haplotype *G-A* in AD cases (21%) compared to controls (1%). Subjects possessing this haplotype versus all other *rs4343–rs4351* haplotypes had 45-fold increased risk of

Table 2

Association of AD with Haplotypes Containing *rs4343* and *rs4351*

Haplotype							Frequency	*P*	Global *P*[a]
rs4329	*rs4335*	*Alu I/D*	*rs4343*	*rs4351*	*rs4362*	*rs4575595*			
			G	**A**			.10	4.0×10^{-8}	7.5×10^{-7}
		D	**G**	**A**			.10	1.8×10^{-9}	1.6×10^{-11}
			G	**A**	C		.10	2.8×10^{-9}	6.4×10^{-7}
	G	D	**G**	**A**			.11	1.5×10^{-9}	3.9×10^{-12}
		D	**G**	**A**	C		.10	1.4×10^{-10}	5.9×10^{-10}
			G	**A**	C	A	.08	3.5×10^{-8}	4.0×10^{-5}
A	G	D	**G**	**A**			.10	1.1×10^{-10}	1.1×10^{-12}
	G	D	**G**	**A**	C		.10	1.4×10^{-10}	2.5×10^{-10}
		D	**G**	**A**	C	A	.08	1.0×10^{-9}	1.9×10^{-10}
			A	**G**			.43	5.3×10^{-4}	7.5×10^{-7}
		D	**A**	**G**			.32	7.8×10^{-8}	8.0×10^{-13}
	G	D	**A**	**G**			.22	6.6×10^{-10}	3.9×10^{-12}

NOTE.—Protective haplotypes are shaded, and risk haplotypes are not.

[a] Global *P* value is based on comparison of frequency distribution of all haplotypes for the combination of SNPs indicated among cases and controls.

developing AD (table 3). Haplotype analyses of *DCP1* using sliding windows excluding *rs4343* showed unremarkable results.

The unusually high prevalence of AD in and the consanguineous nature of Wadi Ara make this population attractive for investigating AD susceptibility genes. Remarkably, AD is not associated with APOE because the frequency of the ε4 allele is very low in both nondemented (2.4%) and demented elders (3.6%).[28] Previously, we carried out an unconventional yet efficient 10-cM genome scan, using a small sample of cases and controls from this community, and confirmed the existence and narrowed substantially the locations of previously reported AD loci on chromosomes 9, 10, and 12.[24]

In this study, we observed in this population significant evidence of association with two adjacent polymorphisms (*rs4343* and *rs4351*) in the *DCP1* gene and AD. One of these markers (*rs4343*) is located 127 bp from an *Alu I/D* polymorphism which has been reported to be associated with AD in some but not all studies.[17–20] Notably, *I/D* is not associated in this population,[29] despite the fact that it is in strong LD ($D' = 1$) with *rs4343* (fig. 1) and that markers encompassing this region are in the same LD block in the African, Asian, and European ancestry samples included in the HapMap project.[27] However, the findings that the minor allele frequencies differ markedly (table 1) and the correlation of alleles at these two sites is low ($r^2 = 0.22$; see fig. 1) are consistent with the observation that AD is associated with only one of two polymorphisms in very close proximity to each other. Explanations for this phenomenon include recent admixture, local variation in recombination rates, gene conversion, and small chromosomal inversions.[30,31]

The observed genetic association between *DCP1* and AD is unlikely to be a consequence of population stratification, because the individuals in our sample descended from a small number of founders in a community which until recently was genetically isolated. It is also possible that the significance of our results is overestimated because we were unable to account for extended familial relationships. However, the impact of population structure on conclusions from genetic association analyses in a genetic isolate, which has been investigated in the context of a genomewide scan,[32] is probably minor in this study because we tested a prior hypothesis and obtained an extraordinarily significant result. It is also unlikely that the association between AD and *DCP1* is due to LD with pathogenic SNPs in a neighboring gene, because the SNPs associated with AD were located within the central portion of *DCP1*, whereas SNPs in the flanking genes *TANC2* and *FTSJ3*, which are in haplotype blocks different from *DCP1*, showed no association.

Although the association between AD and the *I/D* polymorphism has been intensively scrutinized, few studies have examined other variants in *DCP1* in the context of AD susceptibility. Kehoe et al. evaluated eight *DCP1* markers in three late-onset AD and one early-onset AD case-control data sets.[18] Five of these markers (*rs1800764, rs4291, I/D, rs4343,* and *rs4362*) were common to our study, and all were contained within the region of our SNP panel. None of the markers was significantly associated with AD in any of the individual data sets; however, significant evidence was obtained for *rs1800764, rs4291,* and *rs4343* in the combined data sets composed of samples from Sweden, England, and Scotland. Curiously, the most significant result from this previous study was obtained with the promoter SNP

rs4291 ($P = 2 \times 10^{-5}$), which was derived from a weighted odds ratio comparing AA+AT versus TT subjects. We did not detect association with this SNP or its immediate neighbors, *rs1800764* and *rs4295,* which are located 3,665 bp upstream and 2,104 bp downstream, respectively. Moreover, in the Kehoe et al. study, *rs4343* exhibited relatively weak association with AD, and the pattern of association was opposite to that observed in the Wadi Ara sample. These conflicting results might reflect that *rs4343* is in tight LD with another variant with pathogenic alleles or, as has been suggested,[33] that there exist pathogenic alleles at multiple locations within *DCP1*.

Our haplotype analysis suggests that the functional variant responsible for the AD association in this population is located distal to *I/D* and proximate to *rs4343* and *rs4351*. This region of the ACE gene is shared by all three transcript isoforms. Because *Alu* insertions can be associated with alternative splicing, many studies have suggested that the *I/D* polymorphism may explain differences in ACE plasma levels. However, recent in vitro minigene studies have shown that the *I/D* polymorphism and *rs4343* do not affect alternative splicing of *DCP1*.[34] Our *in silico* examination of the *rs4351* using the program RESCUE-ESE[35] suggests that it could affect alternative splicing; however, this has not been demonstrated in vitro or in vivo. There are no common sequence variants between *rs4343* and *rs4351* that have obvious pathogenic alleles. It is possible that *rs4343, rs4351,* or other SNPs in LD with these SNPS affect alternative isoform production, tissue specificity, or ACE activity for substrates (such as Aβ) that have not been studied extensively. This may explain the controversial evidence of association between *I/D* and other SNPs in this region with disease.

In summary, our results indicate that a genetic variant near *rs4343* and *rs4351* has a major influence on AD susceptibility in the Wadi Ara community. In view of (1) the preponderance of data supporting a genetic association between *DCP1* and AD, (2) evidence showing association between CSF Aβ42 levels and both APOE genotype[36] and *DCP1* haplotypes,[18] (3) the observation of significantly smaller hippocampal and amygdalar volumes in women homozygous for the *DCP1 I* allele independent of vascular factors,[16] and (4) the growing recognition that vascular factors including inflammation contribute to the development of AD,[37–41] multiple pathways linking APOE and ACE to AD risk can be proposed (fig. 2). Increased plasma levels of particular ACE and APOE isoforms may lead to increased production of the toxic form of Aβ (Aβ42).[12–14] Alternatively, ACE and APOE might influence AD risk through action on vascular risk factors (including blood pressure, lipids, and the endothelium) that alter metabolism of Aβ or perhaps through another factor leading to inflammatory response associated with AD.[41,42] Our hypothesis predicts that the *DCP1*/AD association may be more evident in populations like Wadi Ara showing a weak influence of APOE genotype. Studies of *DCP1* and APOE in other populations will help to distinguish and elaborate these pathways.

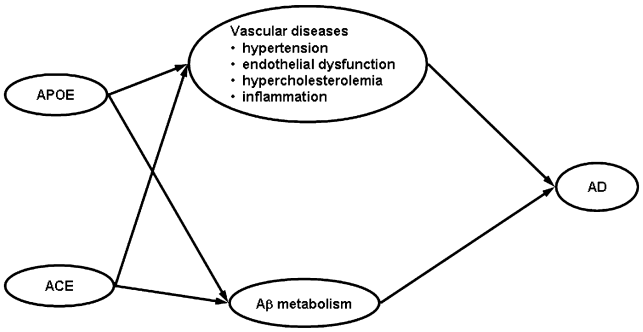

Figure 2 Proposed mechanisms for influence of ACE on development of AD via a pathway leading to increased production of the toxic form of Aβ (Aβ42) or through the action of vascular risk factors (see text for details).

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Table 3

Risk of AD Associated with *rs4343* and *rs4351* Haplotypes

Haplotype		Frequency			
rs4343	*rs4351*	Cases	Controls	Odds Ratio (95% CI)	*P*
A	A	.23	.32		
G	G	.20	.18		
A	G	.35	.50		
G	**A**	**.21**	**.01**	**45.2 (5.95–343.23)**	$\mathbf{3.4 \times 10^{-9}}$

Note.—The first three haplotypes shown are the reference group.

Web Resources

Accession numbers and URLs for data presented herein are as follows:

Daniel J. Schaid's Web site, http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm (for Haplo.stats v1.1.1)
dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/ (primary source for SNP information)
International HapMap Project, http://www.hapmap.org/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for AD, APOE, and ACE)
RESCUE-ESE Web Server, http://genes.mit.edu/burgelab/rescue-ese/

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