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A closely linked gene to apolipoprotein E may serve as an additional risk factor for Alzheimer's disease

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Abstract

The E4 allele of the apolipoprotein E (APOE) gene has been identified as a risk factor for Alzheimer's disease. Immediately downstream from the APOE gene on chromosome 19 is the gene for apolipoprotein CI (APOCI). We have found that the frequency of an APOCI restriction site is 0.45 for Alzheimer's patients and 0.14 for control spouses, which is similar to the frequencies for the APOE4 allele. The APOE4 allele is in linkage disequilibrium with the APOCI restriction site. Thus both the APOE4 allele and the APOCI restriction site may be considered as risk factors for Alzheimer's disease.

Keywords: Alzheimer's disease; Apolipoprotein E (APOE); APOE 4 allele; Apolipoprotein CI

Apolipoprotein E (APOE) 4 has been identified as a risk factor for late onset Alzheimer's disease. However, we have found that the frequency for a restriction site in the apolipoprotein CI (APOCI) gene is similar to that of the APOE 4 allele in probable Alzheimer's patients. Both genes have been shown to be closely linked on chromosome 19 [5]. More interestingly, the APOE 4 allele is in linkage disequilibrium with the restriction site in the APOCI gene. Both genes may be considered to be risk factors for Alzheimer's disease.

Alzheimer's disease is a genetically heterogeneous neurological disorder characterized by progressive dementia. Genetic links on chromosomes 14 and 21 have been found to be associated with early onset Alzheimer's disease, while chromosome 19 has been implicated in late onset disease. APOE is a plasma lipoprotein involved in the transport of cholesterol. The gene for APOE is found on chromosome 19q13.2; it is polymorphic, consisting of three alleles. Between 40 and 50% of late onset Alzheimer's patients have at least one copy of the APOE 4 allele [9,12]; multiple reports have confirmed this finding. In our studies we have found that the APOE 4 allele is associated with both early and late onset Alzheimer's disease [8].

Of the 1100 families enrolled in our DNA bank for genetic studies of Alzheimer's disease, 160 Caucasian patients have had their diagnosis reaffirmed as probable Alzheimer's disease, according to NINCDS-ADRDA criteria [6]. This included a detailed medical history, appropriate blood work, and evidence of cerebral atrophy on CAT scans. The medical history documented the progressive nature of the illness and ruled out cardiovascular problems. Those with possible Alzheimer's disease or with vascular dementia were excluded from this study. The probable Alzheimer's patients consisted of 41 with early onset disease (before the age of 65 years) and 119 with late onset disease. The patients were of European descent who migrated to Texas; they were non-Hispanic, non-Black, and non-Indian. Spouses (94 in number) of similar ethnic background and environmental exposure were used as controls. All participants and those having power of attorney for the patients were advised of the study and gave informed consent.

We also assessed our 20 Alzheimer's families with multiple affecteds (total sample size of affecteds and unaffecteds, 268), Centre d'Etude du Polymorphisme Humain (CEPH) families with an APOE 4 allele [2], and patients from the Indiana University Alzheimer's Disease National Cell Bank for both the APOE 4 allele and the APOCI restriction site.

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Table 1
Correlation of APOE genotype with that of APOCI

	APO E		APO CI		
	Genotype	Number	Genotype*	Number	%
pAD	3/4	78	AB	73	94
Spouses	3/4	13	AB	8	62
Linkage families	3/4	95	AB	94	99
CEPH	3/4	27	AB	26	96
Indiana AD	3/4	20	AB	20	100
pAD	4/4	27	AA	25	93
Spouses	4/4	0	AA	0	–
Linkage families	4/4	20	AA	20	100
CEPH	4/4	8	AA	7	88
Indiana AD	4/4	3	AA	2	67
pAD	3/3	49	BB	44	90
Spouses	3/3	64	BB	51	80
Linkage families	3/3	118	BB	117	99
CEPH	3/3	72	BB	72	100
Indiana AD	3/3	5	BB	5	100

%, percentage of the APOCI genotype compared with the APO E genotype. The various groups are as described in the text.
*A denotes restriction site present; B, absent.

Genomic DNA extracted from blood was used for this study [3]. The primers used for amplification of the APOE gene fragment were as described by Hixson and Vernier [4] and the PCR reaction as described by Saunders et al. [9]. Genotypes were scored by the presence of the upper three bands, a 91 bp fragment for APOE 3, an 83 bp fragment for APOE 2, and a 72 bp fragment for APOE 4. The APOCI primers and the HpaI restriction site at the 5′ end, upstream from the start site, were as described by Nillesen [7]. The fragment with the HpaI restriction site present (AA) is 159 bp and with it absent (BB) is 222 bp.

We examined markers and genes surrounding APOE on chromosome 19 for additional associations with Alzheimer's disease. No associations were found with the BCL3 or the LIPE genes or with the marker D19S47, and only very weak associations with APOCII and D19S178 [1,10]. However, we did find a very strong association with the gene for APOCI. The APOE 4 allele is in linkage disequilibrium with the presence of the APOCI restriction site.

In our study we found 78 probable Alzheimer's patients (pAD) who were heterozygous for the APOE 4 allele and of these, 73 were heterozygous (AB) for the APOCI restriction site (Table 1). Of the 27 probable Alzheimer's patients who were homozygous for the APOE 4 allele, 25 were also homozygous (AA) for the APOCI restriction site. Some 44 of 49 patients homozygous for the APOE 3 allele lacked the APOCI restriction site (BB). The patients were divided into early versus late onset patients and male versus female patients; similar associations were found. When the control spouses were analyzed, 51 of 64 spouses who were homozygous for the APOE 3 allele lacked the APOCI restriction site. Some eight of 13 spouses were heterozygous for both. None of our spouses had the APOE 4/4 phenotype.

We examined other groups for the APOE 4/4 phenotype. Only two out of 74 multiple sclerosis patients had the APOE 4/4 phenotype and both had the APOCI restriction site (AA) (data not shown). In our linkage families, there were 20 who were APOE 4/4, and they all had the APOCI restriction site (AA). Of the 95 who were APOE 3/4, 94 were APOCI AB; 117 of the 118 APOE 3/3 were APOCI BB. The APOE phenotype was assessed in the CEPH families and seven of the eight who were APOE 4/4 were APOCI AA. Most of the patients from the Indiana DNA bank (Indiana AD) were APOE 3/4 and were APOCI AB. Thus APOE 4/4 is tightly linked with APOCI AA and APOE 3/3 with APOCI BB.

This close association of the two genes was not as evident when the frequencies were examined (Table 2). The frequency for the APOCI A (cut) allele was 0.45 for the Alzheimer's patients, 0.43 for the patients from the Indiana DNA bank, and 0.14 for the spouses. Chi-square analysis of patients compared with controls (spouses) gave highly significant results ($P = 0.000$ for the A allele and $P = 0.000$ for the B allele at df = 1). While there is a significant difference in frequencies between the spouses and the affected patients, the close association is not detected. However, the frequency of the APOCI A allele in the probable Alzheimer's patients is similar to the frequency of the APOE 4 allele (0.453 and 0.425, respectively).

Thus we have confirmed that the APOE 4 allele is in linkage disequilibrium with the presence of the APOCI restriction site [1]. The APOCI restriction site has been mapped to the 5′ end of the gene, upstream from the initiation site. Close linkage of the two genes has been found by other investigators [5]. Moreover, this group also found a common regulatory element present in the region between the two genes that directs expression of the two

Table 2
Frequencies of the APOCI and APOE genotypes

	pAD	Spouses	Linkage families	CEPH	Indiana
APOCI					
Allele A	0.453*	0.144*	0.341	0.292	0.429
Allele B	0.547*	0.856*	0.659	0.708	0.571
APOE					
Allele 2	0.019	0.082	0.065	0.131	–
Allele 3	0.556*	0.837*	0.662	0.714	0.536
Allele 4	0.425*	0.082*	0.272	0.155	0.464

*Chi-square analysis revealed significant differences between the pAD and spouses with $P = 0.000$ at df = 1.

genes in various tissues [11]. This regulatory element is currently under investigation.

APOCI is associated with chylomicrons, very low density lipoproteins, and high density lipoproteins in the circulation. The exact function of APOCI is unclear, although it can apparently inhibit the binding of APOE to the low density lipoprotein receptor-related protein [13]. The APOE 4 allele modifies the amino acid sequence of the protein, thus altering its biochemical properties and increasing its avidity of binding to the beta amyloid peptide found in plaques in Alzheimer's disease [12]. Whether the APOCI protein has any effect on the binding or on plaque formation is unknown at this time. Nonetheless, both genes may be considered as major risk factors for Alzheimer's disease.

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