



Gene Dose of Apolipoprotein E Type 4 Allele and the Risk of Alzheimer's Disease in Late Onset Families

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the absence of TCR α , the number of CD4⁺CD8⁺ thymocytes is normal (10, 11), whereas decreased numbers of CD4⁺CD8⁺ thymocytes were reported for TCR β ^{-/-} mice (10), which suggests that efficient entry of CD4⁺CD8⁻ thymocytes into the CD4-CD8 developmental pathway or, alternatively, expansion of the pool of CD4⁺CD8⁺ thymocytes may require expression of both β and ζ subunits.

We also did not expect to find SP T cells in the thymus and periphery of ζ ^{-/-} mice. These cells were not $\gamma\delta$ T cells: they did not express surface TCR δ (7). The generation of SP T cells is thought to require positively selecting TCR signals that are induced in CD4⁺CD8⁺ thymocytes that are TCR^{hi} and CD5^{hi}. The CD4⁺CD8⁺ thymocytes in ζ ^{-/-} mice are CD5^{lo} and express only barely detectable quantities of surface TCR; it was therefore expected that T cell development would be arrested at the CD4⁺CD8⁺ stage of differentiation. We do not yet know whether the unusual peripheral SP T cells detected are derived from the SP cells in the thymus or whether they are generated extrathymically by a cryptic developmental pathway. However, their normal CD4/CD8 ratio is most consistent with their being of thymic origin.

In conclusion, ζ performs a previously unappreciated role in quantitatively promoting the generation or expansion of CD4⁺CD8⁺ thymocytes and is critical for the efficient generation of SP T cells. Surprisingly, some SP T cells can be generated in the thymus and appear in the periphery despite low expression of surface TCR complexes that are devoid of ζ (or η). Understanding the mechanism by which such SP T cells are generated may provide additional insight into the mechanism of positive selection in the thymus.

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- anti-CD3 ϵ (14), or anti- ζ (mixture of mAbs 528 and 551) (6) that had been previously bound to protein A agarose beads (Sigma). Immunoprecipitated samples were solubilized by boiling for 5 min in SDS sample buffer containing 2-mercaptoethanol (3%) and analyzed by one-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) (13%). Gels were fluorographed with dimethyl sulfoxide/2,5-diphenyloxazole, dried, and visualized by autoradiography at -70°C.
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20. We are grateful to A. M. Weissman for providing anti- ζ 551 and 528 and to E. Li and R. Jaenish for providing the J1 ES cell line. We thank Y. Takahama for critical reading of the manuscript.

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The apolipoprotein E type 4 allele (*APOE- ϵ 4*) is genetically associated with the common late onset familial and sporadic forms of Alzheimer's disease (AD). Risk for AD increased from 20% to 90% and mean age at onset decreased from 84 to 68 years with increasing number of *APOE- ϵ 4* alleles in 42 families with late onset AD. Thus *APOE- ϵ 4* gene dose is a major risk factor for late onset AD and, in these families, homozygosity for *APOE- ϵ 4* was virtually sufficient to cause AD by age 80.

Alzheimer's disease (AD) is a devastating neurologic disorder that affects millions of individuals of all races and ethnic backgrounds. Onset before age 60 is infrequent and caused by either a mutation in the amyloid precursor protein (APP) gene located on chromosome 21 or, more commonly, by an unidentified gene on chromosome 14 (1–4). Previous evidence of the involvement of chromosome 19 in late onset AD (5) has been confirmed by the finding of an association between AD and the apolipoprotein E locus (*APOE*) on chromosome 19 (6–8). *APOE* has three

alleles: *APOE- ϵ 2*, *APOE- ϵ 3*, and *APOE- ϵ 4*. A total of 80% of familial and 64% of sporadic AD late onset cases have at least one *APOE- ϵ 4* compared to 31% of control subjects. This finding implicates *APOE- ϵ 4* as an important factor in the etiology of more than half of all AD. Here we examine the effect of *APOE- ϵ 4* gene dose and show that it is correlated with increased risk and earlier onset.

Four of the 46 families tested to date are early onset families; of these, two have chromosome 21 APP mutations and two have the disease state linked to chromo-

some 14. The frequency of APOE-ε4 was not elevated in these and 12 other early onset families (6). Members of 42 late onset

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families diagnosed with AD or examined and found to be unaffected after age 60 with known APOE-ε4 genotype were evaluated. Before enrollment in our study, informed consent was obtained from each subject or, when necessary, their legal guardian. All study protocols were approved by the Duke University Medical Center Institutional Review Board. Age at onset in affected subjects and age at examination in unaffected subjects were similar in men and women (Table 1).

The proportion of affected individuals increased with the number of APOE-ε4 alleles from 20% of subjects with one copy of APOE-ε2 and one of APOE-ε3 (genotype 2/3) or genotype 3/3, to 47% of subjects with genotypes 2/4 or 3/4, and to 91% of subjects with the 4/4 genotype (Table 2). This additive trend was highly significant ($\chi^2 = 33.4$ with 1 df, $P < 0.00001$) (9, 10)

(Table 3). Risk of AD increased by a factor of 2.84 [95% confidence interval (CI) 2.03 to 3.96] for each additional APOE-ε4 (11–13). Hence, subjects with the 4/4 genotype were more than eight times as likely to be affected as subjects with 2/3 or 3/3 genotypes.

We next examined age at onset to determine if it was related to APOE-ε4 gene dose. Onset distributions were constructed from information on age at onset in affected subjects and age when last examined in unaffected subjects and were distinct for each gene dose ($\chi^2 = 53.84$ with 2 df, $P < 0.00001$) (14, 15) (Fig. 1). Each additional APOE-ε4 allele shifted onset to younger age; mean onset was 84.3 [standard error of the mean (SEM) 1.3] years in subjects who did not have APOE-ε4, 75.5 (SEM, 1.0) years in subjects with one APOE-ε4, and 68.4 (SEM, 1.2) years in subjects with two APOE-ε4 alleles.

Similarly, survival distributions were constructed from information on age at death in subjects known to be deceased and from age when last examined in other subjects, regardless of disease status. APOE-ε4 gene dose was related to survival; mean survival was 84.9 (SEM, 1.3) years in subjects without APOE-ε4, 78.8 (SEM, 0.8) years in subjects with one APOE-ε4 allele, and 78.1 (SEM, 1.4) years in subjects with two APOE-ε4 alleles.

The mean difference between the onset and survival distributions was 9.7 years in

Table 1. Descriptive statistics for affected and unaffected subjects.

	Affected*		Unaffected	
	Men	Women	Men	Women
Number	34	61	62	77
Age (years)†				
Mean ± SD	71.8 ± 8.4	70.4 ± 8.0	77.7 ± 8.0	82.6 ± 9.2
Range	60–91	60–90	66–92	72–94

*More than 90% of clinically diagnosed cases were confirmed at autopsy. †For affected individuals, age refers to age at onset; for unaffected individuals, age refers to age at death. The predictive value of clinical examination was nearly 100% when a family member had an autopsy-confirmed diagnosis of AD. Four subjects with a diagnosis of AD and 30 subjects examined before age 60 were excluded from analysis. Late onset families were not linked to chromosome 21 or to chromosome 14 within 19% recombination of the region previously linked to early onset AD. The estimated maximum two-point lod score for AD and APOE was 2.61 at 6% recombination.

Table 2. Percent affected for each APOE genotype. APOE genotype was determined as described (6).

APOE genotype	Percent affected (total n)		
	Men	Women	Combined
2/2	— (0)	— (0)	— (0)
2/3	28.6 (7)	11.1 (9)	18.8 (16)
3/3	7.1 (28)	28.6 (49)	20.8 (77)
2/4	50.0 (2)	0.0 (3)	20.0 (5)
3/4	38.3 (47)	54.5 (66)	47.8 (113)
4/4	91.7 (12)	90.9 (11)	91.3 (23)
Totals	35.4 (96)	44.2 (138)	40.6 (234)

Table 3. Percent of affected subjects and relative hazard according to the gene dose of APOE-ε4.

APOE-ε4 gene dose	Percent affected (total n)			Hazard ratio
	Men	Women	Combined*†	
0	11.4 (35)	25.9 (58)	20.4 (93)	1.00
1	38.8 (49)	52.2 (69)	46.6 (118)	2.84‡
2	91.7 (12)	90.9 (11)	91.3 (23)	8.07‡

*The Mantel-Haenszel correlation statistic (10, 11) stratified by family, was used to evaluate the additive trend in risk with increasing APOE-ε4 gene dose. †Estimates of risk were derived by exponentiation of parameter estimates obtained from a Cox proportional hazard model that allowed risk to differ between men and women (11, 12). ‡Hazard was significantly different from the reference value of 1. Information on each subject between the ages of 60 and 75 was used as the assumption of proportional hazards did not hold after the diagnosis by age 80 of nearly all persons with two copies of APOE-ε4 alleles. Statistically consistent, if not fully efficient, estimates of relative hazard result from proportional hazards models even when related individuals are evaluated (13). Thus, estimates of risk closely approximate estimates which would have been found by sampling just one person from each of a much larger collection of families.

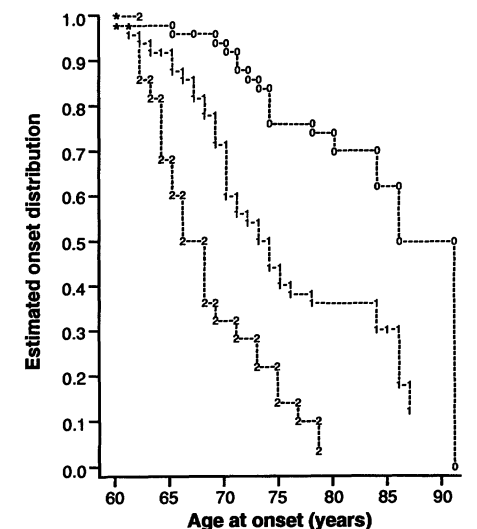


Fig. 1. Age at onset for subjects with 0, 1, and 2 APOE-ε4 alleles. Each curve is labeled by the number of APOE-ε4 alleles. An asterisk indicates multiple diagnoses within a short interval. Onset curves were estimated by Kaplan-Meier product limit distributions (14, 15). For example, at age 75, an estimated 24% of subjects without APOE-ε4 were diagnosed with AD compared to 61% of subjects with one APOE-ε4 allele and 86% of subjects with two APOE-ε4 alleles.

subjects with two APOE-ε4 alleles, 3.1 years in subjects with one APOE-ε4 allele, and 0.6 years in subjects who did not have APOE-ε4 alleles. As subjects with earlier onset and longer duration are more likely to be diagnosed, we suspect that most diagnoses of AD and most prevalent cases are in subjects with one or two copies of APOE-ε4.

It is important to realize that 19 of 95 affected subjects in our cohort of pedigrees and 64 of 176 autopsy-confirmed sporadic AD cases described by Saunders *et al.* (6) had no copies of APOE-ε4. Twelve of 42 late onset families had affected members who did not have APOE-ε4. The fact that these tended to be the largest and, based on simulation studies (16), the potentially most informative families for linkage, suggests that other genetic sources of risk exist. These other genes will only be identified once the effects of APOE-ε4 are included in subsequent analysis. Inclusion of APOE-ε4 as a covariate in ongoing genomic screens will be necessary.

There are two possible mechanisms for generating the allelic association that we observed. The first is through genetic linkage disequilibrium to the actual risk-causing mutation. Genetic linkage disequilibrium arises when two loci are so close together that recombination very rarely occurs. Thus specific alleles may be passed through many generations in cis orientation, leading to an increase in the linked cis allele, despite the fact that it has no biological role in increasing risk. The previous report suggesting genetic linkage of late onset AD to loci residing near APOE (5), and the suggestive evidence for genetic linkage of the APOE locus to AD could support this explanation.

However, biological association, where the risk allele has an actual pathogenetic role, can mimic genetic linkage (17), and would result in similar positive genetic linkage results. In addition, the strong and compelling allelic dose effects are unusual, even in diseases with well-characterized associations (18, 19). Such dose effects are difficult to explain through genetic linkage.

Although the mechanism by which APOE-ε4 participates in pathogenesis is not known, the protein encoded by APOE-ε4 (apoE isoform 4) is immunoreactive in the plaques and neurofibrillary tangles that define the phenotype (20, 21), apoE isoform 4 has higher avidity in vitro for β-amyloid than the apoE isoform 3 (22) and subjects with two APOE-ε4 alleles exhibit greater β-amyloid staining at autopsy than other AD patients (23). The data, in conjunction with the statistical association, support the direct involvement of APOE-ε4 in the pathogenesis of AD.

We should caution that accurate application of these results to the general population, especially the 3% who are homozygous for APOE-ε4, will require population-based epidemiologic studies. However, in this sample of 42 families, the APOE-ε4 allele in a double dose was nearly sufficient to cause AD by age 80, and suggests that APOE-ε4 gene dose is a major risk factor for late onset AD.

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TECHNICAL COMMENTS

Composition Limits of Fe_xO and the Earth's Lower Mantle

The report by C. McCammon (1) addresses a significant problem in planetary geochemistry and should motivate a new generation of experimental and theoretical studies on magnesiowüstite. She concludes that pressure reduces the amount of Fe³⁺ in Fe_xO wüstite equilibrated with Fe (1). Although we would like to agree, her results do not demonstrate this, nor do they support her broader inference that the Earth's lower mantle is out of equilibrium with metallic Fe (for example, that of the core).

An essential difficulty in studying wüstite, a phase of considerable interest in the

materials and geological sciences, is that it is invariably nonstoichiometric (x ≠ 1.0), with poorly characterized deficiencies (vacancies) at both oxygen and Fe sites and variable amounts of Fe²⁺ and Fe³⁺ in crystalline and interstitial-defect sites (1). Therefore, at least three compositional variables are required to describe wüstite, and it is impossible to associate uniquely the deviation from stoichiometry with the use of an Fe³⁺/Fe²⁺ ratio, as McCammon seems to assume. In addition, there is evidence that the stoichiometry varies as a function of composition across the

(Mg,Fe)O magnesiowüstite series that is relevant to the Earth's lower mantle.

The correlation between stoichiometry and lattice parameter assumed by McCammon and her co-workers (1, 3) depends on synthesis conditions and is uncalibrated (indeed, unverified) for wüstites synthesized at elevated pressures (2). Wüstite exhibits an irreversible change in lattice parameter after hydrostatic compression above 12 to 15 GPa at room temperature (4); presumably this phenomenon reflects easy changes in defect structures and is unrelated to stoichiometry. McCammon's report illustrates the significance of wüstite in constraining the Earth's geochemical evolution and thus highlights the need to calibrate simple methods of determining wüstite compositions at high pressures and temperatures.

The new data (1) neither prove nor disprove the earlier contention (3) that x decreases below 0.98 at lower mantle pres-