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Non-familial Alzheimer's disease is mainly due to genetic factors

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Abstract. This team takes the position that what is commonly referred to as non-familial Alzheimer's disease (AD) is predominantly due to genetic factors. Population-based studies suggest that genetic factors cause the majority of cases that begin after age 60. There are several lines of evidence supporting this position:

- Data from the Nun Study suggest that the risk for AD is largely established by early adulthood, implying that later adult exposures likely play only a small role in causation.
- Family studies show that first-degree blood relatives of persons with non-familial AD have a substantially increased risk of AD relative to controls.
- Twin studies suggest that the heritability of AD exceeds 60%.
- Environmental factors, such as socioeconomic status, education, and head injury, are strong risk factors for AD only in individuals with a genetic predisposition.
- The APOE genotype is a powerful risk factor for AD and accounts for as much as 50% of AD in many populations.
- There are numerous other candidate genes with strong associations with AD that presumably explain the remaining population risk.

This paper further reviews the mechanisms associated with AD causation for APOE and other candidate genes and implications for the development of prevention strategies.

1. Introduction

This debate addressed the issue of the causation of non-familial or "sporadic" Alzheimer's disease (AD). Familial AD refers to those small numbers of cases, generally considered to be less than 5% of all cases, in which there is a clear pattern of autosomal dominant inheritance. Such clear patterns usually are associated with an age of onset before 60 years of age. Non-familial AD, which constitutes at least 95% of all cases, includes those cases where there is no clear familial pattern of inheritance. The lack of a clear familial pattern of inheritance can be related to at least four factors. First, there is an increased rate of mortality above age 60, and in many cases the parents or siblings of the patients have not lived through the age of onset for the particular variation of AD in that family to establish the pattern of inheritance. Second, there may have been inadequate diagnosis of family members, particularly parents, grandparents, and earlier ancestors of the proband. Third, particular genetic or environmental factors may lead to variable penetrance of a causative gene, with the result that clinically unapparent cases remain undiagnosed. Finally, the apparent genetic association may be due to factors other than genes. However, data from epidemiologic and twin studies strongly favor a genetic explanation.

This issue is an extension of the classic nature-nurture question. The position of this team is that non-

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familial AD is predominantly due to genetic factors. We do not dispute the possibility that certain environmental stressors may influence the age of onset of clinically apparent AD or that certain environmental measures might reduce the risk of developing the disease, but we contend that such environmental factors modify the presentation of a disease that is largely genetic in origin.

2. Specific genetic factors in familial AD

At the present time, the roles of several genetic factors in AD are well established. The characterized familial genes in AD are autosomal dominant and cause disease that usually starts in the 40's and 50's. These cases are mostly related to point mutations, occurring in the domains of the amyloid precursor protein (APP, chromosome 21), presenilin 1 (PS1, chromosome 14) or presenilin 2 (PS2, chromosome 1) genes and account for about 3% of the cases.

3. Most of the risk for AD is established by early adulthood

From characteristics of autobiographical essays written by Catholic sisters when they were an average of 22 years old, it was possible to predict the occurrence of definite AD (clinically and neuropathologically-confirmed) at an average age of 80 with an extremely high degree of accuracy [56]. This evidence suggests that AD predisposition is determined by late adolescence, diminishing any role of later environmental variations and suggesting that causative factors establish their impact early.

4. Genetic factors account for at least 60% of "non-familial" AD

There is considerable evidence that familial factors play an important role in the etiology of AD. For example, it has long been known that there is a markedly increased cumulative risk of dementia among first-degree relatives of individuals with AD. Life table analyses have shown a cumulative risk of dementia to first-degree relatives of AD cases of approximately 50% by age 90, while relatives of purported control subjects had a much lower cumulative risk [8,45]. Because only about one-third of people meeting neuropathological criteria for AD present with dementia prior to death [1,39,51,57], (for discussion of neuropathological criteria for AD, see [24]; for discussion of predementia and AD neuropathology, see [40]), some individuals classified as controls will also carry the disease. The fact that the first-degree relatives of controls have a 10% chance of expressing dementia by age 90 may well be explained by clinically non-apparent cases among the controls.

Studies of AD among twin pairs over age 70 provide the strongest support for genetic causation. Monozygotic twins show very high concordance rates for AD, and estimates of AD heritability from these studies are in the 60–80% range (Table 1, [5,23]). Given the variable age of onset among monozygotic twins with the disease, these heritability figures may represent underestimates of the true heritability. Although the ratio of the concordance rates in monozygotic and dizygotic twins in these studies was approximately 2:1 as would be expected from a genetically inherited disease, the concordance estimates can be reduced by death prior to diagnosis in unaffected twins, leading to lower heritability estimates.

5. Environmental exposures are strong risk factors for AD only in individuals with a genetic predisposition

Recently, we have shown that low education, low socioeconomic status in youth (a surrogate for poor nutrition) and history of head injury have strong associations with AD only in those individuals with a history of AD in first-degree relatives [41]. For example, among individuals with an affected first-degree relative, the odds ratios associated with these three risk factors were 5.1, 10.8, and 4.2; whereas among those without a positive family history of AD, the odds ratios were 1.7, 1.2 and 1.1, respectively.

6. Indirect effects of genes

Beyond the genes related to familial AD, there is a variety of other ways genes might cause AD. For example, a genetic factor could influence dietary preferences, thus working through the relationship between the individual and the environment. Education, often viewed as an environmental factor protecting against AD, may be a function of earlier genetic influences. Two recent studies [10,34] have shown that individuals

Table 1
Estimated heritabilities of AD from 2 twin studies

Study	Mean age	MZ concordance	DZ concordance	Heritability
Study of Dementia in Swedish Twins [23]	78 years	75.0%	25.9%	.74
Norwegian Twin Registry Study [5]	80 years	83.0%	46.0%	.61

who carry one or more apolipoprotein E (APOE)-ε4 alleles are more likely to stop their education earlier in life. In both of these studies, the effect was evident at a young age, after only a few years of schooling.

Hypertension and hypercholesterolemia also are associated with AD development, and both are strongly determined by genetic factors. In fact, the genetic factor most closely related to non-familial AD, the APOE genotype, is most clearly understood for its role in cholesterol management.

7. The APOE genotype accounts for about 50% of AD in the United States

The clearest genetic factor that has been associated with non-familial or "sporadic" AD is the gene that codes for APOE [49]. This gene has been identified as a major factor in the causation of AD in cases that occur predominantly after 60 years old and do not have an apparent autosomal dominant mode of inheritance. In the U.S., the APOE-ε4 allele, with a prevalence rate of about 13% (ranging from 10% in East Boston [16] to nearly 19% in Cache County, Utah [9]; see [11,33,35,52,64] for several world-wide reports), occurs in 22% of the population (2% with the ε4/4 genotype and 20% with the ε3/4 genotype). Yet this allele occurs in 60% of AD patients (about 15% with ε4/4, 40% with ε3/4 and less than 5% with ε2/4). Those individuals with the ε3/3 genotype constitute 60% of the population but only 35% of the cases [17,29,42,48,50]. In Table 2, numbers of AD cases in the United States are projected for individuals with the observed distributions of APOE genotypes and under the assumption that the APOE-ε4 genotypes do not exist. Both studies show that there would be about half (53–56%) the number of cases in the U.S. if the APOE-ε4 allele did not exist. Therefore, the ε4 allele by itself is likely responsible for about 50% of the "non-familial" AD cases in this country. Other U.S. studies have reported somewhat different results. For example, in Cache County, Utah, a region with an increased frequency of the APOE-ε4 allele relative to other U.S. locations, this allele appears to account for 70% of the population attributable risk for AD [9].

APOE genotype has a substantial effect on age-related prevalence of AD, with the APOE-ε4/4 individuals having an estimated 50% chance of AD onset at 68.4 years old, the APOE-ε3/4 individuals at 75.5, and the APOE-ε3/3 individuals at 84.3 [12]. The APOE-ε4 allele confers its maximal effect on risk before age 70 [6], partly explaining why some studies looking at older populations have not found the full effect of this allele. In the Cache County population, there is a clear relation between the APOE genotype and age of risk for developing AD [9]. The relation to age also appears to be an important factor clinically. In the Lexington (Kentucky) Veterans Affairs Medical Center Memory Disorders Clinic, where 50 probable AD male patients were assessed for age of dementia onset (averaged from estimations derived from chart review, back calculations from Mini-Mental State Exam scores, and analysis of SPECT scans), the APOE-ε4 allele was associated with a significantly younger age of onset (Table 3) (Ashford, Kindy, Shih, Aleem, Cobb, Tsanatos, in preparation).

The two percent of the population with the ε4/4 genotype carries 15 times the risk of the 60% of the population that has ε3/3 genotype and over 20 times the risk of the ε2/3 genotype (see Table 2). By the age of 80 years, 91.3% of patients with the ε4/4 genotype, 47.8% of ε3/4 individuals, and only 20.0% of those without an ε4 allele have AD [12]. The ε4 allele has been referred to as a "susceptibility" gene, but no ε4/4 carrier has been conclusively shown to reach age 90 without having AD. The ε2 carriers are overly represented among centenarians [19]. However, there still has not been an adequate number of ε2/2 carriers examined at late age to define the relationship between this genotype and the classical AD changes at autopsy [43]. With consideration of the variation of risk from ε4/4 to ε2/2, it is possible that more than 75% of the risk of AD may be accounted for by the APOE genotype.

A few studies focusing on population incidence of AD have found substantially lower estimates for population attributable risk associated with the APOE-ε4 allele [14,16,25,54]. This may be due in part to the more advanced age of the populations studied. The study that focused on families with AD [14] was likely enriched in other genetic factors that may mask the association with the APOE-ε4 allele. An important inves-

Table 2
Projected numbers of cases of AD in United States based on number of AD cases and frequency of APOE genotypes in patients and controls from two multi-site studies. Distributions of APOE genotypes are applied to figures from the U.S. 2000 Census (45,797,200 individuals over 60 years of age, www.census.gov) and the estimate that there are 4 million AD cases in the U.S. These numbers are used to calculate the percentages of the population with prevalent AD with each APOE genotype and with and without an $\varepsilon 4$ allele. Also shown are estimates of the number of expected AD cases if all individuals in the U.S. had this specific genotype

Roses, 1995 (from [50]						
Genetic	Sample Population		U.S. Population > 60y/o			if all U.S.
Type	Control %	AD %	General	AD	% with AD	this type
$\varepsilon 22$	1.1%	0.0%	503,266	0	0.0%	0
$\varepsilon 23$	11.0%	3.4%	5,032,659	136,364	2.7%	1,240,909
$\varepsilon 24$	7.7%	4.0%	3,522,862	159,091	4.5%	2,068,182
$\varepsilon 33$	57.1%	33.0%	26,169,829	1,318,182	5.0%	2,306,818
$\varepsilon 34$	20.9%	43.2%	9,562,053	1,727,273	18.1%	8,272,727
$\varepsilon 44$	2.2%	16.5%	1,006,532	659,091	65.5%	29,988,636
N	91	176	45,797,200	4,000,000	8.7%	
no $\varepsilon 4$	69.2%	36.4%	31,705,754	1,454,545	4.6%	2,101,010
an $\varepsilon 4$	30.8%	63.6%	14,091,446	2,545,455	18.1%	8,272,727
Farrer, 1997 (caucasian, clinic/autopsy studies)						
$\varepsilon 22$	0.8%	0.2%	366,378	8,000	2.2%	1,000,000
$\varepsilon 23$	12.7%	4.8%	5,816,244	192,000	3.3%	1,511,811
$\varepsilon 24$	2.6%	2.6%	1,190,727	104,000	8.7%	4,000,000
$\varepsilon 33$	60.9%	36.4%	27,890,495	1,456,000	5.2%	2,390,805
$\varepsilon 34$	21.3%	41.4%	9,754,804	1,656,000	17.0%	7,774,648
$\varepsilon 44$	1.8%	14.8%	824,350	592,000	71.8%	32,888,889
N	6,262	5,107	45,842,997	4,008,000	8.7%	
no $\varepsilon 4$	74.4%	41.4%	34,073,117	1,656,000	4.9%	2,225,806
an $\varepsilon 4$	25.7%	58.8%	11,769,880	2,352,000	20.0%	9,151,751

Table 3
Age of onset and APOE genotype of 50 probable AD male patients seen at the Lexington Veterans Affairs Medical Center over a two-year period (Age of onset for $\varepsilon 3/3$ versus $\varepsilon 4/4$, $p < 0.02$; for $\varepsilon 3/3$ versus $\varepsilon 3/4$, $p < 0.05$)

APOE genotype	Number	Mean age of 0nset (years)	Standard deviation (years)
$\varepsilon 3/3$	20	73.6	4.7
$\varepsilon 3/4$	20	69.5	6.7
$\varepsilon 4/4$	10	68.3	5.6

tigative direction is to define the relationship between apolipoprotein E alleles and AD with respect to the influences of age, other diseases associated with these alleles, other genes, and environmental factors.

8. Over 20 other genes have been associated with AD

Apart from the well established familial genes on chromosome 1,14, and 21, and the APOE gene on chromosome 19, there have been at least 24 other genes or genetic loci associated with AD, identified on nearly every chromosome [58]. Two different approaches have been taken to finding these genes, one examining AD in families, and the other looking at genetic linkages across affected and control populations. Consequently, some studies may identify a particular gene as being associated with AD, while subsequent studies may both confirm and refute the finding. The reason is that families in one study may have one particular genetic factor contributing to the development of AD, while another study may not have families in which this factor is present. In considering new genetic factors that are found in specific families, it is critical to take a broad view. There are estimated to be 4 million individuals with AD in the U.S., and even if there are several individuals affected in a family, there are likely to be a million unrelated families to consider; so a sample that includes even 500 different families may still represent only a small portion of the possible variations. Studies that focus on families also may

overestimate the broader relevance of genetic factors involved in those families or underestimate factors less apparent in familial transmission, such as the APOE genotype [14]. Consequently, a specific genetic factor may itself be associated with an odds ratio of 10, but occur very infrequently and thus contribute only to a small number of cases with AD. Such a genetic factor may not be replicated by subsequent studies. Another important factor in such studies is age of disease onset. If the onset is young, then there appears to be a high penetrance because the disease manifests itself before the population members reach the age where risk of death from other causes is high. However, if a genetic factor predisposes to AD at a later age of onset, then it becomes a progressively less important factor relative to the numerous other conditions that can lead to death. Thus, as a genetic factor becomes less deleterious, contributing to AD at a progressively later age, it is seen as less penetrant, less of a causative agent, and more of a susceptibility agent. Consequently, when considering the multitude of specific genes that may contribute to the development of AD, it is important to consider age, gender, and ethnicity and to account for the major role of the APOE gene.

Of the new genetic loci that have been suggested to have substantial association with AD, regions of chromosomes 9, 10, and 12 have attracted significant interest [59]. On chromosome 12, two loci have been identified, one related to alpha-2-macrogluobulin [7] and the other associated with the low-density lipoprotein receptor. On chromosome 10, there are five separate regions of suspicion (Pericack-Vance, 2001, personal communication, Neuroscience Society Annual Meeting), with particular interest in the region coding for insulysin (the insulin degrading enzyme, which also degrades beta-amyloid) and another associated with the gene encoding the urokinase-type plasminogen activator (Estus and Younkin, 2001, personal communication, Neuroscience Society Annual Meeting) that is also critical in the management of beta-amyloid [60]. On chromosome 9, a significant association has been found with a common polymorphism in the gene encoding the very low-density lipoprotein receptor, and another location some distance away has shown an association with AD exceeding that of the APOE-ε4 allele [44]. These genetic factors have proven to be of great interest because each one seems to have a particular role that it plays in the development of AD. Most of the implicated genes currently are thought to play a role in the metabolism of beta-amyloid [53,59]. Therefore, not only do the genetic factors help us to understand why AD is occurring in a particular family, but they also help us to understand the pathophysiology and point to particular interventions for treatment and possible prevention of AD.

9. Environmental factors have low impact on AD development

While the evidence for genetic factors is strong, there has been considerable study of environmental factors that might be associated with AD. In reviewing these factors, it must be considered that many such studies are fraught with methodological flaws that diminish their relevance. For example, there have been many cross-sectional studies of the effects of cigarette smoking that have suggested that nicotine reduces the risk of AD. However, careful analysis of such studies indicates that the case-control study design probably has produced artifactual associations [32,55,62]. Also, there have been studies of NSAID use among arthritis patients that have indicated that these drugs reduce the risk of AD [61]; although this finding suggests a way to counter genetic risk associated with AD [63], rather than speaking against genetic causation.

The aluminum theory, the first serious environmental theory of AD, is considered because there have been some epidemiological studies that support an association between aluminum and AD. However, the preponderance of evidence is against a significant role for environmental aluminum as a cause for AD (see recent review [20]).

Another environmental factor that has been associated with AD is dietary cholesterol. A small number of studies have shown highly significant correlations across many countries between dietary fat and cholesterol and the prevalence of AD. However, this finding may be the result of other factors associated with diet. In particular, long-term evolution of genetic factors to support survival in particular dietary and energetic environments may be central to Alzheimer risk, with imbalances in the APOE genotype specifically predisposing to AD and arteriosclerotic disease [11]. Controlled comparisons are needed to elucidate the precise role of diet in AD causation. An interesting conundrum is that dietary habits established early in life could mimic genetic influences, and genetic factors could influence dietary preferences. Of great recent interest is the possible relationship between cholesterol and neuroplasticity [31], possibly mediated by an APOE-cholesterol complex [36,47].

This interaction could explain how both of these factors may interact to influence the development of AD.

There are numerous other environmental factors that could be associated with AD. For example, the herpes-simplex virus type 1 (HSV1) has been suggested to have a relationship with AD, and data are developing which link this virus to AD in patients with an APOE-ε4 allele [28]. Homocysteine [27] and traumatic brain injury [46] might also contribute to AD development. However, such environmental factors are more likely to either interact with genetic factors or else play a causative role in a very small number of AD cases.

10. Interaction of genetic and environmental factors throughout evolution

Although the preponderance of data clearly support a largely genetic etiology for AD, two apt analogies provide clear indication that nature and nurture must be seen as interacting. First, the phenylketonuria gene causes mental retardation 100% of the time unless phenylalanine is eliminated from the diet, in which case it has no adverse effect. Second, the sickle-cell gene is harmless at sea level, deadly at high elevations, and protective in regions where malaria is endemic.

To understand the fundamental role of genetic factors in the environmental context, it is frequently enlightening to take an evolutionary perspective. The evolutionary history of the APOE genotype is now becoming clear. The APOE-ε4 allele is the ancestral gene, which existed alone until 300,000 years ago, at which time the APOE-ε3 allele appeared. The APOE-ε2 allele mutated from the ε3 allele about 200,000 years ago [21]. Although the specific environmental pressures that led to the development of the APOE-ε3 and ε2 alleles are not known, current world-wide variation of the frequency of these genes suggest that they are beneficial in agrarian societies, particularly those with greater longevity [11]. It is possible that they provide superior cognitive and cardiovascular function to those individuals who lived beyond 60 years of age and in this way led to the emergence of more complex tribes of early humans. Presumably the human diet changed during this time to include more meat, either because agrarian living conditions made this source of nutrition more abundant or because the enlargement of the human brain led to a greater demand for higher caloric food. There is evidence that growth of tooth enamel changed at that time, distinguishing modern humans from earlier hominins [15]. In the protection of a more organized social environment, elderly individuals could survive, and those elders with retained cognition could provide "wisdom" to foster the success of the tribe, improving the survival of all members of the tribe. Such wise elderly would foster the survival of their own offspring, either as a patriarch who could control his tribe more ably and continue procreating, or as a matriarch who could foster the healthier development of her progeny.

In this evolutionary context with respect to brain development and neuroplasticity, cholesterol is now being understood to have an important role in the building of new synapses. Cholesterol has recently been shown to have a key role in neuroplasticity [31], and this role is mediated by APOE [36,47]. Recent evidence has suggested that cholesterol plays an important role in AD [29,66] and the metabolism of β-amyloid [18,65]. Clearly neuroplasticity has a central role in the evolutionary development of humans, and neuroplasticity also is a critical factor related to vulnerability to AD neuropathology [1,2,3,4,37,38]. As the human life span grew increasingly longer, there would likely have been an increasing pressure on cerebral neurons to store information stably in the face of the numerous physiological stressors associated with aging, including reactive oxygen species (ROS). A point that could bring together many of the theories of AD causation is that neuroplastic mechanisms require intense metabolic activity and may be associated with generation of ROS. With likely changes in diet to accommodate the increasing need for energy and lipid management for supporting brain function and enhancing cognitive function in later life, improved cholesterol management could have been the key development offered by the APOE-ε3 and ε2 alleles.

In the social context, the specific relationship of the APOE genotype to cholesterol metabolism may also have a complex environmental component still evident in modern times. By observation of the frequencies of the APOE alleles across various populations, there is clearly a geographic variation [11]. The APOE-ε4 allele is most common in the African pygmies (41%), least common in Sardinians (5%), and intermediate in most Western populations (9–19%). The APOE-ε4 allele has a rate of 8% in India and China, and this lower rate may account for the lower rate of AD found in India and China compared to Western populations, because this allele seems to have the same association with AD in these countries as it does in Western countries [22,35]. The relationship between the APOE-ε4 allele and AD in Africa has been less clear [26]. This

geographical distribution may indicate that the lifestyle of Africa is subject to different pressures, possibly including a relatively short life span or different diet composition. The APOE-ε3 allele is most common in the Mayans of Central America (91%) and least common in the pygmies (53%). The APOE-ε2 allele did not exist in the aboriginal Americans [for reviews see 11,21].

One point of hope is that if the APOE-ε4 allele is the ancestral form and two specific genetic mutations have led to a considerable decrease in the risk of AD, then it is possible that other effective therapies might be developed to eliminate this disease completely, even in those with the APOE-ε4 allele. Also, by examination of specific genotypes, we can investigate what factors within a particular genotype, such as APOE-ε3/3, play a role in determining which patients are most likely to get a disease and what influences age of onset.

Until we know how to modify or prevent the impact of genetic factors associated with AD, we must watch our diets, cholesterol levels, and blood pressure and put safety first in our lives by preventing traumatic brain injury. At this time, genotyping for diagnosis or risk estimation is not accepted standard medical practice, in spite of the important information that it provides. However, many patients and family members are regularly told their APOE genotype. This information should be given freely along with genetic counseling to those requesting it. Patients and their physicians should use this genetic information to develop strategies to reduce the risk of developing AD, make appropriate plans for the emergence of this disastrous condition, and clarify the diagnosis when the signs first arise. There have been many concerns about the adverse consequences of knowing genotype information. However, dissemination of such knowledge is likely to push research and prevention strategies forward more rapidly.

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