Consensus statement

Apolipoprotein E genotyping in Alzheimer's disease

*National Institute on Aging/Alzheimer's Association Working Group**

Apolipoprotein E (*APOE*=gene; apoE=protein) is the first identified genetic susceptibility factor for sporadic Alzheimer's disease (AD). The application of *APOE* genotyping to the prediction and diagnosis of AD has been a source of controversy for the public and for clinicians and scientists. These issues were explored by a 33 member working group in a two-day conference held in October, 1995, and sponsored by the National Institute on Aging, the Alzheimer's Association (USA), and other organisations. The group's conclusions are:

- The use of *APOE* genotyping to predict future risk of AD in symptom-free individuals is not recommended at this time.
- Insofar as patients with AD are more likely to have an *APOE*-ϵ4 allele than are patients with other forms of dementia or individuals without dementia, physicians may choose to use *APOE* genotyping as an adjunct to other diagnostic tests for AD.
- Since genotyping cannot provide certainty about the presence or absence of AD, it should not be used as the sole diagnostic test.
- In deciding whether or not to carry out *APOE* genotyping for any purpose, physicians and patients should bear in mind that genotype disclosure can have adverse effects on insurability, employability, and the psychosocial status of patients and family members.
- Clinical and research applications of *APOE* genotyping must be linked to adequate pre-test and post-test counselling, education, and psychosocial support.
- Research priorities include large-scale, prospective investigations of dementia incidence as a function of *APOE* genotype, and the development of novel approaches to the prevention and treatment of AD based on knowledge of the role in the disorder played by *APOE* and other factors.

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Introduction

The association between apolipoprotein E (apoE=protein, *APOE*=gene) ϵ4 and risk of Alzheimer's disease (AD) was first reported in 1993,[1] and subsequent confirmations have established *APOE* genotype as the single most important genetic determinant of susceptibility to sporadic and late-onset familial AD yet identified.[2–29] The discovery of this genetic risk factor is an important step forward in Alzheimer research, but has posed dilemmas for individual physicians, patients, and investigators. Since *APOE* genotyping is already commercially available,[30] and tens of thousands of patients have previously undergone testing for cardiovascular risk assessment, patients are now requesting *APOE* genotyping and/or interpretation of previous *APOE* results as an indicator of their risk of AD. No broadly accepted guidelines for the use of *APOE* genotyping for diagnosis and risk assessment are available. By a consensus process (panel 1) a US National Institute on Aging/Alzheimer's Association working group has attempted to fill that gap, with the following position statement.

APOE genotyping may be considered a prototype of genetic risk factor assessment applied to common, late-onset diseases that involve the interaction of several genes and environmental factors. Genetic testing in such diseases is further complicated by uncertainties in predicting and diagnosing multifactorial disorders, and by the potentially far-reaching social, ethical, and medicolegal implications of disclosure of genotype results. In using the knowledge arising from research on *APOE* and its association with AD, the interests of patients, future patients, and their families should be paramount.

Association between *APOE* and AD

ApoE is found in plasma, where it has an important role in the transport of cholesterol and the modulation of atherogenic lipoprotein metabolism. It is also produced by astrocytes in the brain, where its physiological role is less certain. The gene for human apoE in found on the long arm of chromosome 19 (19q13.2) and exists in three common allelic forms (*APOE*-ϵ2, *APOE*-ϵ3 and *APOE*-ϵ4). These three alleles encode forms of the apolipoprotein which differ by aminoacid substitutions at one or both of two sites, imparting distinctive physical and biochemical properties to each isoform. *APOE* genotype is usually determined by a blood test, using leucocyte DNA and polymerase chain amplification, or indirectly by assessing phenotype from isoelectric focusing of apoE in plasma. The six possible *APOE* genotypes differ in their frequency of occurrence. *APOE*-ϵ3/3 is by far the most common genotype and ϵ3 is the most common allele. The *APOE*-ϵ4 allele has a frequency of about 15% in typical populations of European ancestry. *APOE*-ϵ4 allele is found about three times more frequently among AD patients than among age-matched controls, while *APOE*-ϵ2 is slightly underrepresented in

Panel 1: **Consensus methodology**

Under the auspices of the US National Institute on Aging and the Alzheimer's Association (USA) a working group was assembled, with 33 members (researchers, clinicians, bioethicists, epidemiologists, geneticists, genetic counsellors, attorneys, and representatives of government and industry from the United States, Canada, and Europe).

A series of questions on the topics of interest was developed by the conference organisers and revised by the working group members in preparation for the consensus conference. A MEDLINE search spanning the years 1991–95 was done and cross-referenced, providing over 300 publications for review.*

In October, 1995, a two-day conference was convened in Chicago, Illinois, the aim being to review what was known about the association between *APOE* genotype and AD and to recommend guidelines for possible clinical and research applications. Selected participants gave focused presentations and, depending on their expertise, participated in the Scientific and Technical, Predictive and Diagnostic, or Counselling, Ethical and Medicolegal committees. Other working group members acted as advisors to these committees. Dialogue between the committees was encouraged and all working group members had the right to comment on any issue under discussion. A preliminary position statement was presented at the conference; it was then revised and approved by all contributors before submission for publication.

Participants were asked to provide written disclosure of any commercial interests relating to the application of *APOE* genotyping to AD, and to agree to publication of those disclosures.

The full proceedings of the meeting will be published in the *Annals of the New York Academy of Sciences,* and that report will contain more references than can be listed here.

*A complete list is obtainable from NRR.

AD. *APOE* genotype also appears to affect the age-at-onset of AD, with earliest onset associated with *APOE*-ϵ4/4 and latest occurrence with *APOE*-ϵ2/3 and, probably, *APOE*-ϵ2/2.[2,11,31]

To date, no other form of dementia has proven to be as strongly associated with *APOE*-ϵ4 as AD.[32–39] The biological basis of this association is unknown. It has been suggested that the various apoE isoforms may differentially affect amyloid deposition,[40] tangle formation,[41] neuronal plasticity,[42] cholinergic function,[43] and/or other biological aspects of the pathophysiology of AD.[6]

As a group, those possessing an *APOE*-ϵ4 allele can be considered to be at increased risk of AD relative to those without this allele.[1–29] While the absence of *APOE*-ϵ4 does not preclude the development of AD the "zero *APOE*-ϵ4 alleles" group can serve as a reference point (odds ratio=1) for estimating the relative risk of the disease as a function of *APOE*-ϵ4 gene dose. One copy of *APOE*-ϵ4 is associated with a moderately increased risk of AD (reported odds ratios range from 2·2 to 4·4) while two copies convey a high risk (odds ratios ranging from 5·1 to 17·9).[2,3,7,8,16–19,22,31,44] Nevertheless some *APOE*-ϵ4 carriers survive to old age and remain cognitively intact.

Other factors modify *APOE*-related risk and/or convey independent susceptibility to AD. The risk of AD increases with age; the risk attributable to the *APOE*-ϵ4 allele appears to decrease but remains significant with advancing years.[45] Family history is another important factor, and the odds ratios for AD associated with *APOE*-ϵ4 may be higher in persons with relatives who have been diagnosed with AD.[10,50,51] Preliminary evidence suggests that possession of the *APOE*-ϵ2 allele may be associated with a decreased risk for AD[20,31,52–56] and/or a relatively later age of disease onset.[31] Other genetic and epigenetic factors identified or yet to be found are likely to modify overall risk of AD and/or *APOE* gene-associated AD risk.[35,44,57–62]

APOE genotyping to predict AD risk

Susceptibililty factors are sometimes used to assess an individual's future likelihood of developing a particular disease. Physicians prognosticating about the risk of developing AD in cognitively asymptomatic individuals take into account the disease's increasing incidence with age and the greater risk associated with a family history of AD.[63] While a genetic test for susceptibility to AD could be useful, several authors argue against the use of *APOE* genotyping, citing the lack of availability of accurate, prospectively derived estimates of genotype-specific AD risk,[30,64,65] incomplete knowledge of the factors that may modify *APOE*-associated AD risk (such as age, sex, and family history),[64] the questionable value of predictive testing in the absence of preventive treatment,[65,66] the uncertainty of disease prediction afforded by genetic risk factor assessment,[64–66] the competing risk of mortality from other causes[67] and the psychosocial, medicolegal and financial challenges of presymptomatic genetic testing for complex diseases such as AD.[66] Despite these arguments, healthy individuals have continued to request *APOE* genotyping, doing so on the misperception that it provides an objective and personalised means of predicting whether or not they will develop AD.

The usefulness of *APOE* genotyping for predicting risk of AD in asymptomatic individuals has not been established in longitudinal population studies and as such is not recommended by the Working Group at this time.

APOE genotyping for AD diagnosis

APOE genotyping has also been proposed as a possible adjunctive diagnostic test for AD.[65] Risk factors already contribute to the differential diagnosis of dementia when, for example, a patient with a positive family history is considered more likely to have AD than another underlying cause for cognitive impairment. Bayesian analysis shows that possession of *APOE*-ϵ4 similarly increases the likelihood of an AD diagnosis in a patient with dementia by as much as 14% for each allele.[7] Some have expressed doubts about the ultimate usefulness of this adjunctive diagnostic information or the attainable sensitivity/specificity of this approach.[30,64,67]

In clinical settings 43–77% of AD cases have at least one *APOE*-ϵ4 allele,[1,2,4–6,15–19,22,26,31] while in general population-based surveys 49–63% of cases bear *APOE*-ϵ4.[13,23,24] This implies that between 23% and 57% of true AD cases would be misclassified if possession of the *APOE*-ϵ4 allele were the only criterion for diagnosing AD. Genotyping should not be used as the sole diagnostic test for AD.[7,65]

Patients with AD are more likely to have an *APOE*-ϵ4 allele than are patients with other forms of dementia or individuals without dementia. Physicians may therefore choose to use *APOE* genotying as an adjunct to other tests currently employed for AD diagnosis. However, possession of *APOE*-ϵ4 by a patient with dementia does not guarantee the diagnosis of AD; nor does the absence of an *APOE*-ϵ4 allele rule it out. Any gain in diagnostic accuracy afforded by *APOE* genotyping in conjunction

Panel 2: **Factors to be taken into account in pre-test counselling for *APOE* genotyping**

Construction of an adequate pedigree.[69]

Statement of relation between specific genotypes and AD and its age of onset.

Disclosure of risks associated with other disorders related to *APOE*, such as mortality from myocardial infarction.[70,71]

Other known genetic and environmental risk factors for AD.[63]

Discussion of the age-dependent risks of AD *vs* other causes of death.[67,72]

Logical presentation of all possible implications, including the potential discovery of new alleles and possible implications for risk of lipid-related disorders and other diseases.

Possible benefits and burdens of receiving test results, including psychological and psychosocial impact as well as effects on other family members.

Alternatives to testing, including the right to decline genotyping.

Whether, and under what conditions, disclosure of test results to other family members and third parties may take place.

The disposition of DNA samples obtained for testing.[68]

with other dementia tests must be weighed against the possible adverse effects of disclosing the genotype result.

When used for clinical purposes, *APOE* genotyping should be done by a laboratory that can meet accepted standards of specimen handling, genotyping, and confidentiality (eg, in the USA this would be a diagnostic service certified under the Clinical Laboratory Improvement Act). Whether or not genotype results obtained for research purposes will be disclosed to study participants should be discussed and agreed upon in advance as part of the informed consent process. The collection and storage of DNA used for *APOE* genotyping and the clinical data obtained in testing should follow established guidelines for DNA databanking.[68]

Genetic counselling

The complex nature of AD and the competing risks of death and dementia from other causes affect both the interpretation of *APOE* genotype test results and the ensuing counselling. Future clinical applications of genotyping should be offered only when pre-test and post-test counselling, education, and support are available. The pretest assessment and counselling needed for *APOE* genotyping in the research setting (panel 2) may serve as a model for future clinical applications.

Provision for post-test interpretation, counselling, and support services must be made and this includes referral for psychiatric care, support groups, and pastoral care.

Ethical issues in *APOE* research

The use of *APOE* genotyping in AD research raises concerns about privacy and informed consent that are associated with almost all DNA-based tests,[73] as well as others which arise when cognitively impaired AD patients are involved. Investigators should be aware of local and national standards for ethical conduct in clinical research. For example, testing in the context of federally sponsored clinical research in the USA is subject to institutional review board approval and oversight and oversight by the Office of Protection from Research Risks (Department of Health and Human Services, National Institutes of Health). Some of the ethical issues related to clinical research on *APOE* genotyping are set out in panel 3.

For studies involving the application of *APOE* genotyping to patients with dementia, researchers should comply with national or regional standards for obtaining informed consent from the mentally impaired. Unless the patient is too impaired to participate his/her wishes should be paramount. If the patient is not capable of giving informed consent, consent should be obtained from a legally authorised guardian or appropriate substitute decision-maker, in conjunction with assent from the patient.

Medicolegal and insurance issues

APOE genotyping for the diagnosis of or for the prediction of AD is not part of the current standard of health care. Research may one day produce evidence that genotyping is cost-effective and valuable in guiding diagnostic and treatment decisions relating to AD but until that time, *APOE* genotyping will not represent a just claim on public health resources outside the research setting. It should be noted that no other diagnostic test for dementia has been held to this standard.

APOE genotype information sought for its value in assisting diagnosis and assessing medical risk may at the same time cause social harm to those who seek it. Concerns include access to insurance (especially for private long-term care), employment, social stigma, and family dynamics. These effects are not only worrisome for those tested, but also can raise issues for family relatives and have social implications for caregivers as well. The disclosure of information and access to DNA samples should therefore respect principles of privacy and confidentiality, and may require further protection through legislation.

Recommended future directions in *APOE* research

It is important to elucidate the biological mechanisms underlying the association between *APOE* and AD, especially the nature and significance of interactions between apoE and other molecular constituents of senile plaques (including β-amyloid) and neurofibrillary tangles (including tau protein), as well as the effects of *APOE* polymorphism on neuronal plasticity. These investigations must be integrated with studies of other identified genetic factors including the neurobiology and neuropathology of

Panel 3: **Issues to be taken into account in ethical review and oversight of clinical research projects on *APOE* genotyping**

Whether genotype information is to be disclosed to participants.

The impact that genetic information can have on participants and on others, especially family members.

Other potential risks of disclosing genotype information, such as use by third parties (eg, insurers, employers, and family members).

Ensuring confidentiality of test results.

The source of payment for genotyping and related services.

Whether participants will be recontacted for subsequent studies, and what will be done with clinical data and samples when study is over or when funding expires.

Plans for pre-test assessment, education, and counselling, as well as post-test interpretation and support.

amyloid precursor protein,[74] and presenilin 1 and 2.[75,76] Characterisation of new disease genes and susceptibility factors that may interact with or modify risk of AD associated with *APOE* genotype require investigation. The possibility that apoE plays a direct role in the aetiology and/or pathogenesis of AD needs to be further explored, as does the possibility that apoE plays a part in non-Alzheimer's dementias and in recovery from other brain diseases. To the extent that plaque and tangle pathology may be influenced by *APOE* genotype, the current criteria for necropsy diagnosis of AD may require re-examination.

APOE genotype should be considered in the design of most patient-based research on AD. We need large-scale, prospective, population-based studies to determine more accurately the relative risk of AD associated with the six *APOE* genotypes. Such studies should also address how *APOE*-related relative risks are modified by age, sex, ethnicity, family history of AD or Down's syndrome, head trauma, other environmental and epigenetic factors, as well as other genetic factors. The risk-modifying effects of *APOE*-ε2 deserves particular attention, especially if its possible protective effects are confirmed. The biological basis of *APOE*-ε2's effects should be thoroughly explored. Further studies should address the nature of *APOE*'s influence on the age of onset of AD and the extent to which genotype influences the rate and course of disease progression. When accurate risk estimates and characterisation of major risk modifiers are available the value of using *APOE* genotyping to assess the likelihood of AD developing in asymptomatic individuals, especially family members of AD patients, should be re-examined.

The value of *APOE* genotyping as an adjunct to established diagnostic criteria for AD needs further evaluation in terms of cost-effectiveness and impact on AD diagnostic accuracy and clinical care. We need to know how big an improvement in the accuracy of AD diagnosis genotyping can provide and how genotyping should be best integrated into the diagnostic process. The possibility that genotype results could substitute for one or more of the tests currently used should be investigated. The utility of combining *APOE* genotyping with other biological markers for AD differential diagnosis and with other technologies (eg, neuroimaging)[77] for early detection and for monitoring disease progression should be further explored.

Specific protocols are needed for the counselling and education of those who are candidates for *APOE* genotyping. Huge numbers of people are potential recipients of these services but the number of genetic counsellors is limited so physicians and other professionals involved in delivering health care to the elderly should be trained to deliver counselling. The efficacy of counselling protocols will require assessment in controlled studies.

The use of *APOE* genotyping to predict response to drug treatments for AD also warrants further investigation.[43] It is strongly recommended that the results of basic and clinical *APOE* research be applied to the development of novel therapeutic interventions for AD.

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Declarations of interest
A patent for *APOE* testing for diagnostic uses in AD has been allowed to Duke University Medical Center and licensed by Athena Neurosciences. Of the conference participants, Allen Roses, Warren Strittmatter, and Margaret Pericak-Vance are among the inventors listed on the patent. Judes Poirer is a founder of Nova Molecular Diagnostics, which owns rights for patents filed linking *APOE*-ε4 to AD response to pharmacotherapy. Allen Roses serves as a consultant to Athena Neurosciences. Dale Schenk is a shareholder in Athena Neurosciences, and is employed by Athena. Dennis Selkoe is a co-founder of Athena Neurosciences and serves as a consultant to Athena.

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