Rarity of the Alzheimer Disease–Protective APP A673T Variant in the United States

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**IMPORTANCE** Recently, a rare variant in the amyloid precursor protein gene (APP) was described in a population from Iceland. This variant, in which alanine is replaced by threonine at position 673 (A673T), appears to protect against late-onset Alzheimer disease (AD). We evaluated the frequency of this variant in AD cases and cognitively normal controls to determine whether this variant will significantly contribute to risk assessment in individuals in the United States.

**OBJECTIVE** To determine the frequency of the APP A673T variant in a large group of elderly cognitively normal controls and AD cases from the United States and in 2 case-control cohorts from Sweden.

**DESIGN, SETTING, AND PARTICIPANTS** Case-control association analysis of variant APP A673T in US and Swedish white individuals comparing AD cases with cognitively intact elderly controls. Participants were ascertained at multiple university-associated medical centers and clinics across the United States and Sweden by study-specific sampling methods. They were from case-control studies, community-based prospective cohort studies, and studies that ascertained multiplex families from multiple sources.

**MAIN OUTCOMES AND MEASURES** Genotypes for the APP A673T variant were determined using the Infinium HumanExome V1 Beadchip (Illumina, Inc) and by TaqMan genotyping (Life Technologies).

**RESULTS** The A673T variant genotypes were evaluated in 8943 US AD cases, 10 480 US cognitively normal controls, 862 Swedish AD cases, and 707 Swedish cognitively normal controls. We identified 3 US individuals heterozygous for A673T, including 1 AD case (age at onset, 89 years) and 2 controls (age at last examination, 82 and 77 years). The remaining US samples were homozygous for the alanine (A673) allele. In the Swedish samples, 3 controls were heterozygous for A673T and all AD cases were homozygous for the A673 allele. We also genotyped a US family previously reported to harbor the A673T variant and found a mother-daughter pair, both cognitively normal at ages 72 and 84 years, respectively, who were both heterozygous for A673T; however, all individuals with AD in the family were homozygous for A673.

**CONCLUSIONS AND RELEVANCE** The A673T variant is extremely rare in US cohorts and does not play a substantial role in risk for AD in this population. This variant may be primarily restricted to Icelandic and Scandinavian populations.

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The amyloid precursor protein gene (APP; GenBank NC_011512) encodes a transmembrane protein of unknown normal function. In the normal processing of the APP protein, proteolytic cleavage yields a 39- to 43-amino acid peptide called β-amylloid (Aβ). Release of Aβ from APP is catalyzed by the β-site APP cleaving enzyme 1 (BACE1) that cleaves APP at the N-terminal end of Aβ and by the γ-secretase complex that cleaves at the C-terminal end. Together, these 2 proteases generate the Aβ peptide. In Alzheimer disease (AD), Aβ accumulates in extracellular amyloid plaques that are characteristic of this disease. Mutations in APP cluster around the N-terminal and C-terminal sequences that encode Aβ and cause early-onset AD. Likewise, early-onset AD is also caused by mutations in the presenilin 1 gene (PSEN1) and presenilin 2 gene (PSEN2), which encode protease subunits of the γ-secretase complex. Genetic studies and a large body of functional evidence convincingly show that Aβ is a toxic molecule critical to the pathogenesis of AD. As a result, multiple drug trials are in progress designed to stimulate Aβ cleavage using immunological approaches or to inhibit Aβ production using small-molecule inhibitors of γ-secretase or BACE1.

Recently, Jonsson et al. reported that the APP coding mutation A673T, in which alanine is replaced by threonine at position 673, is protective against late-onset AD. This rare variant was enriched in Icelandic elderly controls compared with AD cases from the same population. The frequency was 0.13% in AD cases and ranged from 0.45% to 0.79% in controls, depending on age. The A673T variant was also observed in an individual with ischemic cerebrovascular disease but not AD and in a 104-year-old patient with dementia who had hippocampal sclerosis and little Aβ accumulation. The A673T variant is located at position 2 of Aβ and thus is immediately downstream of the BACE1 cleavage site. Ex vivo and in vitro experiments show that this variant inhibits BACE1 cleavage and results in reduced Aβ production. Another mutation at this site, A673V, enhances BACE1 cleavage activity and is a recessive mutation causing early-onset AD. Likewise, the K670N/M671L mutation that affects the 2 amino acids immediately upstream of the BACE1 cleavage site also enhances BACE1 cleavage, increases Aβ production, and causes early-onset AD. Thus, multiple mutations in close proximity to the BACE1 cleavage site influence risk for AD and Aβ production.

We genotyped a large number of AD cases and controls to determine whether the A673T mutation is an important protective variant in cognitively intact elderly US white individuals and in patients with AD from the same population. We found that this variant is extremely rare and does not have a discernible impact on AD risk in the US population.

**Methods**

**Genotyping**

We genotyped the samples listed in Table 1 using the Infinium HumanExome V1 Beadchip (Illumina, Inc.). Genotyping was performed for 8410 individuals at the Robert S. Boas Center for Genomics and Human Genetics, Feinstein Institute for Medical Research, Manhasset, New York, 1990 individuals at the John P. Hussman Institute for Human Genomics, University of Miami, Miami, Florida, and 6166 individuals at the Center for Applied Genomics, The Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania. Genotypes were initially called using the default clustering profile from Illumina and recalled using clustering profiles generated by Gnenetech using data from 30,000 samples. We also genotyped the individuals listed in Table 2 for single-nucleotide polymorphisms rs63750847 using the TaqMan assay C_89522366_10 (Life Technologies) and a 384-well plate format. Each plate contained samples from 2 heterozygotes from the exome array experiments. For both the exome array assay and the TaqMan assay, manual inspection of clustering indicated both were valid assays (eFigure 1 in the Supplement).

**Participants**

The National Institute on Aging Alzheimer’s Disease Centers case-control sample, the University of Toronto/GlaxoSmithKline (also called Gen ADA) case-control sample, the Vanderbilt/Miami/Mount Sinai case-control sample, the National Institute on Aging-Late-Onset Alzheimer’s Disease multiplex family-based sample, the National Cell Repository for Alzheimer’s Disease multiplex family-based sample, the Multi-institutional Research in Alzheimer’s Genetic Epidemiology family-based sample, and the Adult Changes in Thought (ACT) prospective cohort were described previously. The Genetics Differences cohort is a population-based prevalent case-control study from the same population as the ACT study. The Washington Heights–Inwood Columbia Aging Project sample is a multiethnic prospective cohort; for this study, only white individuals were genotyped. The Washington University cohort is a white case-control cohort. The Miami multiplex families and the National Institute of Mental Health multiplex families were as previously described. The Cache County Study on Memory Health and Aging is a population-based study with 4 assessments of cognitive function since 1994. The Swedish cohorts are case-control studies recruited from neuropsychiatric clinics in Sweden as described previously. For the family-based sample, we genotyped a single affected individual from each kindred. All studies were approved by institutional review boards at the respective universities involved in each study, and the overall study was approved by the University of Pennsylvania institutional review board. The participants provided written informed consent.

**Analysis**

The exome chip genotyping data (16,525 samples total) were first preprocessed using quality check steps adapted from Naj et al. Briefly, we excluded 881 samples and markers with a missing call rate higher than 2%, samples with genotype-imputed or reported sex mismatch, and markers significant ($P < 10^{-6}$) in either Hardy-Weinberg or informative missingness tests. We pruned 969 samples by relatedness test ($\pi > 0.4$ using 15,086 linkage disequilibrium–pruned autosomal markers with a minor allele frequency >0.1) and compared samples with HapMap 3 data to exclude nonwhite individuals. We checked population substructure in 15,644 samples with good call rates using 5848 single-nucleotide polymorphisms that
Table 1. Samples Genotyped for A673T (rs63750847) With the Exome Array That Passed Quality Checking

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>University of Toronto/GSK</th>
<th>Miami</th>
<th>NIMH</th>
<th>NIA-LOAD</th>
<th>NCRAD</th>
<th>MIRAGE</th>
<th>Genetic Differences</th>
<th>ACT</th>
<th>WHICAP</th>
<th>Washington University</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Cohort</td>
<td>ADC</td>
<td>Miami</td>
<td>NIMH</td>
<td>NIA-LOAD</td>
<td>NCRAD</td>
<td>MIRAGE</td>
<td>ACT</td>
<td>WHICAP</td>
<td>Washington University</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples, No.</td>
<td>(A673T heterozygotes, No.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cases</td>
<td>3930 (0)</td>
<td>152 (0)</td>
<td>936 (0)</td>
<td>354 (0)</td>
<td>749 (0)</td>
<td>395 (0)</td>
<td>576 (0)</td>
<td>239 (0)</td>
<td>282 (1)</td>
<td>54 (0)</td>
<td>554 (0)</td>
</tr>
<tr>
<td>Controls</td>
<td>2326 (0)</td>
<td>0 (0)</td>
<td>995 (0)</td>
<td>0 (0)</td>
<td>458 (0)</td>
<td>0 (0)</td>
<td>12 (0)</td>
<td>216 (0)</td>
<td>1445 (1)</td>
<td>322 (0)</td>
<td>360 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>6256 (0)</td>
<td>152 (0)</td>
<td>1931 (0)</td>
<td>354 (0)</td>
<td>1207 (0)</td>
<td>395 (0)</td>
<td>588 (0)</td>
<td>455 (0)</td>
<td>1727 (2)</td>
<td>376 (0)</td>
<td>914 (0)</td>
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<td>Male, %</td>
<td>43.6</td>
<td>46.1</td>
<td>38.5</td>
<td>28.0</td>
<td>31.9</td>
<td>38.4</td>
<td>35.8</td>
<td>43.4</td>
<td>39.9</td>
<td>40.4</td>
<td>41.1</td>
</tr>
<tr>
<td>Case age at onset</td>
<td>Mean (SD), y</td>
<td>72.4 (9.3)</td>
<td>77.8 (6.8)</td>
<td>72.6 (7.1)</td>
<td>71.6 (8.1)</td>
<td>73.8 (7.2)</td>
<td>71.0 (8.5)</td>
<td>68.6 (8.7)</td>
<td>76.4 (6.2)</td>
<td>83.7 (4.7)</td>
<td>84.5 (7.2)</td>
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<tr>
<td>No.</td>
<td>3930</td>
<td>152</td>
<td>885</td>
<td>354</td>
<td>749</td>
<td>393</td>
<td>572</td>
<td>239</td>
<td>282</td>
<td>54</td>
<td>554</td>
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<tr>
<td>Control age at last examination</td>
<td>Mean (SD), y</td>
<td>77.2 (9.3)</td>
<td>NA</td>
<td>73.5 (7.9)</td>
<td>NA</td>
<td>79.6 (8.7)</td>
<td>NA</td>
<td>76.1 (7.8)</td>
<td>80.8 (6.6)</td>
<td>81.6 (6.1)</td>
<td>81.0 (6.1)</td>
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<tr>
<td>No.</td>
<td>2326</td>
<td>NA</td>
<td>991</td>
<td>NA</td>
<td>458</td>
<td>NA</td>
<td>12</td>
<td>216</td>
<td>1445</td>
<td>322</td>
<td>360</td>
</tr>
<tr>
<td>Cohort type</td>
<td>Case-control</td>
<td>Case-control</td>
<td>Case-control</td>
<td>Multiplex families</td>
<td>Multiplex families</td>
<td>Multiplex families</td>
<td>Multiplex families</td>
<td>Multiplex families</td>
<td>Population-based incident cases with matched controls</td>
<td>Prospective cohort</td>
<td>Prospective cohort</td>
</tr>
</tbody>
</table>

Abbreviations: ACT, Adult Changes in Thought; ADC, Alzheimer’s Disease Centers; GSK, GlaxoSmithKline; MIRAGE, Multi-institutional Research in Alzheimer’s Genetic Epidemiology; NA, not applicable; NCRAD, National Cell Repository for Alzheimer’s Disease; NIA-LOAD, National Institute on Aging-Late-Onset Alzheimer’s Disease; NIMH, National Institute of Mental Health; WHICAP, Washington Heights–Inwood Columbia Aging Project.

Results

A total of 8221 AD cases and 6134 elderly cognitively normal controls were genotyped using Illumina exome arrays (Table 1). These arrays have single-nucleotide polymorphism assays for more than 240 000 exonic variants that are nonsynonymous, nonsense, or splice-site variants. The exome array has an assay for rs63750847, which is the A673T variant discussed earlier (eFigure 2 in the Supplement). We identified 2 heterozygotes, one in a sample from an AD case (minor allele frequency = 0.0061%) of Russian ancestry who had onset of AD symptoms at age 89 years and an APOE genotype of ε3/ε4 and the second in a sample from a cognitively intact 82-year-old control (minor allele frequency = 0.0082%) born in Iceland with an APOE genotype of ε3/ε3 (Table 3). Both were from the ACT study based in Seattle, Washington. Population principal components showed both to be of northern European ancestry when compared with HapMap 3 samples (eFigure 2 in the Supplement).

To validate the array genotyping results, we used a TaqMan assay to regenotype 1610 of the same samples, including the 2 heterozygotes. We also genotyped 983 additional samples from the ACT study (Table 2). All genotypes derived from the exome array genotyping were confirmed by the TaqMan assay, including the 2 heterozygotes initially observed. All additional ACT samples were homozygous for the normal A673 allele. TaqMan genotyping was also used to survey 506 AD cases, 387 individuals with other dementias, and 383 controls from the Cache County Study on Memory Health and Aging. All dementia cases were homozygous for the normal A673 allele. One of the 383 individuals without dementia carried the A673T allele (Table 2). This individual was aged 77 years at last assessment, had an APOE genotype of ε3/ε4, and had ancestors from Denmark, Ireland, Scotland, and England (Table 3). Because the minor allele of the A673T variant was observed in a Swedish
sample at a frequency of 0.42%, we also genotyped 862 AD cases and 707 cognitively normal controls from Sweden (Table 2). Three controls were heterozygous for A673T. All AD cases were homozygous for the common A673 allele.

Previous work reported that an affected individual in a small family with late-onset AD was heterozygous for A673T. Because an affected individual from the same family was genotyped using the exome array as one of the individuals from the National Institute on Aging-Late-Onset AD sample, we genotyped all other available family members for the A673T variant. This included 6 AD cases, 4 married-in spouses, and 7 blood relatives of affected individuals. We observed 1 spouse and the child of that spouse as heterozygous for A673T. The child is a blood relative of affected individuals in the family. Both the child and parent were unaffected individuals (ages at last visit, 84 and 72 years, respectively). The heterozygous individual originally reported as an AD case was actually the unaffected child described here. This family is from the United States with a Scandinavian background.

### Discussion

The APP A673T variant is overrepresented in Icelandic controls when compared with Icelandic AD cases. Therefore, like the APOE ε2 allele, the A673T variant appears to protect against late-onset AD, defined as disease onset after age 60 years. Studies on the functional consequences of the A673T substitution showed that this amino acid substitution inhibited BACE1 cleavage of APP, potentially reducing or eliminating the production of Aβ peptide from APP encoded by this allele. That this allele appears to be protective provides additional genetic evidence that Aβ is a critical toxic molecule contributing to AD. Overproduction of Aβ (eg, from the APP K670N/M671L mutation) causes early-onset AD, and a variant associated with reduced Aβ production, A673T, protects against AD. Amino acid 673 appears to be critical for BACE1 cleavage since a different allele at the same amino acid (an alanine change to a valine, A673V) enhances Aβ production and causes recessive early-onset dementia. However, the A673T variant is exceedingly rare in the white individuals tested here (carrier frequency = 0.011% in US individuals with AD and 0.018% in cognitively normal controls), so it could not have a substantial role in risk for AD in the US population. The frequency in Swedish controls tested here was somewhat higher (0.42%) and is in line with the frequency in the Icelandic population. The effect size (Fisher exact test 99% CI, 3.97–110) is weaker than in the Icelandic population (odds ratio = 5.29), although this observation is suggestive and a larger sample size is needed.

The protective effect of A673T against late-onset AD reported in the Icelandic study supports the hypothesis that Aβ not only plays a critical role in early-onset familial AD but also is important for late-onset AD. This hypothesis is also supported by earlier work showing that the APOE ε4 allele not only increases risk for late-onset AD and lowers the age at onset but similarly also modifies AD risk in carriers of PSEN1 and PSEN2 mutations. Mutations in PSEN1 and PSEN2 alter the C-terminal cleavage γ-secretase site and thus cause AD by an Aβ-related mechanism. The fact that APOE genotype influences age at onset of this Aβ-driven process suggests that late-onset AD and early-onset familial AD share at least 1 common mechanism. The age cutoff distinguishing early- and late-
Table 3. Heterozygotes for A673T

<table>
<thead>
<tr>
<th>Cohort and Participant</th>
<th>Age, y</th>
<th>APOE Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89</td>
<td>ε3/ε3</td>
</tr>
<tr>
<td>Control</td>
<td>77</td>
<td>ε3/ε4</td>
</tr>
<tr>
<td>Cache County Study on Memory Health and Aging control</td>
<td>82</td>
<td>ε3/ε4</td>
</tr>
<tr>
<td><strong>Swedish cohort 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>72</td>
<td>ε3/ε3</td>
</tr>
<tr>
<td>Control 2</td>
<td>55</td>
<td>ε4/ε4</td>
</tr>
<tr>
<td>Control 3</td>
<td>59</td>
<td>ε3/ε3</td>
</tr>
<tr>
<td><strong>Family 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, mother</td>
<td>72</td>
<td>ε3/ε3</td>
</tr>
<tr>
<td>Control, daughter</td>
<td>84</td>
<td>ε3/ε3</td>
</tr>
</tbody>
</table>

Abbreviation: ACT, Adult Changes in Thought.

* Age indicates age at onset for the ACT case, age at last examination for the ACT, Cache County Study on Memory Health and Aging, and family 1 controls, and age at sampling for the 3 Swedish controls.

Conclusions

We show that the APP A673T allele is extremely rare in US white populations and thus does not play a substantial role in risk of developing AD in this group. Our study of a Swedish cohort showed a higher carrier frequency of APP A673T, and thus this variant appears primarily present in Icelandic and Scandinavian populations. Our results are consistent with this mutation being protective because carriers in our study were mostly controls. However, because of the rarity of this mutation in our populations, we could not independently verify that APP A673T is a protective allele.
Rarity of Alzheimer Disease–Protective APP Hyd


Drafting of the manuscript: Wang, Naj, Schellenberg.

Critical revision of the manuscript for important intellectual content: All authors.

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Study supervision: Munger, Leverenz, Hakonarson, Pericak-Vance, Mayeux, Schellenberg.

Conflict of Interest Disclosures: Drs Graham and Behrens are full-time employees of Genentech, Inc. Dr Deloskoy is a consultant for Jazz, Biogen-Inningheml, Navidea Biopharmaceuticals, and Pimall Healthcare. Dr Petersen is chair for the data monitoring committee for Pfizer and Janssen Alzheimer Immunotherapy and is a consultant for GE Healthcare and Roche. Dr Wright receives royalties from UpToDate for 2 chapters; has done legal consulting for the law firms of Aball, Mine, and Faegre Baker Daniels; is a consultant for Merck and Co; and does adjudication for a National Institutes of Health clinical trial. No other disclosures were reported.

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Author Contributions: Drs Wang and Schellenberg had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Wang, Naj, Cruchaga, Zetterberg, Blennow, Kauwe, Haines, Farrar, Pericak-Vance, Mayeux, Schellenberg.

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