Resistance to autosomal dominant Alzheimer’s in an APOE3-Christchurch homozygote

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SUMMARY

We identified a PSEN1 mutation carrier from the world’s largest autosomal dominant Alzheimer’s disease kindred who did not develop mild cognitive impairment until her seventies, nearly three decades after the expected age at onset. She had two copies of the APOE3 Christchurch (R136S) mutation, unusually high brain amyloid, and limited tau/tangle and neurodegenerative measurements. Our findings have implications for APOE’s role in the pathogenesis, treatment, and prevention of Alzheimer’s disease.

MAIN TEXT

We have identified about 1,200 Colombian Presenilin 1 (PSEN1) E280A mutation carriers and 4,600 non-carriers, who together compose the world’s largest known autosomal dominant Alzheimer’s disease (ADAD) kindred with a single mutation1,2. The mutation carriers develop mild cognitive impairment (MCI) and dementia at the respective median ages of 44 (95% CI, 43-45) and 49 (95% CI, 49-50) years3,4. Variability in the age at onset and clinical presentation has been reported in this population and other ADAD pedigrees5,6. Studying autosomal dominant AD (ADAD) mutation carriers who remain cognitively unimpaired until older ages could help in the discovery of risk-reducing gene variants7. We identified a PSEN1 E280A mutation carrier who did not develop MCI until her seventies, nearly three decades after the median age at onset. In this article, we describe findings from this case, summarize subsequent in vitro experimental and case-control findings, and consider potential implications for the understanding, treatment and prevention of AD.

This study was conducted with the participant’s informed consent following Institutional Review Board guidelines (her exact age and other identifying information are omitted to protect her anonymity and confidentiality). The participant was found to carry the amyloid-β42 (Aβ42)-overproducing PSEN1 E280A mutation, confirmed by family informants to be cognitively unimpaired until her seventies, and subsequently met criteria for MCI8 during a 24-month period of annual assessments. She continued to live independently and had no apparent decline in her activities of daily living. Her memory deficits were limited to recent events and her neurological exams were normal. Her age and education-adjusted neuropsychological test scores indicated a preferential impairment in recall memory, relatively preserved recognition memory, initial learning, naming, visuospatial abilities and verbal fluency skills, and relatively stable cognitive performance during the 24-month assessment period (Supplementary Table 1). Because the participant was not assessed prior to the onset of clinical symptoms and we relied on family informants to reconstruct her history we cannot rule out the possibility of a slow progressive AD.26

Whole exome sequencing corroborated her PSEN1 E280A mutation and discovered that she also had two copies of the rare APOE3 Christchurch (APOEch) mutation. Sanger DNA sequencing confirmed the latter finding (Supplementary Figure 1). Whole genome sequencing and a Genomizer analysis were used to comprehensively identify and rank all potentially significant rare and common variants9. Using this approach,
the PSEN1 E280A mutation was confirmed to be the participant’s primary risk factor and APOE3ch homozygosity was identified as the most likely genetic modifier. To our knowledge, there are no other studies previously reporting on the effect of the ApoE3ch on risk to dementia. We ruled out the presence of other mutations in PSEN1, tau, APP and the chemokine cluster—previously reported as a modifier in this pedigree. Single cell RNA sequencing of peripheral blood mononuclear cells from this subject also confirmed allelic-specific expression of the E280A PSEN1 variant (Supplementary Table 2). A post hoc analysis of a previously reported cohort from the same Colombian pedigree revealed a frequency of the Christchurch variant of 6%, though carriers were closely related, including four PSEN1 E280A mutation carriers heterozygotes for the Christchurch variant who progressed to MCI at the median age of 45. For this reason, we postulate that APOE3ch homozygosity is required to dramatically lower the risk and postpone the clinical onset of AD.

APOE, the major susceptibility gene for late-onset AD, has three common alleles (APOE2, 3, and 4). Compared to the most common APOE3/3 genotype, APOE2 is associated with a lower AD risk and older age at dementia onset, and each additional copy of APOE4 is associated with a higher risk and younger age at onset. The APOEch variant, an arginine-to-serine substitution at amino acid 136 (136Arg→Ser), corresponding to codon 154, can reside on any of the common APOE alleles, including this participant’s two APOE3 alleles. Homozygosity for rare APOE variants, including APOE2 homozygosity, is very rare in the general population for unknown reasons hence we were unable to identify any additional homozygote carriers of the ApoE3ch that also carry the PSEN1 E280A variant.

Carriers of APOEch and other rare mutations in APOE’s low density lipoprotein receptor (LDLR) binding region commonly have hyperlipoproteinemia type III (HLP-III), similar to that observed in 5-10% of APOE2 homozygotes. The participant in this report was confirmed to have HLP-III, including APOEch and elevated triglyceride and total cholesterol levels (Supplementary Table 3), though at the time of the initial evaluation she had no history of a diagnosis of her condition nor was she receiving any treatment for it.

While several mechanisms have been proposed to account for the impact of APOE variants on AD risk, most studies have focused on their differential effects (APOE2<3<4) on Aβ plaque burden and plaque plaque burden. Neuroimaging measurements were used to clarify whether the participant’s resistance to the clinical onset of AD was associated with a) relatively little Aβ plaque burden despite more than seventy years of Aβ overproduction or with b) relatively high Aβ plaque burden but limited downstream measurements of paired helical filament (PHF) tau (neurofibrillary tangle burden) and neurodegeneration.

The participant’s neuroimaging findings are shown in Figure 1. She had unusually high positron emission tomography (PET) measurements of Aβ plaque burden, as indicated by a higher mean cortical-to-
cerebellar Pittsburgh Compound B (PiB) distribution volume ratio (DVR=1.96) than in PSEN1 E280A carriers who developed MCI in their forties (DVRs 1.49-1.60). Despite her high Aβ plaque burden, the magnitude and/or spatial extent of her PHF tau burden and neurodegeneration were relatively limited: Her flortaucipir (tau) PET measurements were within the range of mutation carriers who developed MCI in their forties, but were restricted to medial temporal and less commonly affected occipital regions with relative sparing of other regions that are characteristically affected in the clinical stages of AD (Figure 1a). Her fluorodeoxyglucose PET measurements of the cerebral metabolic rate for glucose (CMRgI, Figure 1b) showed preserved metabolism in brain regions that are known to be preferentially affected by AD, including higher precuneus-to-whole brain measurements than in PSEN1 E280A mutation carriers who developed MCI at younger ages and many younger, cognitively unimpaired mutation carriers. Her MRI-based hippocampal-to-whole brain volume, a hippocampal atrophy measurement that can be affected by AD and/or normal aging, was within the range of mutation carriers who developed MCI in their forties (Figure 1c). Her plasma neurofilament light concentration (a blood biomarker for neurodegeneration in familial AD (ref: Preische O et al., Nat Med 2019) was within the normal range (her result and the mean and SD of non-mutation carriers in the same age range). Our findings suggest that this APOE3ch homozygote’s resistance to the clinical onset of AD is mediated through a mechanism that limits tau pathology and neurodegeneration even in the face of high Aβ plaque burden.

To study functional consequences of the APOE3ch variant we compared Aβ42 aggregation in vitro in the presence of the bacteria-derived human ApoE3 protein, presence of ApoE3ch protein, and absence of ApoE. Aβ42 aggregation was highest in the presence of human ApoE3 protein (C-terminus domain), lower in the presence of human APoE3ch (similar to that observed in the presence of ApoE2), and lowest in the absence of any ApoE (Supplementary Figure 2). This finding was confirmed using a sensitive split-luciferase complementation assay in which luciferase signal is reconstituted once amyloid forms oligomers, some of the most toxic amyloid species. Full-length ApoE3ch expression in mammalian cells triggered significantly less oligomerization of Aβ42 compared to wild type ApoE3. Even though these assays provided important insights on the kinetic of aggregation of amyloid in presence of both ApoE variants, we cannot exclude that the in vivo effect of ApoE might result in a more complex modulation of the amyloid aggregation kinetic toward a protective off-pathway aggregation kinetic leading to an accumulation of larger amyloid deposits. The latter phenomenon would be in agreement with physiological mechanisms of synaptic resilience observed in non-demented individuals with Alzheimer neuropathology, where reduced synaptic accumulation of toxic oligomeric species is observed in the presence of amyloid deposits. It also remains possible that the research participant may have had even greater Aβ plaque deposition had she survived to her seventies without the APOEch/3ch genotype and that the ApoE3ch altered the morphology of Aβ aggregates in ways that limited downstream neuroinflammation, tau pathology, neurodegeneration and
cognitive decline.

The R136S mutation impacts a region of APOE known to play a role in binding to the LDL receptor and interactions with heparin sulfate proteoglycans (HSPG\textsuperscript{21}). In fact, previous reports showed that ApoE\textsubscript{3ch} reduced LDLR binding to 40\% whereas ApoE\textsubscript{2} reduced it more substantially to 2\% compared to APOE\textsubscript{3}\textsuperscript{22,23}. The potential impact of the R136S mutation on HSPG had not been tested before and could be potentially relevant because protein-protein interactions mediated via HSPGs play a critical role in a multitude of processes relevant to AD pathology including amyloid and tau pathology and neurodegeneration\textsuperscript{24,25}. Our analyses of heparin binding confirmed that ApoE\textsubscript{4} had the highest affinity for heparin followed by ApoE\textsubscript{3} and ApoE\textsubscript{2}\textsuperscript{21} whereas ApoE\textsubscript{3ch} displayed the lowest heparin binding ability. To confirm this result, we raised a monoclonal antibody (1343A) against amino acids 130 to 143 of ApoE and tested its effect on the binding between full-length ApoE\textsubscript{3} protein and heparin. Our monoclonal antibody was able to reduce binding of wild type ApoE\textsubscript{3} to heparin producing an affinity profile similar to that of ApoEch. This proof of principle experiment demonstrates the relevance of the ApoE sequence including R136 for heparin binding and suggests that antibodies or other molecules binding to this region of ApoE\textsubscript{3} could reproduce the effects of ApoE\textsubscript{3ch}.

Our data support a model in which APOE variants differ in the extent of their pathogenic functions (\textit{APOEch} and \textit{APOE2<3<4}) and \textit{APOE3ch} and \textit{APOE2} are associated with greatest functional loss. We postulate that interventions that safely and sufficiently edit APOE, lower its expression, or modulate its pathogenic functions could have a profound impact on the treatment and prevention of AD. Together, our findings suggest that homozygosity for \textit{APOE3ch} is associated with a profound resistance to the clinical onset of AD; that this genotype exert its beneficial effects by directly or indirectly limiting downstream tau pathology and neurodegeneration, \textbf{possibly through the ability to bind differently to HSPGs and to LDL receptor}. While this mutation may not be deterministic, and more investigations are needed to better clarify causal relationships between APOE variants, non-genetic protective factors, and AD, our data strongly suggest that the Christchurch variant of ApoE\textsubscript{3} is not neutral to the AD phenotype as it would be expected for the wild type ApoE\textsubscript{3}. We surmise that the ApoE\textsubscript{3ch} is the best candidate that we can clearly identify as a genetic modifier in this subject and fully recognize that other factors (i.e. female gender, low level of education, lack of reported brain trauma) not only could but also must have played a role to achieve such strong resistance phenotype. Our findings have implications for APOE’s roles in the understanding, treatment, and prevention of AD, and may galvanize interest in developing APOE-modifying genetic and drug therapies for this disorder.

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Competing interests
Based on findings described in this case report, the Massachusetts General Hospital has filed a provisional patent application on methods and materials for the prevention and treatment of AD and related disorders diseases listing Drs. Quiroz, Arboleda-Velasquez, Reiman and Lopera as inventors. All other authors declare no competing financial interests.

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**Data Availability**

The data that support the findings of this study are available on request from the corresponding author Y.T.Q. The data are not publicly available because they contain information that could compromise research participant privacy and anonymity.
FIGURE 1
a

PSEN1 mutation carrier with two APOE3 ChC mutations and late onset MCI

Amyloid Plaque Burden (PIB PET DVR)

PHF Tau Burden (FTP PET SUVR)

b

PSEN1 mutation carrier with MCI onset at typical age

MRI and FDG PET (SUVR)

c

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<th>Amyloid burden</th>
<th>Tau burden</th>
<th>Hippocampal volume</th>
<th>Glucose metabolism</th>
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- Older MCI Carrier
- Younger MCI Carriers
- Unimpaired Carriers
Figure 1. Brain imaging. (A) Measurements of amyloid plaque burden and PHF tau burden in a PSEN1 E280A mutation carrier with two APOE3ch alleles and the exceptionally late onset of MCI (left) and a PSEN1 E280A carrier with kindred’s typical age at MCI onset (right). In each row, PET images are superimposed onto the medial and lateral surfaces of the left hemisphere. The top row shows PET measurements of amyloid plaque burden (PiB DVRs). The bottom row shows PET measurements of paired helical filament (PHF) tau (i.e., neurofibrillary tangle) burden. The person with late-onset of MCI is in her seventies; the person with the typical age at MCI onset is 44 years old. (B) 18F-fluordeoxyglucose Positron Emission Tomography (PET)–Measured Cerebral Metabolic Rate for Glucose (CMRgl) and Volumetric Magnetic Resonance (MRI) in a PSEN1 E280A mutation carrier with two APOE3ch alleles and the exceptionally late onset of MCI (left) and a PSEN1 E280A carrier with kindred’s typical age at MCI onset (right). Shown is relatively preserved levels of CMRgl levels in parietal regions (pointed by arrow/circle) in a PSEN1 E280A mutation carrier with two APOE3ch alleles. (C) Brain imaging measurements of mean cortical amyloid plaque burden, inferior temporal cortex PHF tau burden, hippocampal volume, and precuneus glucose metabolism in the PSEN1 E280A mutation carrier with two APOE3ch alleles and exceptionally late-onset of MCI (red dots, n=1), PSEN1 E280A mutation carriers with MCI at the kindred’s typical, younger age at MCI onset (black dots, n=7-11), and PSEN1 E280A mutation carriers who have not yet developed MCI (gray dots, n=13). Amyloid plaque burden is expressed as mean cortical-to-cerebellar distribution volume ratios (DVRs). Paired helical filament (PHF) tau burden is expressed as entorhinal cortex-to-cerebellar flortaucipir (FTP) standard uptake value ratios (SUVRs). Hippocampal volumes, which may be reduced by hippocampal atrophy, are expressed as hippocampal-to-whole brain volume ratios. Cerebral glucose metabolism, which is reduced in AD-affected brain regions with synaptic dysfunction and loss, is reflected as precuneus-to-whole brain cerebral metabolic rate for glucose (CMRgl) ratios. While the PSEN1 E280A mutation carrier with two APOE3ch alleles had by far the highest amyloid plaque burden, she did not have comparably severe PHF tau burden or hippocampal atrophy, and she had no evidence of precuneus glucose hypometabolism. All MCI carriers were at similar cognitive status as confirmed by the analysis of their cognitive tests.
**Figure 2.** The APOE3 Christchurch mutation. (A) Subject’s genealogy, with circles representing females, squares representing males, diamonds representing individuals whose gender has been masked for privacy, arrowhead depicts proband individual with MCI, and shading indicates individual with history of dementia. Deceased individuals are marked with a crossed bar. The individual APOE and PSEN1 genotypes are indicated as appropriate to preserve anonymity. (B) Model of the structure of the wild-type ApoE3 protein.
N-terminal (residues 1-191) and C-terminal (residues 201-299) domains are highlighted in red and blue, respectively. The amino acid positions for ApoE4 (C112R), ApoE3ch (R136S) and ApoE2 (R158C) variants are shown. (C) Western blotting of fractions eluted using an increasing NaCl gradient (0.0-0.65M NaCl) from each of the His Tagged APOE isoforms revealed impaired heparin binding of APOE3ch. B) ELISA measurement of APOE isoforms eluted from heparin column fractions obtained with an increasing NaCl gradient (0.0M-0.725M NaCl), APOE2 (Blue) (n=3), APOE3 (Magenta) (n=3), APOE4 (Black) (n=3) and APOE3ch (Cyan) (n=3) confirms impaired heparin binding of ApoE3ch. C&D). The use of an antibody targeted to the R136 region of APOE3 reduces APOE3 affinity for heparin binding and mimics APOE3ch bimodal distribution. C) Western blotting fractions from heparin binding columns obtained with an increasing NaCl gradient (0.0-0.5M NaCl) with and without incubation with an monoclonal antibody targeted to the amino acid sequence 130-143 of ApoE referred to as A1343 (Ab). D) ELISA measurement of APOE isoforms elution from heparin column fractions during an increasing NaCl gradient (0.0M-0.725M NaCl), Control (Magenta) (n=3) and APOE3 incubated with A1343 (Cyan) (n=3) shows reduced heparin binding of ApoE3 in the presence of A1343.
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