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Lack Of *APOE* Christchurch Variant In Five Age Of Onset Outliers With *PSEN1, PSEN2* Alzheimer Disease And *MAPT* Frontotemporal Dementia

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Abstract

Introduction: Age of onset modifiers are of considerable importance in Alzheimer and related dementias. Arboleta-Valesquez et al, reporting on a single *PSEN1* subject, suggested that homozygosity for the Christchurch variant of *APOE* could represent such a modifier.

Methods: We studied *APOE* Christchurch and Kloth-VS genotypes of five dementia age of onset outliers who carried their families' pathogenic variant, but were asymptomatic at ages beyond the families' average age of onset.

Results: Four age of onset outliers with *PSEN1/2* and *MAPT* mutations did not carry the Christchurch variant and a fifth individual was also determined to not be homozygous for this variant. Among them, only one subject (*APOE* ε3/ε3) carries the Klotho-VS heterozygous genotype.

Discussion: From a small but informative sample of five age of onset outliers we show that neither the *APOE* Christchurch nor the Klotho-VS variant is a common age of onset modifier for three genetic forms of dementia. Larger studies of this association and further research is required to identify additional genetic modifiers.

Keywords

Dementia; Alzheimer Disease; APOE; Christchurch mutation; modifiers

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Introduction

Factors influencing the age of onset of Alzheimer disease (AD) are considered highly important but largely unidentified. Onset age of sporadic late onset AD ranges from the late 60s to greater than 95 years. Likewise, onset age in the autosomal dominant familial forms of AD caused by mutations in the *PSEN1, PSEN2,* and *APP* genes varies from the 30s to the 70s. Similarly, onset age in the genetic forms of frontotemporal dementia (FTD) varies from the 30s to the 80s. The single strongest and best recognized genetic factor influencing age of onset in dementia is the *APOE* gene in which the ε4 allele (especially 4/4 homozygotes) confers the highest risk and a lower age of onset in both sporadic AD and familial forms of AD. Arboleta-Valesquez et al have recently associated homozygosity of the Christchurch variant of *APOE* (R136S) to resistance to early onset of dementia in a single individual in the large Colombian family with the *PSEN1* E280A mutation.[1] This Christchurch variant has been suggested to provide protective effect for AD. In addition, the VS heterozygous variant of the Klotho gene (KL) also has been suggested to provide protective effect for AD in *APOE* ε4 carriers[2]. In the present study we screened for both the *APOE* Christchurch and the KL-VS variants in five age of onset outliers from families with *PSEN2* (N141I), *PSEN1* (A79V) and *MAPT* (V337M) forms of genetic dementia.

Methods

Human subjects

Blood samples were obtained from family members with informed consent and IRB approved protocols. Because of the uniqueness of the outliers investigated in this study they have been previously been described in detail or referred to in several previous publications.[3,4,5,6]

APOE and Klotho-VS genotyping by Sanger sequencing

For each subject, 50 ng of blood-isolated genomic DNA was used for genotyping. Initially, an *APOE* exon 4 fragment (574 bp) and an KL exon 2 fragment (399 bp) were separately PCR amplified using HotStar Taq Master Mix (Qiagen), 1.25 M betaine (Sigma) and 0.2 μM of primers in a final volume of 20 μl. Nucleotide sequence of the four primers are: (1) APOE_Ex4-F: TCGGAACTGGAGGAACAACT, (2) APOE_Ex4-R: GCTCGAACCAGCTCTTGAGG, (3) KL_Ex2-F: CACTCAGGGAGGTCAGGTGT, and (4) KL_Ex2-R: CCTGAGACAAACCAGCCATT. The thermo-cycling program was set at 1 cycle for 5 min at 95°C, 30 cycles of 20 s at 95°C, 20 s at 55°C, and 2 min at 72°C using a 9700 thermocycler (Applied Biosystem). Next, PCR mixtures were incubated with 1μl of ExoSAP-IT (USB) at 37°C for 2 hrs to remove residual primers and dNTPs and followed by heating at 80°C for 10 min to inactivate enzymes. The ExoSAP-treated PCR fragments were then subjected to DNA sequencing using the BigDye terminator cycle sequencing kit (Applied Biosystems) with forward PCR primer in a final volume of 10 μl. Post PCR cleanup was performed using standard ethanol precipitation procedure. The nucleotide sequences were then determined using a SeqStudio DNA sequencer (Thermo Fisher) and manually inspected using Sequencher software (GeneCodes).

Results

Description of subjects

Subject 1 is a 70 yo man from a family with FTD who has the V337M mutation found in this family. He and his family have been previously reported.[3] He remains asymptomatic more than 2 SD beyond the mean age of onset in his family (51.5 years).

Subject 2 comes from a family with the A79V mutation in *PSEN1* which he carried. He had onset of dementia at age 79, died at age 85 and brain autopsy demonstrated typical changes of AD.[4] Five other affected persons in his family had onset ages ranging from 58-63 with a mean of 60 years. This particular *PSEN1* mutation is known to be associated with a generally later age of onset than other mutations in this gene.

Subject 3 is from a large Volga German family with the N141I mutation in *PSEN2*.[5] The mean age at onset in his family is 49.5 years. He had the onset of mild cognitive impairment in his mid-60s and is presently living independently at home with his wife at age 70 years. He has the N141I mutation.

Subject 4 was a member of the HB family with the N141I mutation in *PSEN2*.[6] Mean age of onset in this family was 59.9 years. This individual died at the age of 80 of cancer without any cognitive difficulties noted by his family. He had no formal evaluation. He had an affected mother and two affected children onset ages 56 & 58. He carried the N141I mutation as did his 2 affected children.

The fifth individual is from the H family also with the N141I mutation in *PSEN2*.[6] She is the affected daughter of a family member who died at age 89 of cancer and also had no cognitive deficits according to his family. That man had an affected parent and three affected children with dementia all of whom carried the N141I mutation, including the person we are now testing. DNA was not available from other children. Mean age of onset in this family was 60.3 years.

APOE and Klotho-VS genotyping results

Subjects 1-4 had the ε3/ε3 *APOE* genotype, and subject 5 was ε3/ε4. None of the subjects carried the *APOE* R136S Christchurch variant nor did we identify additional genetic variants in the sequenced region (Table 1). The fact that subject 5 did not carry the Christchurch variant implies that his 89 yo father could not have been homozygous for that variant. Among the five tested subjects, only one (#3; *APOE* ε3/ε3) was heterozygous for Klotho-VS (Table 1).

Discussion

The main finding from this study is that four age of onset outliers from families with a variety of genetic dementia (*PSEN1*, *PSEN2,* FTD-*MAPT*) did not carry the Christchurch variant (R136S) of *APOE*. In addition, we find evidence that an 89 yo person from another *PSEN2* family with AD could not have been homozygous for the Christchurch variant. The subject with the mutation in *MAPT* was included because he is clearly an age of onset

outlier and *APOE* has been reported to influence age of onset in FTD.[7] Thus, this data suggests that the *APOE* Christchurch variant is not a common modifier of age of onset in a variety of genetic forms of dementia. Additional larger studies are required to investigate this phenomenon.

Carriers of *APOE* R136S commonly have hyperlipoproteinemia similar to *APOE ε2* homozygotes.[8] This R136S variant was suspected to potentially provide resistance to AD because of its involvement with heparin sulfate proteoglycans and beta amyloid aggregation. *APOE* R136S was shown to have a lower ability to trigger Aβ42 aggregation than *APOE* ε3 alone.[1] The ApoE region containing the R136S variant is known to have a role in binding to lipoprotein receptors and heparin sulfate proteoglycans,[7] and this variant has been shown to have a 60% reduction in LDLR binding[8,9]. However, the actual role of ApoE R136S in the pathogenesis of AD remains speculative. Although the frequency of this variant is rare (0.00001283; 0.00007843 (Latino allele frequency))[10], if it is an important modifier of age of onset in familial dementia one might expect it to be enriched in a group of "onset outliers". While it is possible that this variant does modify age of onset in certain families such as the Colombian pedigree with a unique genetic background besides the E240A mutation in *PSEN1,* our present findings suggest the occurrence of such modification must be uncommon.

Heterozygosity for Klotho-VS has been reported to reduce the risk of AD in individuals that carry *APOE* ε4[2]. Subject 3 in the present study was heterozygous for Klotho-VS. However, the subject was *APOE* ε3/ε3 so this finding is not likely to have influenced this individual's risk for AD.

Research will obviously continue on searching for additional genetic and environmental modifiers of onset age in AD and factors influencing resistance and resilience to the underlying pathology[11].

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Highlights:

- Klotho-VS genotyping completed and addressed.
- Explain why a subject with a mutation in MAPT is included
- Corrected the frequency of the CH allele
- Toned down one of the sentences in the Conclusion.
- We explain that even an outlier with MCI is important.

Table 1.

Characteristics of Five Outliers

#	Sex	Age	Mean Family Onset Age	Gene	Mutation	APOE	APOE3 R136S	KL-VS (het)
1	M	70	51.5	MAPT	V337M	3/3	Neg	No
2	M	85	60.0	PSEN1	A79V	3/4	Neg	No
3	M	70	49.5	PSEN2	N141I	3/3	Neg	Yes
4	M	80	59.9	PSEN2	N141I	3/3	Neg	No
5*	F	64 (89/father)	60.3	PSEN2	N141I	3/3	Neg	No

*Affected daughter of unaffected father who lived to 89 years.