Targeting Neuroplasticity, Cardiovascular, and Cognitive-Associated Genomic Variants in Familial Alzheimer's Disease

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July 20, 2018

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The authors have declared they have no financial interests to disclose.

Running Title: Targeted Genetic Analysis in Familial Alzheimer's disease
Number of Characters: 90 (title), 57 (running title)
Number of Words: 251 (abstract), 3304 (main text)
Number of Figures: 2
Number of Coloured Figures: 2

Number of Keywords: 8
Number of Tables: 1
Number of References: 76
Online Supplementary Material: Tables 1; Figures 1

ABSTRACT

Background: The identification of novel genetic variants contributing to the widespread in the age of onset (AOO) of Alzheimer's disease (AD) could aid in the prognosis and/or development of new therapeutic strategies focused on early interventions. ***Methods:*** We recruited 78 individuals with AD from the Paisa genetic isolate in Antioquia, Colombia. These individuals belong to the world largest multigenerational and extended pedigree segregating AD as a consequence of a dominant fully penetrant mutation in the *PSEN1* gene and exhibit an AOO ranging from the early 30s to the late 70s. To shed light on the genetic underpinning that could explain the large spread of the age of onset (AOO) of AD, 64 single nucleotide polymorphisms (SNP) associated with neuroanatomical, cardiovascular and cognitive measures in AD were genotyped. Standard quality control and filtering procedures were applied, and single- and multi-locus linear mixed-effects models were used to identify AOO associated SNPs. A full two-locus interaction model was fitted to define how identified SNPs interact to modulate AOO. ***Results*:** We identified two key epistatic interactions between the *APOE*E2* allele and SNPs *ASTN2*-rs7852878 and *SNTG1*-rs16914781 that delay AOO by up to ~8 years (95%CI: 3.2-12.7, P=1.83x10^{-3}) and ~7.6 years (95%CI: 3.3-11.8, P = 8.69x10^{-4}), respectively, and validated our previous finding indicating that *APOE*E2* delays AOO of AD in *PSEN1* E280 mutation carriers. ***Discussion*:** This new evidence involving *APOE*E2* as an AOO delayer could be used for developing precision medicine approaches and predictive genomics models to potentially determine AOO in individuals genetically predisposed to AD.

***Keywords*.** Alzheimer's disease, *APOE*E2*, Age of Onset, *ASTN2*, Genetic Isolate, *PSEN1*, Extreme phenotypes, *SNTG1*.

INTRODUCTION

The prevalence of Alzheimer's disease (AD) continues growing at an alarming pace. In 2006, the number of patients with AD was reported to be over 26.6 million worldwide, and it could rise by approximately four-fold to over 106.2 million by 2050 (1). This neurodegenerative condition is incurable and constitutes a massive burden for patients, their families, and the public health system.

Genetic isolates have shown to be a powerful tool for the genetic mapping of inherited diseases (2). For more than three decades, we have been studying the world's largest known pedigree segregating AD in which the E280A (p.Glu280Ala) mutation in the *Presenilin-1* (*PSEN1*) gene causes early-onset AD (3, 4). This pedigree is genetically homogeneous, exhibits a high degree of endogamy, and originated centuries ago as a consequence of a founder effect during the colonizing of Colombia by Spaniards (2-6). To date, more than 5000 individuals descend from the original founder, 1784 have been enrolled in a comprehensive ongoing clinical monitoring study, and 1181 individuals have been genotyped (459 carry the *PSEN1* E280A mutation) (3). Although the median Alzheimer's disease age of onset (ADAOO) in this mentioned pedigree is ~49 years (3), it varies from the early 30s to the late 70s in some individuals (3, 7-10). It is hypothesized that this substantial variation in the ADAOO is the result of interactions between *PSEN1* and other key genes to modify ADAOO, and that this modification results in some members of this pedigree developing signs and symptoms of AD at an earlier or later age than other members (that is, these gene interactions with *PSEN1* either accelerate or decelerate ADAOO).

In a recent study, we performed a pooling/resampling-based genome-wide association study (GWAS) and successfully identified both known and novel loci associated with ADAOO in individuals with the E280A mutation, including *DAOA*, *NPHP1*, *CLUAP1*, *EXOC2*, *CADPS2*, *GREM2* and *CD44* (7). Subsequent genetic studies in *PSEN1* E280A mutation carriers identified functional exonic variants within some of these genes (9) and demonstrated that the *APOE*E2* allele (rs7412, P=5.44x10^{-35},

P_{FDR}=2.13x10^{-30}) delays ADAOO by ~12 years (8). Interestingly, in a separate study, we also reported an exonic missense mutation in the *DAOA* gene (rs2391191, $P = 1.94\times10^{-4}$) that was found to delay the ADAOO in patients from the Paisa cohort in ~4 years (9). It is also noteworthy to remark that the variant *SH3RF3*-rs6542814, flanking *NPHP1*, delays ADAOO by ~9 years (11), and the presence of two copies of the rare allele in *NPHP1*-rs906815 (rs906815, $P = 4.51\times10^{-6}$) accelerates ADAOO by ~21 years compared to the common allele in Caribbean Hispanic families carrying the *PSEN1* G206A mutation (12).

Since cognitive function and decline are highly polygenic traits where a large number of genetic factors of small effect are involved, it is difficult to find associations between these factors and clinical outcomes assessing cognition or cognitive decline (13, 14). One of the standard methods to overcome this issue is to increase the sample size and subsequently increase the power to detect small effect sizes. Another possible approach is to perform targeted analysis by employing specific genetic markers that could be relevant to AD.

In the present study, we screened 78 individuals from the above-described pedigree and genotyped 65 single nucleotide polymorphisms (SNPs) previously reported to be associated with dementia and cognition. These SNPs showed association with neuroanatomical differences in brain areas that play essential roles in cognition such as the hippocampus, or that were related with hypertension because common genetic links appear to occur between AD and cardiovascular disease (Supplementary Table 1). We successfully replicated the association between the *APOE*E2* allele and ADAOO, found two novel variants that also delay the age of onset of this pathological condition, and identified epistatic interactions between the *APOE*E2* allele and variants within the *Astrotactin 2* (*ASTN2*) and *Syntrophin, Gamma 1* (*SNTG1*) genes that dramatically delay the ADAOO in *PSEN1* E280A mutation carriers.

METHODS

Subjects

Seventy-eight individuals with AD (47 [60%] women, 31 men [40%]) carrying the *PSEN1* E280A mutation from the Metropolitan Area of Medellin in Antioquia, Colombia, were included in this study. Genetic studies have shown that this community has not been subject to microdifferentiation (2, 5). Clinical, neurological and neuropsychological assessments at the Group of Neurosciences AD Clinic used a Spanish version of The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) evaluation battery (15) adapted for the cultural and linguistic characteristics specific to this population (3, 16-18). Mild cognitive impairment (MCI) and AD affection status were defined based on Petersen's and DSM-IV criteria, respectively (19, 20). The Ethics Committee of the University of Antioquia approved this study (Protocol 1115-408-20543). Informed consent was obtained from all participants.

DNA extraction and SNP genotyping

Genomic DNA was extracted from peripheral blood, and whole-genome amplified, fragmented, hybridized, fluorescently tagged, and scanned using the Infinium assay (21). Sixty-four SNPs were selected based on previous associations with dementia, cognition, neuroanatomical differences and blood pressure (**Supplementary Table 1**), and further selected in our sample. Genomic DNA was normalized to a concentration of ~50 ng/µl, and 2.5 µL of genomic DNA was mixed with 2.5 µL TaqMan OpenArray Master Mix. The resulting samples were dispensed using the OpenArray® AccuFill™ System onto OpenArray plates with each plate containing 48 samples and 65 SNP assays per sample. The QuantStudio™ 12K Flex instrument (Applied Biosystems, Carlsbad, CA, USA) was used to perform the real-time PCR reactions on the loaded OpenArray plates. The fluorescence emission results were read using the OpenArray® SNP Genotyping Analysis software v1 (Applied

Biosystems), and the genotyping analysis performed using TaqMan® Genotyper v1.3 with the auto call feature and the default settings.

Genetic association analysis

Genotypes for the selected SNPs were processed, subject to quality control and association analysis performed using in Golden Helix® SNP Variation Suite (SVS) 8.3.2 (Golden Helix, Inc. Bozeman, MT, USA). Quality control exclusion criteria included (*i*) deviations from Hardy-Weinberg equilibrium with $P < 0.05/m$ (where m is the number of markers included for analysis), (*ii*) a minimum genotype call rate of 90%, (*iii*) the presence of one or more than two alleles, and (*iv*) a minor allele frequency (MAF) < 1% to exclude rare variants (22). Genotype and allelic frequencies were estimated by maximum likelihood, and the identity by descent (IBD) matrix between all pairs of individuals was used for quality control.

Single- and multi-locus additive, dominant and recessive linear mixed-effect models (LMEMs) with up to 10 steps in the backward/forward optimization algorithm (23-25) were used to study the association between ADAOO and the aforementioned SNPs. The advantage of these models is the inclusion of both fixed (sex and years of education) and random effects, the latter to account for potential inbreeding (which, in our case, was estimated using the IBD matrix described above). A single-locus LMEM assumes that all loci have a small effect on the trait, while a multi-locus LMEM assumes that several loci have a large effect on the trait (25). The optimal model was selected using a comprehensive exploration of multiple criteria (see (8-10) for more information). After the estimation procedure completed, the *P*-values associated with the LMEM coefficients $\hat{\beta}_1, \hat{\beta}_2, \ldots, \hat{\beta}_m$ were extracted and corrected for multiple testing using the false discovery rate (FDR) (26) and a method based on extreme-values theory (27).

Effect of SNP × SNP interactions on ADAOO

We evaluated potential SNP × SNP interactions between markers modifying ADAOO in carriers of the E280A mutation using a modified version of the full two-locus epistatic model (28-30). Conceptually, the analysis of SNP × SNP interactions intends to determine whether the joint effect of two SNPs on the ADAOO is greater than that of either marker alone. For each pair of markers found to modify ADAOO in our patients, the ADAOO was compared at each genotype combination after correcting for potential confounding variables. Since the maximum number of genotype combinations is nine, it is likely that the sample size at each of these combinations is small. To overcome this, a nonparametric bootstrap (31, 32) procedure with B=10,000 replicates was implemented to derive permutation-based P-values for these comparisons.

RESULTS

ADAOO distribution

The average ADAOO in all *PSEN1* E280A mutation carriers was 48.8±4.9 years (blue line, **Figure 1a**). Mean ADAOO did not differ significantly by gender (P=0.55, **Figure 1b**). A total of 37 patients (20 women [54%] and 17 men [46%]) had an ADAOO < 48 years (7). Years of education ranged between 0-16 years; four patients (5%) never attended school, 43 (55%) finished elementary school (grades 1 to 5), 26 (34%) finished high school (grades 6 to 11, inclusive), and 5 (6%) had tertiary education. The average ADAOO differed across education groups ($F_{3,74}$ = 3.724, P = 0.015) (**Figure 1b**). However, closer inspection of the data revealed that this effect was a consequence of the *APOE*E2* allele in a 66 years old male who never attended school. After excluding individuals that did not attend school, the effect of education groups on the ADAOO was no longer statistically significant ($F_{2,71}$ = 0.373, P = 0.690). Thirty-seven (47%) individuals developed AD earlier than the average for this population (ADAOO < 48 years; early-onset) and 41 developed late-onset AD (ADAOO ≥ 48 years). The average ADAOO was statistically different between these groups (early-onset: 44.8±1.9, late-onset: 52.5±3.9, $P < 2.5\text{x}10^{-16}$, **Figure 1b**). No association between gender (P = 0.979, **Figure 1b**) or years of education was found (R^2=0.028, P=0.076, **Figure 1b**).

ADAOO associated SNPs

A dominant multi-locus LMEM with three steps in the forward/backward selection algorithm (25) was selected based on the *mPPA* and pseudo-heritability criteria. This oligogenic model includes variants rs7412 (*APOE*, $P=1.94\text{x}10^{-4}$, $P_{FDR}=9.34\text{x}10^{-3}$, **Table 1a**), rs7852878 (*ASTN2*, $P=1.94\text{x}10^{-4}$, $P_{FDR}=9.34\text{x}10^{-3}$, **Table 1a**) and rs16914781 (*SNTG1*, $P=1.94\text{x}10^{-4}$, $P_{FDR}=9.34\text{x}10^{-3}$, **Table 1a**), which explains ~43% of the ADAOO variance. The proportion of the ADAOO variance explained by each

marker is ~24%, ~13% and ~8% for rs7412, rs7852872 and rs16914781, respectively. No gender- or education-specific effect of these SNPs was found (**Table 1b**). Because all estimated β coefficients from this model are positive (**Table 1a**), these alleles delay the ADAOO in our sample of *PSEN1* E280A mutation carriers. In particular, individuals with the C/T genotype in *APOE*-rs7412 (that is, the *APOE*E2* allele) have an ADAOO ~8 years later than that of individuals with the C/C genotype ($\hat{\beta} = 8.21, \widehat{\text{SE}}_{\hat{\beta}} = 1.5$; **Table 1a** and **Figure 2a**). Likewise, *PSEN1* E280A mutation carriers with C/G or G/G in *ASTN2*-rs7852878 have an ADAOO ~3.7 years later compared to that of C/C individuals ($\hat{\beta} = 3.68, \widehat{\text{SE}}_{\hat{\beta}} = 0.88$; **Table 1a** and **Figure 2a**). In addition, members of this pedigree with the G/G genotype in *SNTG1*-rs16914781 have a ~3.3 years delay in the ADAOO compared to those with A/A or A/G ($\hat{\beta} = 3.27, \widehat{\text{SE}}_{\hat{\beta}} = 0.872$; **Table 1a** and **Figure 2a**).

Effect of the *APOE*E2*×*ASTN2* and *APOE*E2*×*SNTG1* interactions on ADAOO

The presence of the *APOE*E2* allele in E280A mutation carriers was found to delay ADAOO by ~8.1 years (95%CI: 4.65-11.58, P=1.37x10^{-5}) (**Figure 2b**). A similar effect was observed when this same allele interacts with markers *ASTN2*-rs7852878 and *SNTG1*-rs16914781, which suggests an epistatic mechanism between *APOE*E2* and *ASTN2* (**Figure 2b**), and between *APOE*E2* and *SNTG1* (Figure 2b) to modify the ADAOO in carriers of the E280A mutation. In particular, the ADAOO in individuals with the *APOE*E2* allele and C/G genotype in *ASTN2*-rs7852878 is ~8 years (95%CI: 3.2-12.7, P=1.83x10^{-3}) later than that of individuals lacking the *APOE*E2* allele (**Figure 2b**). Similarly, those with C/C in *ASTN2*-rs7852878 carrying the *APOE*E2* allele have an ADAOO ~6.6 years (9%CI: 1.2-11.9, P = 0.017) later compared to non-carriers (**Figure 2b**). Conversely, individuals with the A/A genotype in *SNTG1*-rs16914781 carrying the *APOE*E2* allele have an ADAOO ~7.6 years (95%CI: 3.3-11.8, P = 8.69x10^{-4}) later than that observed in non-carriers (**Figure 2b**), and the presence of the

*APOE*E2* allele delayed the ADAOO in ~11 years (95%CI: 6.6-15.2, P=1.7x10^{-5}) in individuals with the A/G genotype in *SNTG1*-rs16914781 (**Figure 2b**). We found no effect of the *ASTN2*×*SNTG1* interaction on the ADAOO.

DISCUSSION

In this study, we targeted neuroanatomical, cardiovascular and cognitive-associated markers in familial AD from the Paisa community, a genetic isolate from Antioquia, Colombia. Even though several GWAS studies have provided a potential list of a handful of putative candidate genes for sporadic AD (i.e., an age of onset > 65 years) most of those genes failed in their replication. It is well known that heterogeneity of genetic and environmental background could largely account for this apparent discrepancy. Thus, to increase power in our analyses, our approach was aimed at performing a targeted analysis in a multigenerational family from a local community that is exposed to a quite homogenous environment. More specifically, we employed 65 genetic markers related to Alzheimer's disease in a large family from the local Paisa community that originated from a common ancestor from Northern Spain during the 1500s. In this community, Alzheimer's disease is quite common as a result of the high frequency of the autosomal dominant and fully penetrant *PSEN1* E280A allele. Our main goal was to shed light on the genetic underpinning that could explain the large spread of the age of onset of AD that ranks from the early 30s to late 70s.

This cohort was also subjected to two earlier preliminary studies in which smaller sample sizes were employed, and different outcomes were observed (33, 34). Since the time those studies were performed, more E280A carriers have been identified. Hence, here we expanded the sample to detect new genes that could explain the widespread of the ADAOO observed in E280A carriers. Our present data show that the presence of the *APOE*E2* allele confers protection by delaying the ADAOO by ~8.2 years (95%CI: 5.2-11.2, P=4.21x10^{-5}; **Figure 2a**), which confirms our most recent reported finding in a sample of 71 *PSEN1* E280A mutation carriers displaying an extreme ADAOO (8). Basically, by increasing the sample size to 78 patients carrying the E280A mutation, in the present study, we corroborated the decelerating *APOE*E2* effect on ADAOO previously shown in individuals from the

Paisa community (8). Power analyses indicate that, overall, the ADAOO can be safely tested using our current sample size (see **Supplementary Material**).

Collectively, previous and current work in this genetic isolate suggests that the ADAOO accelerating and decelerating effects conferred by the *APOE*E4* and *APOE*E2* alleles, respectively, become evident. Therefore, our results provide convincing evidence that not only does the *APOE*E2* allele exert a protective role in the onset of AD in sporadic patients (35, 36), but also in the *PSEN1* E280A familial cases.

The role of beta-amyloid (Aβ) in AD has been openly challenged (37-39). One of the primary reasons is that there is evidence showing that Aβ deposition rises with healthy aging and its increase is not necessarily correlated with the onset of dementia and the progression to AD (39, 40). However, it is noteworthy to remark that patients with familial Alzheimer's disease display fibrillar Aβ pathology several years before symptoms onset (41). For instance, by employing florbetapir PET analyses, Fleisher et al. showed that individuals from the Antioquia cohort carrying the *PSEN1* E280A mutation showed evident accumulation of fibrillar Aβ at a mean age of 28.2 years, which was approximately 16 and 21 years before the expected MCI and dementia onset, respectively (42). Thus, it appears that fibrillar Aβ pathology could represent an early preclinical stage of AD. Another piece of evidence supporting that Aβ is involved in the pathogenesis of AD is the fact that the three well-known genes that cause a dominant Mendelian form of familial AD (*APP*, *PSEN1,* and *PSEN2*) are involved in the processing of Aβ peptides (43-45). Aβ peptides vary between 37-43 amino acids in length depending on the γ-secretase cleavage site. Mounting evidence suggests that the majority of early-onset familial AD mutations in *APP, PSEN1,* and *PSEN2* elevate the Aβ1-42: Aβ1-40 ratio, which favors the aggregation of neurotoxic oligomeric assemblies of Aβ. It is considered that Aβ1-42 is more amyloidogenic than other Aβ peptides, which assemble into soluble Aβ oligomers that are thought to cause synaptic loss and a progressive cognitive decline in AD (46). Aβ1-42 oligomers can elicit an

inflammatory cascade by triggering the activation of microglia (47). Moreover, Aβ oligomers associate with membrane proteins in synapses (48) and astrocytes (49). In post-synaptic neurons increase the Ca^{2+} concentration causing inflammation and cell death (48). Post-mortem studies carried in brain tissue from the E280A kindred suggest that their *PSEN1* mutation selectively increases the processing of the amyloidogenic peptide Aβ1-42 (45). Mounting evidence suggests that there are links between Aβ and tau in the pathogenesis of AD (50-52). Aβ promotes abnormal tau phosphorylation and aggregation into neurofibrillary tangles, which is associated with neuronal toxicity and impaired cognition in AD. For instance, in functional studies employing transgenic animal models and neuronal cell culture, it was found that a 56-kDa amyloid oligomer elicited an influx in intracellular Ca2+ that triggered phosphorylation of tau at a site that promoted its aggregation (53). This recent finding expands previous evidence supporting a possible link between Aβ and tau in the pathogenesis of AD (50-52).

In this context, it can be argued that the *APOE*E2* variant might cause a beneficial impact on AD by improving the clearance of central Aβ, and consequently delay the onset of AD (54). On the other hand, the *APOE*E4* variant accelerates the ADAOO since it performs poorly in the clearance of Aβ peptides thereby favoring the formation of aggregates and the occurrence of the disease (55, 56).

Marker rs7852878, harbored in *ASTN2*, was also found to delay ADAOO in individuals with AD carrying the E280A mutation. ASTN2 is an integral membrane protein that participates in glial-guided neuronal migrations and is largely expressed within the hippocampus (57). Genomic variants in genes engaged in neuronal migration processes have been linked to several neurocognitive and psychiatric disorders. For instance, genes casually linked to schizophrenia such as *Disrupted in schizophrenia-1* (*DISC1*), *Reelin, neuroregulin* (*NRG*) and its receptor, *ERBB4*, control neuronal migration during brain development (58). Likewise, genes linked to ADHD (*LPHN3*), (59, 60) autism (*YWHAZ*) (61), and depressive behavior (*BDNF*) (62) also control neuronal fate within different brain regions. Interestingly, SNPs within *ASTN2* have been associated with cognitive decline and reduced

hippocampal volume (63, 64) and several psychiatric conditions such as schizophrenia (65, 66), ADHD (67), and bipolar disorder (66). More recently, genetic variants within *ASTN2* have been associated with ADAOO in late-onset AD (68).

We found that marker rs16914781 within *SNTG1* delays ADAOO by ~3.2 years in individuals carrying the *PSEN1* E280A mutation (Table 1a). SNTG1 belongs to the syntrophin family; it is an adapter protein that participates in the subcellular organization of several proteins. It also mediates gamma-enolase trafficking to the plasma membrane and is involved in neurotrophic signaling (69). SNTG1 is expressed exclusively in neurons, including Purkinje cells, hippocampal pyramidal cells, and in multiple cortical regions, where it could be playing important roles in the pathophysiology of AD and other neurodegenerative/neuropsychiatric conditions (57, 70). *SNTG1* has been reported as a highly penetrant recessive locus in schizophrenia (70), and as AOO modifier gene in AD (7). More recently, a circular RNA hotspot involving *SNTG1* has recently been identified in multiple system atrophy (MSA) (71), a neurodegenerative disorder causing parkinsonism, cerebellar ataxia, and autonomic, urogenital, and pyramidal dysfunction in various combinations. Previously, a case report displayed an association of MSA and AD (72). *SNTG1* has also been implicated in obstructive sleep apnea (73), a condition that is highly prevalent in patients with Alzheimer's disease (74).

To the best of our knowledge, we are the first to demonstrate a significant association between variants within *ASTN2* and *SNTG1*, and ADAOO in individuals with familial AD caused by a fully penetrant mutation. Our study suggests that the genetic variants described here exert a protective effect by delaying ADAOO up to ~3.7 years (**Table 1a**); this value increases to ~11 years when the *APOE*E2* allele is present (**Figure 2a**). Future studies need to be performed to address the underlying action mechanism describing the interaction between *ASTN2* and *PSEN1*, and between *STNG1* and *PSEN1*.

Acknowledgements

The authors express their highest appreciation to the patients and relatives enrolled in this study for more than 25 years. This study was financed by a research-grant from Australian National University (ANU) to MAB to launch his laboratory; and COLCIENCIAS and the University of Antioquia, grant 1115-408-20543. JIV and CAM were supported by grant R42100-2 from the John Curtin School of Medical Research, Canberra, ACT, Australia. MAB and CAM are currently supported by internal funds provided by the Universidad del Rosario. JIV is partially supported by research-grant FOFICO 32101 PE0031 from Universidad del Norte. MLC-H is a doctoral student at Universidad del Norte, Barranquilla, Colombia. Some of this work is to be presented in partial fulfilment of the requirements for her PhD degree.

Competing Interest

None to declare.

Role of funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the paper. JIV, CAM and MAB have full access to all the data in the study and are responsible for submitting this work for publication. Data are available from the authors by request.

FIGURE LEGENDS

Figure 1. (a) ADAOO distribution in 78 patients with Alzheimer's disease carrying the *PSEN1* E280A mutation. Notice the presence of two hidden groups with an average ADAOO of ~46 and ~51 years old, respectively. To identify these groups, a mixture of two Gaussian distributions was fitted as implemented in the mixtools (75) package for R (76); the number of hidden groups was determined based on the log-likelihood criterion (the lowest the better). The blue vertical line is at ~48 years, which corresponds to the average ADAOO in our sample. Box- and violin-plots for the ADAOO by **(b)** gender, **(c)** early-onset, and **(d)** education group. Only differences in the average ADAOO were found by AD status. **(e)** ADAOO as a function of the years of education. AD = Alzheimer's disease. ADAOO = Alzheimer's disease age of onset.

Figure 2. (a) Effect of the presence of the *APOE*E2* allele, and the genotypes in rs7852872- *ASTN2* and rs16914781- *SNTG1* on ADAOO. A two-sample *t*-test indicates the presence of the *APOE*E2* allele increases the ADAOO by ~ 8.1 years (t_{72} = 4.67, 95%CI: 4.6-11.6, P=1.37x10^{-6}). Pink, blue and dotted horizontal lines are, respectively, the within genotype average ADAOO, the individuals' ADAOO and the global average ADAOO in our sample. **(b)** Effect of the *APOE*E2*×*ASTN2* and *APOE*E2*×*SNTG1* interactions on ADAOO. Green lines symbolize protection, red lines susceptibility and the grey line the average ADAOO in our sample. Note that the *APOE*E2* allele delays ADAOO regardless of the interacting marker. Abbreviations as in Figure 1.

TABLES

Table 1. (a) Results of the association analysis for ADAOO in 78 patients with *PSEN1* E280A Alzheimer's disease. **(b)** Proportion of variance explained and gender- and education-specific effects of ADAOO associated SNPs.

(a)

Chr	SNP[a]	Position	Gene	Marker information				Multi-locus linear mixed-effects model		
				Ref/Alt	MA (Freq)	CR	Change	β (SE$_\beta$)	*P*	P_{FDR}
19	rs7412	45,412,078	*APOE*	C/T	T (0.046)	0.974	p.Arg176Cys	8.213 (1.505)	6.48×10^{-7}	4.21×10^{-5}
9	rs7852872	119,249,338	*ASTN2*	C/G	G (0.396)	0.987	Intronic	3.684 (0.881)	8.10×10^{-5}	2.63×10^{-3}
8	rs16914781	51,287,481	*SNTG1*	A/G	G (0.339)	1.000	Intronic	3.273 (0.872)	3.52×10^{-4}	7.62×10^{-3}

(b)

SNP[a]	PVE	Sex			Education group		
		χ^2	*df*	*P*	χ^2	*df*	*P*
rs7412	0.239	0.023	2	0.989	4.303	6	0.636
rs7852872	0.133	1.041	1	0.308	2.681	3	0.443
rs16914781	0.076	0.939	2	0.625	6.331	6	0.387

[a] UCSC GRCh37/hg19 coordinates. ADAOO = Alzheimer's diease age of onset; Chr: chromosome; SNP = Single Nucleotide Polymorphism, Ref/Alt = Reference/Alternate allele; MA = Minor allele; Freq = Frequency; CR = Call rate; β = Regression coefficient; SE$_\beta$ = Standard error of β; *P* = *P*-value; FDR = False discovery rate; PVE = Proportion of variance explained; χ^2 = Test statistic; *df* = degrees of freedom.

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