Genetic analyses of *SERPINA5* in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is the most common cause of dementia. Our previous studies have shown that increased expression of the *SERPINA5* gene is associated with hippocampal vulnerability in AD, and that the SERPINA5 protein binds to tau and co-localizes within neurofibrillary tangles. To determine if genetic variants in the *SERPINA5* gene may be contributing to this phenotype, we sequenced 103 autopsy-confirmed young-onset AD cases with a positive family history of cognitive decline. We observed one individual with a rare missense variant (rs140138746) in the *SERPINA5* gene, resulting in an amino acid change (p.E228Q). We screened a further 1170 neuropathologically diagnosed AD cases and identified an additional 5 carriers of this variant, resulting in an allelic frequency of 0.002141 within our AD validation cohort, which was comparable to online genomic databases. Although not significant, *SERPINA5* p.E228Q variant carriers were found to be younger at age of onset and age of death than non-carriers. *SERPINA5* p.E228Q variant carriers had a longer disease duration than non-carriers, which approached significance. To further elucidate possible neuropathologic contributions of the *SERPINA5* p.E228Q variant, we carried out descriptive neuropathologic burden analysis on a variant carrier that was matched to a non-carrier for age, sex, disease duration, Braak tangle stage, TDP-43 positive status, and who possessed an *APOE* ϵ 4 risk allele. Interestingly, SERPINA5 burden was lower in the *SERPINA5* p.E228Q carrier than the non-carrier in 9 corticolimbic brain regions studied, which exaggerated the tau:SERPINA5 immunohistochemical ratio. The *SERPINA5* p.E228Q carrier was observed to have more severe neuronal loss in several brain regions compared to the non-carrier. Together, we cautiously interpret these findings to suggest that the *SERPINA5* p.E228Q variant may stall tangle maturity and slow AD disease progression, thus prolonging disease duration in these individuals.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia, accounting for 60–90% of cases [9, 10, 17]. Today, AD affects approximately 5.8 million Americans aged 65 and older, a number that is expected to rise to 13.8 million by 2050 [1]. In 2020, healthcare costs associated with the treatment of AD totaled an estimated \$305 billion [1]. With such a large social and economic burden, understanding the molecular mechanisms that underlie this devastating disease is ever pressing.

AD is neuropathologically defined by the aberrant accumulation of two proteins; amyloid- β ($A\beta$), which is deposited extracellularly to form $A\beta$ plaques, and hyperphosphorylated tau, which accumulates intracellularly to form neurofibrillary tangles (NFTs) [23]. In 2011, Murray *et al.* defined 3 distinct AD subtypes characterized by the differential regional distribution of corticolimbic NFTs; hippocampal sparing (HpSp) AD, typical AD, and limbic predominant AD [13, 25]. The HpSp AD subtype has vast NFT accumulation in the cortex with the hippocampus being relatively spared. In comparison, limbic predominant AD has a severely affected hippocampus with a relatively spared cortex. Typical AD falls between these two extreme AD subtypes, with the expected corticolimbic NFT burden that is widely recognized in the Braak staging scheme [4]. Alongside differences in their neuropathologic distributions, the AD subtypes contrast in their demographic and clinical characteristics [13, 25]

As the hippocampus is differentially affected in AD, it is important to understand what mechanisms may be contributing to this selective vulnerability. Through a unique translational neuropathology approach, our group observed that hippocampal gene expression changes are associated with hippocampal neuropathologic changes in AD [8]. Using transcriptomic analysis and machine learning, *SERPINA5* was identified as the top gene predictive of hippocampal vulnerability. Further, *SERPINA5* expression was increased in all AD subtypes, with its expression correlating with an advanced tangle maturity marker (i.e. Ab39 [40], which recognizes mature tangles and ghost tangles [22]) [8]. Immunofluorescence microscopy showed that SERPINA5 colocalizes with tau in NFTs and interestingly, co-immunoprecipitation experiments of postmortem brain tissue indicated that SERPINA5 interacts with tau in a disease-specific manner. Together, these findings suggest that SERPINA5 may play an important role in the development and progression of AD.

SERPINA5, also known as protein C inhibitor, is a predicted 47kDa secreted serine protease inhibitor that plays an important role in the blood coagulation and thrombosis [32]. It interacts with and inhibits many blood coagulation factors, including activated protein C, thrombin, factor XI, factor Xa, and plasma kallikrein [14, 21, 32, 33]. In humans, SERPINA5 was shown to be expressed by many organs, most predominantly the liver [31], though also in the brain [8, 15]. However in rodents, SERPINA5 is expressed only in the reproductive organs [38, 41]. Genetically, the *SERPINA5* gene is positioned on chromosome 14q32.1 [3] and consists of six exons; two untranslated regions and 4 coding regions. As SERPINA5 expression is increased in AD and associates with pathology, we sought to determine if genetic variants in the *SERPINA5* gene may be a contributing disease factor.

Methods

Study samples for discovery cohort

The FLorida Autopsied Multi-Ethnic (FLAME) cohort [18, 29] is a set of 2809 autopsied brains based at Mayo Clinic in Jacksonville, Florida and is derived from the State of Florida brain bank, a component of the Alzheimer's Disease Initiative supported by the State of Florida Department of Elder Affairs (<http://elderaffairs.state.fl.us/does/alz.php>). The FLAME cohort was queried for neuropathologically diagnosed AD cases with an age of onset younger than 65 (i.e. young-onset AD) and a positive family history of cognitive decline. We excluded 1163 cases that were not neuropathologically characterized as having AD, 101 cases that lacked NFT data and 42 cases with a Braak stage < V. We excluded 20 cases with known autosomal dominant AD mutations. We excluded 276 cases with limited availability of clinical history. Of the remaining cases, 242 were excluded for unevaluated family history and a further 458 cases were excluded for negative family history of cognitive decline. Out of the remaining 507 AD cases, we excluded 21 cases for missing age of onset and 378 cases that were classed as late-onset AD. Of the 108 remaining, 5 cases were excluded for unavailable brain tissue and/or DNA. Thus, our final cohort consisted of 103 young-onset AD cases with a positive family history of cognitive decline. Demographically, the cohort consisted of 44/103 (44%) females, 59/103 (57%) males. The average age of onset was 59 years (range; 43–65 years) and the average age at death was 71 years (range; 54–86 years). Disease duration ranged from 4 years to 27 years, with an average of 12 years. All brains and DNA samples were acquired with appropriate ethical approval, and the research performed on postmortem samples was approved by the Mayo Clinic Research Executive Committee.

Genetic screening

To undertake genetic screening of the 105 cases, DNA was extracted from frozen brain tissue using standard protocols. *APOE* genotype rs7412 and rs429358 was determined using TaqMan products (Invitrogen), as previously described [16, 25]. To determine the presence of *SERPINA5* genetic variants, each case was sequenced for all 6 exons of *SERPINA5* (RefSeq: NM_000624.6). DNA fragments were PCR amplified by Apex products and 7 primer sets stated in **Supplementary Table 1** using a 60 – 50 Touchdown program. The subsequent PCR products were purified using the Agencourt AMPure system (Agencourt Bioscience Corporation, Beverly, MA, USA). Sequencing was performed with Big Dye Terminator V3.1 products (Applied Biosystems, Foster City, CA, USA) and purified with Agencourt CleanSEQ (Agencourt Bioscience Corporation). Sequences were run on an ABI3730 DNA-analyzer (Applied Biosystems) and subsequently analyzed with Sequencher software (Gene Codes, Ann Arbor, MI, USA). A copy number assay for *SERPINA5* (Hs02961512_cn) was also performed.

Rare variant frequency validation cohort

To determine the frequency of the *SERPINA5* p.E228Q variant, we performed a TaqMan genotyping assay (C_162935990_10, rs140138746) on a cohort of 1170 autopsied-confirmed AD cases. Genotypes were generated and analyzed on Applied Biosystems QuantStudio 7 real-time PCR instruments and software (Applied Biosystems). Demographically, the cohort consisted of 638/1170 (55%) female and 532/1170 (45%) male. The average age of death was 80 years (range; 48–103 years).

Statistical analysis

Sigma Plot Version 13 (Systat Software, San Jose, CA) was used to perform statistical analyses. The Mann-Whitney Rank Sum Test was used to evaluate non-parametric, pair-wise differences in median values and interquartile range (i.e. 25th -75th percentile). Statistical significance was considered for 2-sided $p < 0.050$.

Immunohistochemistry

Standardized neuropathologic procedures were performed by a single board-certified neuropathologist, as previously defined [29]. 5 μ m thick sections were cut from formalin-fixed, paraffin-embedded tissue blocks and mounted onto glass slides. Thioflavin-S fluorescent microscopy was used to assess AD neuropathologic changes, as well as to assign a Braak tangle stage [4], Thal amyloid phase [34], and AD subtype [25]. Lewy body pathology was diagnosed using the NACP antibody (1:3000 [2]) and assigned a subtype according to the fourth dementia Lewy body consortium [20]. Hippocampal sclerosis of a TDP-43 etiology (MC2085, 1:2500 [42]) was diagnosed when neuronal loss in the CA1 or subiculum of the hippocampus exceeds that for the extent of tangle pathology observed [24, 27]. Significant cerebrovascular disease was included in the neuropathologic diagnosis if 2 or more of the following pathologies were observed: large infarct, lacunar infarct, microinfarct, white matter infarct, moderate-to-severe white matter rarefaction, hippocampal sclerosis of a vascular etiology, or severe cerebral amyloid angiopathy.

To explore neuropathologic differences between a *SERPINA5* p.E228Q variant carrier and non-carrier, we performed digital pathology. We matched for age at death (carrier = 75 years vs. non-carrier = 76), sex (both males), disease duration (16 years vs. 17 years), Braak tangle stage (both VI), presence of TDP-43 pathology, and presence of *APOE* ϵ 4 allele. Digital pathology was performed on 9 brain regions that included entorhinal cortex, limbic regions (amygdala and 2 hippocampal subsectors [CA1, subiculum]), association cortices (superior temporal, inferior parietal, and middle frontal), and primary cortices (visual and motor). These 9 regions and the pons were stained with the following commercially-available antibodies: *SERPINA5* (R&D, MAB1266, 1:100), AT8 (Thermo, MN1020, 1:2500) which recognizes “earlier” aspects of tangle maturity (pretangles, mature tangles), and 2E9 (Novus, NBP2-25162, 1:100,000) which recognizes “advanced” aspects of tangle maturity (mature tangles, ghost tangles). The stained slides were digitally scanned at 20X magnification on an Aperio AT2 scanner (Leica Biosystems, Buffalo Grove, IL, USA). ImageScope x64 software v12.4.2.700 (Leica Biosystems) was used to annotate regions of interest, which were subsequently analyzed in Aperio eSlide Manager (Leica Biosystems) using three custom-designed color deconvolution digital pathology macros to assess neuropathologic burden. Routine hematoxylin and eosin (H&E) stained slides were also digitized and used to evaluate neuronal loss on a semi-quantitative scale: none, mild, moderate, severe.

Results

Analyses of known AD genes

Sequencing analyses of *SERPINA5*

In our young-onset AD cohort with a positive family history of cognitive decline, 44/103 (43%) individuals were heterozygote for the *APOE* ϵ 4 allele and 23/103 (22%) were homozygote for the *APOE* ϵ 4 allele. We observed common variants in *SERPINA5* (Table 1) at comparable frequencies to publicly available online genomic variant databases. In addition, we observed one individual with a rare missense variant in exon 5 of the *SERPINA5* gene, resulting in a C > G transversion (Chr14: 95056440 G > C; p.Glu228Gln) (Fig. 1). The allelic frequency within our initial screening cohort was 0.004854. The reported allelic frequency in gnomAD v2.1.1 for this variant (dsSNP ID: rs140138746) is 0.002599, with a minor allele frequency of < 1%. The combined annotation dependent depletion (CADD) score for this variant is 17.95 and thus is predicted to be pathogenic, scoring within the top 10% most deleterious substitutions. For the remainder of this paper, this rare missense variant will be referred to as *SERPINA5* p.E228Q. We observed no copy number variants in the *SERPINA5* gene.

Table 1
Common variants in *SERPINA5* observed in our cohort of young-onset AD with positive family history of cognitive decline.

dbSNP ID	Position	Allelic frequency
SERPINA5 Exon 1		
rs2144076	Chr14:95047686 C > T	0.2222
rs8019792	Chr14:95047810 A > G	0.3065
rs8020223	Chr14:95047837 G > A	0.3046
rs8019810	Chr14:95047847 A > C	0.3045
SERPINA5 Exon 3		
rs6113	Chr14:95053849 T > C	0.1517
rs6118	Chr14:95053863 C > T	0.1523
rs6115	Chr14:95053890 G > A	0.6132
rs6111	Chr14:95053891 C > T	0.1507
rs6119	Chr14:95054012 A > G	0.1498
rs6112	Chr14:95054176 T > C	0.6130
SERPINA5 Exon 6		
rs6109	Chr14:95058360 A > G	0.6512
rs6116	Chr14:95058462 A > C	0.3916
rs6108	Chr14:95058631 A > T	0.5323
Reference assembly: GRCh37. Genomic database used to obtain allelic frequency: gnomAD v2.1.1		

To further assess *SERPINA5* p.E228Q variant frequency, we screened 1170 neuropathologically diagnosed AD cases. In this validation cohort, 576/1170 (49.2%) of individuals carried one copy of the *APOE* ϵ 4 allele and 173/1170 (14.8%) carried two copies of the *APOE* ϵ 4 allele. We identified a further five individuals with the *SERPINA5* p.E228Q variant, resulting in an allelic frequency of 0.002141 within the validation cohort.

Demographic and clinicopathologic characteristics of *SERPINA5* p.E228Q carriers and non-carriers

Table 2 shows the demographic and clinicopathologic characteristics of the 6 *SERPINA5* p.E228Q variant carriers and 1213 non-carriers in this study. Although significance was not reached in the analyses due to small sample size, we did observe some interesting patterns. *SERPINA5* p.E228Q carriers were younger at age of death (median = 79 years) than non-carriers (81 years). Similarly, *SERPINA5* p.E228Q carriers were younger at age of onset (66 years) than non-carriers (71 years). *SERPINA5* p.E228Q carriers had a longer disease duration (12 years) than non-carriers (9 years), which approached significance ($p = 0.079$). Braak tangle stage (both VI) and Thal amyloid phase (both 5) did not differ between *SERPINA5* p.E228Q carriers and non-carriers.

Table 2
Comparison of demographic and clinicopathologic characteristics of non-carriers and carriers of the *SERPINA5* p.E228Q variant from our cohort.

Characteristic	<i>SERPINA5</i> p.E228Q carriers (n = 6)	Non-carriers (n = 1213)	Overall p-value
Age at death, yr.	79 (74,85)	81 (75,87)	0.682
Age at onset, yr.	66 (60,73)	71 (63, 77)	0.351
Disease Duration, yr.	12 (10,15)	9 (6,12)	0.079
Braak NFT stage	VI (IV, VI)	VI (V,VI)	0.978
Thal amyloid phase	5 (5,5)	5 (5,5)	0.266
Data shown as median (25th, 75th percentile).			
Statistical analysis was performed using the Mann-Whitney Rank Sum Test.			

Further characteristics of the 6 *SERPINA5* p.E228Q carriers are summarized in Table 3. Amongst the six variant carriers, there were 4 males (67%) and 2 females (23%). In terms of AD subtype, there was 1 HpSp AD subtype (17%), 3 typical AD subtypes (50%) and 2 limbic predominant subtypes (33%). Family history of cognitive deficits was described in 2 (23%) carriers; father, brother, and sister in case #1 and mother in case #3. Lewy body pathology was observed in 5 carriers (83%) and significant cerebrovascular disease was found in 3 carriers (50%). There were 5 carriers (83%) who possessed at least one copy of the *APOE* ϵ 4 allele.

Table 3
Complete characteristics of each individual *SERPINA5* p.E228Q variant carrier in our series.

ID	Neuropathologic diagnosis	Sex	Age at death (years)	AD subtype	FHx of cognitive deficits	Brain weight (grams)	Lewy body pathology	TDP-43 pathology	Vascular disease	<i>APOE</i> genotype
1	AD/VaD/ALB	M	75	Typical	Yes	1020	Yes - ALB	Yes	Yes	ϵ 3 ϵ 4
2	AD/VaD	F	72	HpSp	No	920	No	No	Yes	ϵ 2 ϵ 3
3	AD/TLBD/HpScl	M	82	LP	Yes	1040	Yes - TLBD	Yes	No	ϵ 3 ϵ 4
4	AD/ALB	F	89	LP	No	960	Yes - ALB	No	Yes	ϵ 3 ϵ 4
5	AD/ALB	M	86	Typical	No	1100	Yes - ALB	Yes	No	ϵ 4 ϵ 4
6	AD/ALB/HpScl/VaD	M	86	Typical	No	1300	Yes - ALB	Yes	Yes	ϵ 3 ϵ 4
HpSp – hippocampal sparing AD, LP – limbic predominant AD. FHx – family history										

Immunohistochemical evaluation of the *SERPINA5* p.E228Q variant

Digital pathology was used to measure immunohistochemical burden of *SERPINA5* and explore differences with two tau markers that recognize predominantly more pretangles/mature tangles (AT8) or mature tangles/ghost tangles (2E9) (Fig. 2). Overall, the *SERPINA5* p.E228Q carrier (Case #1 in Table 3) had a lower *SERPINA5* burden (0.65%) compared to the non-carrier (1.0%) across all 9 brain regions. Apart from the entorhinal cortex, the limbic and cortical structures had a lower, or nearly even, *SERPINA5* burden in the carrier compared to the non-carrier. AT8 burden was higher overall in the carrier (14%) compared to the non-carrier (12%), while 2E9 burden was comparable (1.7% vs. 1.6%).

Semi-quantitative neuronal loss analysis was carried out across the locus coeruleus and 9 corticolimbic structures (Supplementary Table 2). Representative neuropathologic images of the carrier and non-carrier are shown in Fig. 3 and Fig. 4, respectively. Upon microscopic investigation on routine H&E, both the carrier and non-carrier had severe neuronal loss in the entorhinal cortex. The higher *SERPINA5* burden in the entorhinal cortex of the carrier was comprised of more neuropil thread pathology than was observed in the non-carrier. The amygdala and hippocampal subsectors (CA1, subiculum) of the carrier had severe neuronal loss compared to

moderate neuronal loss observed in the non-carrier. Neuropil threads and SERPINA5-positive neurofibrillary tangles were observed in pyramidal neurons across limbic structures in both cases. The superior temporal and middle frontal cortex had moderate neuronal loss in the carrier compared to mild neuronal loss in the non-carrier. Inferior parietal cortex had moderate neuronal loss in both cases. SERPINA5-positive neurofibrillary tangles were predominantly found in layer V with more SERPINA5-positive neuropil threads and neuritic plaques found in upper layers of the superior temporal, inferior parietal, and middle frontal cortices. Primary visual and primary motor cortex had no detectable neuronal loss and rare SERPINA5-positive neurofibrillary tangles. As the site considered to be affected earliest by tau pathology [5, 19], the locus coeruleus was additionally examined in stained sections of the pons. Severe neuronal loss was observed in the locus coeruleus of the carrier compared to moderate neuronal loss in the non-carrier. Sparse SERPINA5-positive neurofibrillary tangles and extensive 2E9-positive ghost tangles were observed in the locus coeruleus in both cases.

Discussion

In this study of 103 young-onset AD cases with a positive family history of cognitive decline we observed 13 common variants in the *SERPINA5* gene at a similar frequency to that previously reported. In addition, we observed one individual with the rare missense variant *SERPINA5* p.E228Q (rs140138746). To increase our cohort size, we screened a further 1170 neuropathologically diagnosed AD cases and observed another 5 individuals carrying this variant (< 1%). This revealed an allelic frequency in AD (0.002141) comparable to that observed in the general population (0.002599).

Although the discovery cohort of young-onset AD cases with a positive family history was used to identify *SERPINA5* p.E228Q variant carriers, age was not used to select the validation cohort. Despite this, we continued to see a non-significant pattern of a median age of onset of 66 years in carriers, which was 5 years younger than the non-carriers in our cohort. Previous studies have indicated that disease duration in AD dementia ranges between 3 to 10 years [6, 39]. Interestingly, *SERPINA5* p.E228Q carriers were found to have a longer-than-average disease duration of 12 years, which approached significance as this was 3 years longer than non-carriers. Additionally, case #2, a HpSp AD subtype, had a disease duration of 12 years, which is much longer than the reported average of 9 years for this subtype [13]. Together, these findings suggest that the *SERPINA5* p.E228Q variant may be associated with prolonged disease duration, though more work is needed to understand on the association of *SERPINA5* genetic variants and age.

Global neuropathology measures, including Braak tangle stage [4] and Thal amyloid phase [34], did not differ between *SERPINA5* p.E228Q carriers and non-carriers. The limbic predominant AD subtype was observed in 33% of the *SERPINA5* p.E228Q variants, which is higher than reported in neuropathology-based series (14%) but exactly at the frequency observed in recently validated tau imaging-based classification [13, 37]. We observed a high frequency of Lewy body pathology in 5/6 (83%) of the *SERPINA5* p.E228Q carriers. Lewy pathology exists concurrently with AD pathology, occurring most frequently in the olfactory bulb and amygdala, as compared to the substantia nigra that is more commonly seen in Parkinson's disease [10, 30]. Studies have shown that Lewy body pathology is detected in 60% of AD cases [12] and amygdala predominant Lewy bodies is detected in 18% [35]. The longer disease duration may account for the greater frequency of amygdala predominant Lewy bodies, as it is more commonly observed in severe end-stage AD brains.

It is well established that variants in the *APOE* gene are strongly associated with risk of sporadic late-onset AD [7]. The strongest risk is associated with the *APOE* ϵ 4 allele, followed by *APOE* ϵ 3 and finally by the *APOE* ϵ 2 allele [36]. Approximately 40% of late-onset AD patients have the *APOE* ϵ 4 allele, compared to about 14% in the general population [11]. The presence of one copy of the *APOE* ϵ 4 allele increases the risk of late-onset AD by about 3 times and 2 copies by 12 times [36]. Interestingly, the presence of one or two copies of the *APOE* ϵ 4 allele is associated with an earlier age of onset by about 10–20 years, as compared to non-carriers [7]. We observed a high frequency (83%) of at least one *APOE* ϵ 4 allele in our *SERPINA5* p.E228Q carriers, which intersects well with the younger age of onset in these cases.

In addition, we sought to assess the SERPINA5 and tau burden across 9 different brain regions, including the entorhinal cortex, limbic regions (amygdala and hippocampus), association cortices (temporal, parietal and frontal) and primary cortices (visual and motor). This allowed us to assess neuropathologic burden in regions routinely used to indicate AD disease progression in the Braak tangle staging scheme [4]. Using digital pathology to quantify immunohistochemical burden, SERPINA5 was overall observed to be lower in the *SERPINA5* p.E228Q variant carrier than the non-carrier. To examine neurofibrillary tangle changes, we used two antibodies (AT8 and 2E9) to encompass the dynamic lifespan of tangle maturity [22]. The tau:SERPINA5 ratio was much higher in the carrier compared to the non-carrier. Although unable to test the hypothesis in the current study, our recent data suggests SERPINA5 may be a “tipping point” in the tangle maturation process that could contribute to associated neurodegeneration [8]. The observed lower levels

of SERPINA5 immunohistochemical burden in the variant may have allowed for more time for neurofibrillary pathology to accumulate before maturing into a ghost tangle upon the death of the neuron. To examine an area outside of the vulnerable corticolimbic regions, the locus coeruleus was evaluated as this region may have the longest-standing tau accumulation [5]. We observed only moderate neuronal loss in the non-carrier compared to severe neuronal loss in the carrier. As we more commonly observed greater neuronal loss in the carrier, we cautiously speculate that the *SERPINA5* p.E228Q variant requires less SERPINA5 to promote more extensive neurofibrillary tangle maturity, leading to the death of the neuron. Taken together with the trend for longer disease duration observed overall, it is interesting to consider that the longer time to accumulate pathology corresponds to greater neuronal loss in the context of similar 2E9 (advanced tangle) burden.

There are several limitations to this study. First, we did not sequence the promotor and regulatory regions of the *SERPINA5* gene. It is well established that variants in these regions can affect gene expression, and can contribute to disease [26, 28]. Therefore, it is possible that regulatory region variants may be contributing to the increased expression of *SERPINA5* that we observe in AD, although more thorough sequencing must be undertaken before such conclusion can be made. In addition, it will be important to sequence more cases for *SERPINA5* variants. Future directions include screening cases of other tauopathies, such as primary age-related tauopathy, corticobasal degeneration, and progressive supranuclear palsy, which will assist in determining if *SERPINA5* variants contribute to the development of tau pathology in general. Furthermore, we were unable to carry out statistical analysis for our neuropathologic assessment due to lack of power, and therefore any observations made are descriptive and interpreted with caution.

Conclusion

To summarize, we sequenced a cohort of 103 young-onset AD patients with positive family history of cognitive decline for coding variants in the *SERPINA5* gene. We observed no copy number variants in the *SERPINA5* gene but identified common variants at comparable frequencies to online genomic databases. In addition, we identified one individual with a rare missense variant (rs140138746), a G > C transversion that resulted in an amino acid change from a glutamic acid to a glutamine at position 228 in the SERPINA5 protein. To assess the prevalence of this variant, we screened a further 1170 neuropathologically diagnosed AD cases and observed an additional 5 cases with the *SERPINA5* p.E228Q variant. We identified that carriers of the *SERPINA5* p.E228Q variant were younger at age of onset and age of death than non-carriers. In addition, variant carriers had a longer disease duration than non-carriers. Neuropathologically, the variant carrier had lower SERPINA5 burden and more severe neuronal loss compared to the non-carrier. Together, these findings suggest that the *SERPINA5* p.E228Q variant may modify disease progression, although further investigation is necessary.

Abbreviations

A β
amyloid- β
AD
Alzheimer's disease
CADD
Combined annotation dependent depletion
FLAME
FLorida Autopsied Multi-Ethnic
HpSp
hippocampal sparing
IQR
interquartile range
NFT
neurofibrillary tangles
PCR
polymerase chain reaction

Declarations

Ethics approval and consent to participate

All brains were acquired with appropriate ethical approval, and the research performed on postmortem samples was approved by the Mayo Clinic Research Executive Committee.

Consent for publication

All authors have reviewed the contents of the manuscript being submitted, approved of its contents, and consented to publication.

Availability of data and material

We will provide anonymized data to any qualified investigator for purposes of validation and/or replication.

Competing interests

None

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Authors' contributions

B.J.M., S.J.L. and M.E.M. designed and supervised the entire study. K.M.H., M.D.T., and M.E.M. dissected frozen brains. K.M.H. and M.B. performed DNA extraction and quality control. M.B. designed the *SERPINA5* sequencing primers. B.J.M., S.J.L. and M.B. screened known AD genes. B.J.M., S.J.L., M.B. and N.T. performed *SERPINA5* sequencing, copy number variant assays and analysis. M.B. carried out *SERPINA5* p.E228Q variant screening. D.W.D. and M.E.M. performed neuropathologic evaluation. S.A.L. and J.C-R. performed the digital pathology analysis. N.E.T., O.A.R. and R.R. provided DNA for this study. B.J.M. wrote the manuscript. All authors edited and reviewed the manuscript.

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Figures

neuropathologic burden. b) The early tangle marker, AT8, to SERPINA5 burden ratio. c) The late tangle marker, 2E9, to SERPINA5 burden ratio.

Figure 3

Photomicrographs of a 75-year-old male SERPINA5 p.E228Q carrier neuropathologically diagnosed with Alzheimer’s disease. Representative images of the locus coeruleus, entorhinal cortex, limbic structures (CA1 hippocampal subsector), association cortices (superior temporal), and primary cortices (visual) are shown. Case characteristics— Disease duration = 16 years, Braak stage = VI, Thal phase = 5, TDP-43 positive, APOE ε4 allele positive. Immunohistochemistry— SERPINA5, Early Tau marker: AT8, Late Tau marker: 2E9. Scale bar— 50µm.

Figure 4

Histopathological analysis of a 76-year-old male non-carrier neuropathologically diagnosed with Alzheimer's disease. Case characteristics— Disease duration = 17 years, Braak stage = VI, Thal phase = 5, TDP-43 positive, APOE ϵ 4 allele positive. Immunohistochemistry— SERPINA5, Early Tau marker: AT8, Late Tau marker: 2E9. Scale bar— 50 μ m.

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