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Gene-based association study of genes linked to hippocampal sclerosis of aging neuropathology: *GRN, TMEM106B, ABCC9*, and *KCNMB2*

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Abstract

Hippocampal sclerosis of aging (HS-Aging) is a common neurodegenerative condition associated with dementia. To learn more about genetic risk of HS-Aging pathology, we tested gene-based associations of the *GRN, TMEM106B, ABCC9*, and *KCNMB2* genes, which were reported to be associated with HS-Aging pathology in previous studies. Genetic data were obtained from the Alzheimer's Disease Genetics Consortium (ADGC), linked to autopsy-derived neuropathological outcomes from the National Alzheimer's Coordinating Center (NACC). Of the 3,251 subjects included in the study, 271 (8.3%) were identified as an HS-Aging case. The significant gene-based association between the *ABCC9* gene and HS-Aging appeared to be driven by a region in which a significant haplotype-based association was found. We tested this haplotype as an expression Quantitative Trait Locus (eQTL) using two different public-access brain gene expression databases. The HS-Aging pathology protective *ABCC9* haplotype was associated with decreased *ABCC9* expression, indicating a possible toxic gain of function.

Keywords

CARTS; GWAS; PGRN; KATP; rs704180

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1. Introduction

Hippocampal sclerosis of aging (HS-Aging) is a high-morbidity brain disease in people of advanced age (Corey-Bloom, et al., 1997). The prevalence of HS-Aging pathology ranges from 5 to 30% in older people in large autopsy series (Dickson, et al., 1994; Leverenz, et al., 2002; Nelson, et al., 2013; Zarow, et al., 2012). Clinical signs and symptoms of HS-Aging are similar to those of Alzheimer's disease (AD) with amnestic memory deficits (Pao, et al., 2011; Zarow, et al., 2008). Because of the overlapping symptomology, HS-Aging is often clinically misdiagnosed as AD (Brenowitz, et al., 2014; Pao, et al., 2011; Zarow, et al., 2008). AD is characterized by the accumulation of amyloid plaques and neurofibrillary tangles (Hyman, et al., 2012), while HS-Aging is pathologically characterized by neuronal cell loss and gliosis in the hippocampus seen by hematoxylin and eosin (H&E) stain, which can occur unilaterally (~50%) or bilaterally (Nelson, et al., 2013; Zarow, et al., 2008). Whatever the laterality on H&E stain, the large majority of cases with HS-Aging show bilateral TAR DNA-binding protein 43 (TDP-43) pathology in limbic structures (Amador-Ortiz, et al., 2007; Nelson, et al., 2011b). Awareness of this common cause of dementia is rapidly increasing, and we recently recommended a revision of the terminology for describing this disease to cerebral age-related TDP-43 with sclerosis (CARTS) (Nelson, et al., 2016b). However, here we will maintain use of the term HS-Aging because the neuropathologic databases we assessed did not include TDP-43 pathologic information until quite recently.

Genetic risk factors for HS-Aging have been recently identified. Unlike AD, the apolipoprotein E (*APOE*) ε4 allele is not a risk factor for HS-Aging (Brenowitz, et al., 2014; Leverenz, et al., 2002; Nelson, et al., 2011b; Pao, et al., 2011; Troncoso, et al., 1996). By contrast, the following four genes (in the chronological order they were so identified) have been reported to harbor risk alleles associated with HS-Aging pathology: Granulin (*GRN*) on chromosome 17q, Transmembrane protein 106B (*TMEM106B*) on chromosome 7p, ATP-binding cassette subfamily member 9 (*ABCC9*) on chromosome 12p, and potassium channel subfamily M regulatory beta subunit 2 (*KCNMB2*) on chromosome 3q (Aoki, et al., 2015; Beecham, et al., 2014; Dickson, et al., 2010; Murray, et al., 2014; Nelson, et al., 2014; Nelson, et al., 2015b; Pao, et al., 2011).

Alleles near the coding portions of the *GRN* and *TMEM106B* genes were shown to have an association with HS-Aging using an allele test, following the known relationship of those two genes to frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP). Specifically, HS-Aging pathology was associated with the T-allele of the *GRN* single nucleotide polymorphism (SNP) rs5848 (Dickson, et al., 2010; Murray, et al., 2014; Pickering-Brown, et al., 2008; Rademakers, et al., 2008). For the other FTLD-related gene, *TMEM106B*, persons with eventual autopsy-proven HS-Aging pathology were more likely to have the T-allele than controls (Aoki, et al., 2015; Murray, et al., 2014; Rutherford, et al., 2012). We confirmed an increase in HS-Aging odds for each copy of the T-allele of *TMEM106B* rs1990622 (Nelson, et al., 2014).

The connections of the *ABCC9* and *KCNMB2* genes to HS-Aging risk were discovered via genome-wide association studies (GWAS), which are neither helped nor biased by prior

mechanistic hypotheses. The association of *ABCC9* SNP rs704178 with HS-Aging pathology was demonstrated in a GWAS using a recessive mode of inheritance (MOI) (Nelson, et al., 2014). The relationship of this locus with HS-Aging was subsequently tested in a different group of research subjects, and the association was replicated (Nelson, et al., 2015b). Beecham and colleagues reported the *KCNMB2* SNP rs9637454 as the top SNP for HS pathology, although this association was not genome wide significant (Beecham, et al., 2014), and has not been replicated to date.

In the present study, we examined the associations of these four putative risk SNPs with HS-aging pathology, using genetic data obtained from Alzheimer's Disease Genetics Consortium (ADGC) linked to neuropathological outcomes from the National Alzheimer's Coordinating Center (NACC) (Nelson, et al., 2014; Nelson, et al., 2015b). Here we aggregated those data sets to attain greater statistical power for gene-wide association analyses, for the purpose of understanding better the association of multiple (often co-inherited) gene variants with disease development. Thus, we tested *GRN, TMEM106B, ABCC9*, and *KCNMB2* for gene-based associations with HS-Aging pathology by aggregating SNPs and indels (small insertions or deletions) on each of those genes. In addition, we focused on the interesting region located around intronic SNP rs704178 on the *ABCC9* gene that was identified in the previous work, and analyzed haplotype associations of the region with HS-Aging pathology and *ABCC9* gene expression.

2. Material and methods

2.1 Study subjects

ADGC genotype data were linked to data from the National Institute on Aging (NIA)-funded 36 AD Centers (ADCs) and NACC registry phenotype information. Of 3,730 subjects with both genotype and autopsy information available to us, those who died at age 60 years or older were included in this study. Cases of HS-Aging were identified as patients who met at least one of the following criteria at autopsy; 1) the primary pathologic diagnosis was hippocampal sclerosis, 2) there was a contributing pathologic diagnosis of hippocampal sclerosis, or 3) medial temporal lobe sclerosis was present at autopsy. We then excluded 180 individuals who had FTLD with ubiquitin-positive inclusions, FTLD with no distinctive histopathology, FTLD-tau, or prion associated disease (Figure 1).

2.2 Quality control of the ADGC genotype data

Standard quality control (QC) procedures were performed on the ADGC genotype data using PLINK v1.90a (Purcell, et al., 2007). Markers were excluded based on the following criteria: (1) minor allele frequency (MAF) < 1%; (2) call rate per variant (SNPs and indels) < 95%, (3) Hardy-Weinberg equilibrium test in controls < 10^{-5}. (Supplemental Table 1). Samples were excluded based on the following criteria: (1) call rate per individual < 95%, (2) a high degree of relatedness per an estimated proportion of identical by descent (IBD) > 0.1875, (3) excess of ± 3.0 standard deviations of heterozygosity rate. Of the 3,407 individuals after the inclusion and exclusion criteria were applied, 3,330 passed the QC (Figure 1).

2.3 Identifying ethnic outliers

We performed principal component analysis (PCA) in EIGENSTRAT (Price, et al., 2006) using a linkage disequilibrium (LD) pruned subset of markers (pairwise $r^2 < 0.2$) from our data merged to 1000 Genomes Project Phase 3 (1000 Genomes) (1000 Genomes Project Consortium, 2010) data after removing symmetric SNPs and flipping SNPs discordant for DNA strands between the two datasets. We then plotted the first and second principal components (PCs) for each individuals (n = 5,834: 2,504 from 1000 Genomes and 3,330 from the study) using the ggplot2 R package (version 2.2.0) (Wickham, 2009) in R (version 3.2; http://www.r-project.org). Based on the PC plot, 79 study subjects were removed as ethnic outliers (Figure 1 and Supplemental Figure 1). We reran the PCA for the remaining 3,251 European ancestries to derive orthogonal PCs which were used as covariates in the subsequent analyses.

2.4 Statistical analysis

2.4.1 Gene-based association analysis—Prior to gene-based association analyses, we performed the single variant association testing using logistic regression assuming each of the three most commonly used MOI (additive, dominant, and recessive) adjusted for age at death, sex and the top three PCs using PLINK v1.90a (Purcell, et al., 2007). Gene-based association analyses were conducted using GATES (Gene-Based Association Test Using Extended Simes Procedure) (Li, et al., 2011) as implemented in the open-source software Knowledge-Based Mining System for Genome-wide Genetic Studies (KGG; version 3.5) (Li, et al., 2011). GATES is a gene-based association test that combines the p-values of variants within a gene obtained from single variant association testing described above. We assigned variants to genes based on their physical positions at the UCSC Genome Browser GRCh37/hg19 human assembly (https://genome.ucsc.edu/) (Kent, et al., 2002), and defined gene boundaries as ± 5kb from 5' and 3' untranslated regions (UTRs). This gene-based association test adjusts for LD in European super population genotype data from the 1000 Genomes (1000 Genomes EUR) (1000 Genomes Project Consortium, 2010). The input data files to KGG contained four columns: chromosome number, marker ID, marker position, and single variant association p-value. We then obtained overall p-values for the associations of the target genes. Since those who live to advanced old age have a higher risk of HS-Aging pathology (Nelson, et al., 2011a; Nelson, et al., 2011b), there is a possibility that those who died earlier would be always identified as a control even if they have a genetic risk. Therefore, for sensitivity analysis against these possible misclassifications, we further performed these gene-based association tests in cases and controls who died at age 80 years or older. For the gene-based association test, statistical significance level was defined using the Bonferroni correction, yielding $\alpha = 0.05/(4 \text{ genes} \times 3 \text{ MOI} \times 2 \text{ age groups}) = 0.0021$ for the four examined genes and three MOI.

2.4.2 Haplotype-based association analysis for HS-Aging—After identifying the HS-Aging risk-associated region on the *ABCC9* gene by generating a regional association plot using LocusZoom software (Pruim, et al., 2010), we performed additional post hoc haplotype analysis for the variants on the region. First, we selected tag variants using a pairwise SNP tagging approach with $r^2 \geq 0.8$ based on the 1000 Genomes EUR in Haploview version 4.2 (Barrett, et al., 2005). Maximum likelihood estimates of haplotype

frequencies were computed using an expectation-maximization (EM) algorithm implemented in the functions haplo.em (for overall subjects) and haplo.group (for HS-Aging cases and controls) of the haplo.stats R package (version 1.7.7) (Sinnwell and Schaid, 2016) using R (version 3.2; http://www.r-project.org). The associations between common haplotypes (the estimated frequencies greater than 1% in entire subjects) and HS-Aging status assuming a recessive MOI were then tested with a haplotype score test adjusted for age at death, sex, and the top three PCs (Schaid, et al., 2002) implemented in the function haplo.score. The global and haplotype-specific empirical p-values were obtained via 10^7 Monte-Carlo simulations.

2.4.3 Haplotype-based expression Quantitative Trait Locus (eQTL) analysis for ABCC9 gene expression—We examined the association of the haplotypes with *ABCC9* gene expression, focusing on the haplotypes that were identified in association analysis for HS-Aging pathology. We retrieved *ABCC9* gene expression values in human brain and genotype data from two independent datasets: North American Brain Expression Consortium (NABEC) (Hernandez, et al., 2012) and United Kingdom Brain Expression Consortium (UKBEC) (Trabzuni, et al., 2011).

In the NABEC dataset, the expression data were available at Gene Expression Omnibus (GEO) public repository (http://www.ncbi.nlm.nih.gov/geo/) under the GEO accession GSE36192, consisting of two brain regions (cerebellum and frontal cortex) from 228 neurologically normal donors. The genotype data were obtained from the database of Genotypes and Phenotypes (dbGaP: http://www.ncbi.nlm.nih.gov/gap) under the dbGaP study accession phs000249.v2.p1. After the QC procedure with the same settings as we did for the ADGC genotype data was applied, the genotype data were imputed using Michigan Imputation Server (https://imputationserver.sph.umich.edu/start.html) (Das, et al., 2016) with the following parameters: 1000 Genome Phase 3 v5 reference panel, Eagle v2.3 phasing (Loh, et al., 2016), and EUR population. The imputed genotype with posterior probabilities < 0.9 were labeled as missing. Among the 228 NABEC subjects, 130 who died at age 30 years or older and passed the QC were included in the analysis (all of them were US Caucasians).

In the UKBEC dataset, gene expression for ten brain regions (cerebellar cortex, frontal cortex, hippocampus, medulla, occipital cortex, putamen, substantia nigra, thalamus, temporal cortex, and white matter) and genotype data from 134 “neuropathologically normal” individuals were obtained at BRAINEAC website (http://www.braineac.org/). The dosage files downloaded from the website (accessed 6/28/2016) were converted into PLINK file format using Genome-wide Complex Trait Analysis (GCTA) software version 1.24.4 (Yang, et al., 2011). The haplotype-based association analyses on *ABCC9* gene expression were performed for the five haplotypes that were identified in the haplotype-based association analysis for HS-Aging assuming an additive MOI.

The analyses were carried out separately in the two datasets. We focused on *ABCC9* gene expression through Illumina probe ID ILMN_1751453 in frontal cortex of the NABEC and through Affymetrix transcript ID t3446919 in the average of all ten regions of the UKBEC dataset. Expression data were quantile normalized and log2-transformed.

3. Results

Of the 3,251 included subjects from ADGC/NACC, 271 (8.3%) met at least one of the HS-Aging case criteria. Figure 2 shows the proportion of participants with HS-Aging pathology increased with age at death, from 3.1% (95% confidence interval (CI) is 1.6 to 5.4%) in those aged less than 70 years to 15.7% (95% CI is 12.8 to 19.0%) in those aged 90 years or older. The mean age at death in the cases was significantly higher than that in the controls (84.8 ± 8.4 years in the cases and 80.5 ± 8.8 years in the controls). No statistically significant differences were noted by case status and sex, *APOE* ε4 and microtubule-associated protein tau (*MAPT*) haplotype (H1 haplotype tagging rs8070723 A-allele and H2 tagging G-allele) frequencies (Table 1).

3.1 Single variant-based association

Table 2 shows the most associated variants on each of the four genes defined gene boundaries as ± 5kb from 5' and 3' UTRs. The highest association signals came from SNPs on the *ABCC9* gene (rs7966849; $p = 7.1 \times 10^{-6}$ with an assumed recessive MOI and $p = 4.4 \times 10^{-5}$ with an assumed additive MOI) and on the *KCNMB2* gene (rs73183328; $p = 8.2 \times 10^{-5}$ with an assumed additive MOI and $p = 1.6 \times 10^{-4}$ with an assumed dominant MOI). There was a series of small signals in high LD with the top SNP on the *TMEM106B* gene, and there was an associated region with small effects in low-to-moderate LD with the top SNP on the *ABCC9* gene.

3.2 Gene-based association

In the gene-based association analyses, 20, 222, 259 and 939 variants were mapped to the *GRN, TMEM106B, ABCC9* and *KCNMB2* genes, respectively. Table 3 shows the results of the gene-based association test in people aged 60 years. The *ABCC9* gene had a significant gene-based association with HS-Aging assuming a recessive MOI when applying the Bonferroni correction ($p = 2.4 \times 10^{-4}$). There were nominally significant gene-based associations for the *GRN* gene assuming a recessive MOI, the *TMEM106B* gene assuming a recessive and an additive MOI, the *ABCC9* gene assuming an additive MOI, and the *KCNMB2* gene assuming an additive and a dominant MOI. For sensitivity analysis in people aged 80 years or older (n = 1,883: 203 in HS-Aging cases and 1,680 in controls), we confirmed the same results that the *ABCC9* gene had a significant gene-based association with HS-Aging assuming a recessive MOI (p = 0.0017) (Supplemental Table 2).

3.3 Haplotype-based association with HS-Aging

The single-variant-based association plots (Figure 3) imply that the significant gene-based association of the *ABCC9* gene is driven by the region in which the most significant variants were located on the position 21,982,262 – 22,015,114 (all chromosomal positions we describe are referent to human assembly GRCh37/hg19). The top SNP (rs7966849) in this study is in high LD with rs704180 ($r^2 = 0.926$) which was identified as the predominant risk SNP of HS-Aging (Nelson, et al., 2014; Nelson, et al., 2015b). Assuming a recessive MOI, there were 33 variants (30 SNPs and 3 indels) associated with HS-Aging pathology (each with $p < 1.0 \times 10^{-3}$) in this region, all of which are intronic. We selected four tag SNPs between exon 18 and 29 (Figure 3) of the *ABCC9* gene when assuming a recessive MOI.

The most frequent haplotypes were “Hap1” T-A-G-T (from 5’ to 3’) estimated to be present in 40.1% of observed chromosomes (32.1% in cases and 40.8% in controls), and “Hap2” C-C-A-C (36.8%; 43.7% in cases and 36.2% in controls). Hap1 was significantly associated with a lower risk of HS-Aging (score statistic = −2.747 and p = 0.0061) and Hap2 with a higher risk of HS-Aging (score statistic = 4.277 and $p = 3.3 \times 10^{-5}$).

3.4 Haplotype-based expression Quantitative Trait Locus (eQTL) association with ABCC9 gene expression

In haplotype-based association tests assuming an additive MOI, Hap1 was significantly associated with *ABCC9* gene expression in both datasets (p = 0.0026 in the NABEC and p = 0.024 in the UKBEC). Compared with the association with rs704180 only, Hap1 had a stronger association with *ABCC9* gene expression in the NABEC.

4. Discussion

In the large autopsy dataset derived from multiple research centers, we evaluated the genetic associations of four candidate genes (*GRN, TMEM106B, ABCC9*, and *KCNMB2*) for HS-Aging pathology. We found significant gene- and haplotype-based associations of the *ABCC9* gene with HS-Aging, and these approaches provide new insights into the other candidate genes and variants that are associated with HS-Aging. The haplotype made up of the risk alleles at the region (Hap2: C-C-A-C) was significantly overrepresented in HS-Aging cases, and thus could be a risk haplotype, while the opposite haplotype (Hap1: T-A-G-T) was significantly overrepresented in controls, and thus could be a protective risk factor. We further revealed that the protective haplotype (i.e., Hap1) was associated with down-regulation of *ABCC9* gene expression, and the results were consistent in two independent datasets.

Unlike the *TMEM106B* and *GRN* genes, the association between the *ABCC9* gene and FTLD-TDP has never been reported. That is, the *ABCC9* gene could potentially be a key gene on the distinction between FTLD-TDP and HS-Aging pathogenesis. The *ABCC9* gene encodes a transmembrane protein, a part of an ATP-sensitive potassium (K_{ATP}) channel complex. K_{ATP} channel consists of two distinct subunits: an inwardly rectifying K^+ channel (Kir6.x) and a regulatory sulfonylurea receptor (SURx) (Quast, et al., 2004). When the ATP levels drop due to hypoxia/ischemia or other stressor, vascular smooth muscle cell K_{ATP} channels open to increase K^+ efflux, voltage-activated calcium channels close to block Ca^{2+} entry, and in turn, vasodilatation is induced (Sun and Feng, 2013; Sun and Hu, 2010). Given the critical roles in regulation of vascular tone, K_{ATP} channel dysfunction may be involved in cardio- and cerebrovascular diseases. In mouse experiments, knock-out Kir6.1 (encoded immediately downstream from *ABCC9* on chromosome 12) and *Abcc9* led to hypertension, coronary artery vasospasm, and sudden cardiac death (Chutkow, et al., 2002; Miki, et al., 2002). In addition, Leverenz and colleagues found in their community-based study that HS-Aging cases were more likely to have history of stroke, small vessel disease, and hypertension than AD cases (Leverenz, et al., 2002). Our group also reported that brains with HS-Aging pathology tended to have arteriolosclerosis in multiple cortical and subcortical regions (Neltner, et al., 2014). We note that known mutations in the human

ABCC9 gene lead to a toxic gain of function ("Cantu syndrome") also are associated with human cerebrovascular pathology - a phenotype of "tortuous cerebral vessels" detected on neuroimaging (Leon Guerrero, et al., 2016). These prior studies imply that cerebrovascular factors might be involved in developing HS-Aging via the K_{ATP} channel-dependent activity (Nelson, et al., 2015a). In addition, we recently reported that human brain gene expressions that are triiodothyronine (T3) responsive were correlated with the *ABCC9* gene expression, and total T3 levels in cerebrospinal fluid (CSF) were significantly higher in HS-Aging cases than in controls (Nelson, et al., 2016a). Prior studies showed links between thyroid hormone (TH) levels and dementia (Annerbo and Lokk, 2013; Pasqualetti, et al., 2015; Rieben, et al., 2016), as well as TH levels and vascular diseases (Delitala, et al., 2015; Gao, et al., 2015; Sara, et al., 2015). Therefore it is possible that the *ABCC9* gene variants may help mediate links between TH dysregulation, cerebrovascular disease, and HS-Aging pathology.

The *TMEM106B* gene did not have a significant gene-based association with HS-Aging when applying the Bonferroni correction, but nominal significance was found assuming a recessive and an additive MOI. Van Deerlin and colleagues identified rs1990622 T-allele as a risk factor for FTLD with TDP inclusions (FTLD-TDP) (Van Deerlin, et al., 2010). Here we report that rs3823612, which is in strong LD with rs1990622 ($r^2 = 0.975$), is the variant on the *TMEM106B* gene that is most strongly associated with risk for HS-Aging pathology assuming a recessive and an additive MOI. However, there are 108 gene variants (96 SNPs and 12 indels) in near perfect LD with the top SNP rs3823612 over the gene (the range of r^2 was from 0.930 to 0.996). Of the 108 variants, rs3173615 is a missense variant on exon 6, rs6460901 is a splice region variant, rs2302634 and rs2302633 are non-coding transcript exon variants, 19 variants are 5' or 3' UTR variants, 10 variants are upstream or downstream gene variants, and the remaining variants are intronic. Yu and colleagues reported that rs1990622 A-allele was associated with more advanced TDP-43 pathology which is the dominant feature of HS-Aging (Yu, et al., 2015). TDP-43 is also a major disease protein of other neurodegenerative diseases including FTLD and amyotrophic lateral sclerosis (ALS) (Neumann, et al., 2006). Nicholson and colleagues showed that rs3173615 (missense variant on exon 6), dictating the amino acid at codon 185 of threonine (ACC: T185) or serine (AGC: S185), was associated with higher TMEM106B protein levels in *GRN* mutation carriers (Nicholson, et al., 2013). Aberrant TDP-43 immunoreactivity is seen in both HS-Aging and FTLD-TDP, and rs1990622 A-allele is reported to be a risk allele of both HS-Aging and FTLD-TDP. However, these two diseases differ in clinical symptoms and pathological characteristics (Ighodaro, et al., 2015; Nelson, et al., 2011b).

The SNP on the *KCNMB2* gene that was identified as a possible risk factor is rs9637454 (Beecham, et al., 2014), while in the current study we found that rs73183328 was the most strongly associated variant assuming an additive and a dominant MOI. Nominally significant gene-based association of the *KCNMB2* gene with HS-Aging were found assuming an additive and a dominant MOI, although the gene-based associations were not significant when applying the Bonferroni correction. The KCNMB2 protein is the transmembrane β2 subunit of the large-conductance Ca^{2+}- and voltage-activated K^+ (BK) channel. The channel is formed by poreforming α-subunit encoded on the *KCNMA1* gene (chromosome 10) and four β-subunits (β1 to β4) (Wu and Marx, 2010). The β2 subunit induces the BK channel inactivation with the coexpressed α-subunit leading to neuronal excitability by inhibiting K^+

currents (Wallner, et al., 1999). Since inactivating BK channels are found in CA1 hippocampal neurons (Hicks and Marrion, 1998), HS-Aging may be related to the *KCNMB2* gene via a process involving BK channel activation. It seems remarkable that both GWAS-identified putative HS-Aging risk genes (*ABCC9* and *KCNMB2*) encode proteins that modify potassium channels.

There are limitations in this study. Since NACC data are derived from ADCs, the study design is not population-based. Also, HS pathologic diagnoses vary across calendar time and ADCs. Thus, there was probably some misclassification of HS-Aging diagnosis. However, neuropathologic evaluation is the gold standard for HS diagnosis, and thus the problem of misclassification, while ever-present, was minimized as much as possible. We did not obtain dense genetic information on the *GRN* gene. The previously identified SNP rs5848 as a HS-Aging risk SNP was removed in the process of the QC due to high missing rate. Therefore, we could not evaluate the *GRN* gene well in this study.

In summary, we confirmed that the *ABCC9* gene had the significant gene-based association with HS-Aging when assuming a recessive MOI. The significant gene-based association of the *ABCC9* gene is driven by the region in which a significant haplotype-based association was found. Although we did not find statistically significant gene-based associations of the other three genes (i.e., *GRN, TMEM106B*, and *KCMNB2*) with HS-Aging in this study, it does not mean that these genes are not associated with HS-Aging. Single variants may independently affect HS-Aging pathology rather than the entire gene, or there may be interactions between these genes conferring HS-Aging risk via other mechanisms, such as TDP-43 proteinopathies or ion channel dysfunction. In the future, we plan to examine what role the intronic region of the *ABCC9* gene plays in developing HS-Aging pathology, and whether there are single variant-based and gene-based gene-gene interactions among these four genes to HS-Aging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- Gene-based association of the ABCC9 gene with HS-Aging is significant
- *ABCC9* gene-based association is driven by a region with significant haplotypes.
- Protective *ABCC9* haplotype is associated with decreased *ABCC9* expression.

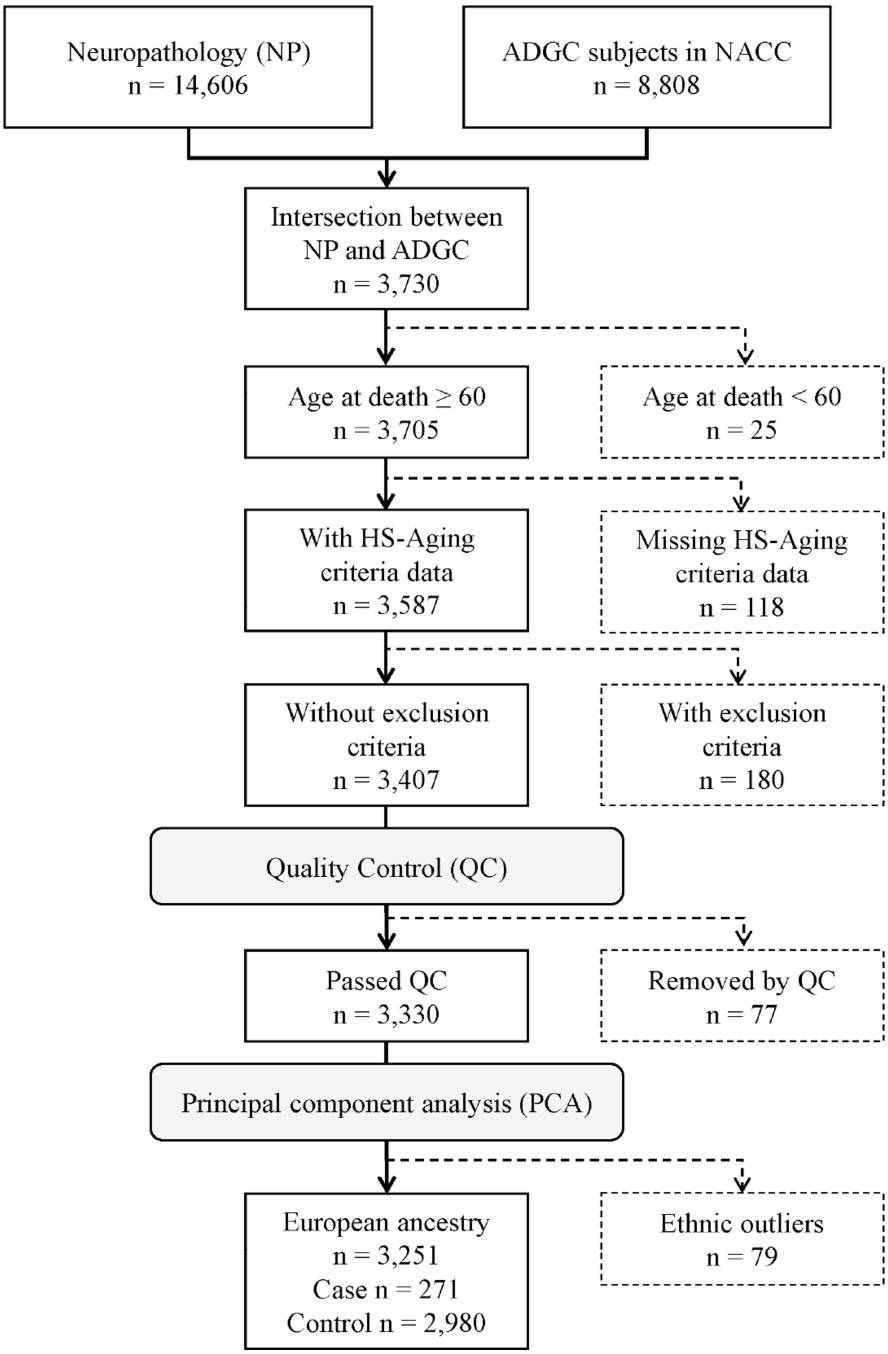


Figure 1.

Flow diagram of the subjects included in the analyses. Genetic data were obtained from subjects in ADGC who had the NACC individual IDs. Phenotype data were available from the neuropathological dataset in NACC. The inclusion/exclusion criteria, quality control and removal of ethnic outliers were applied in order.

Key: ADGC, Alzheimer's Disease Genetics Consortium; NACC, National Alzheimer's Coordinating Center; NP, neuropathological dataset; HS-Aging, hippocampal sclerosis of aging

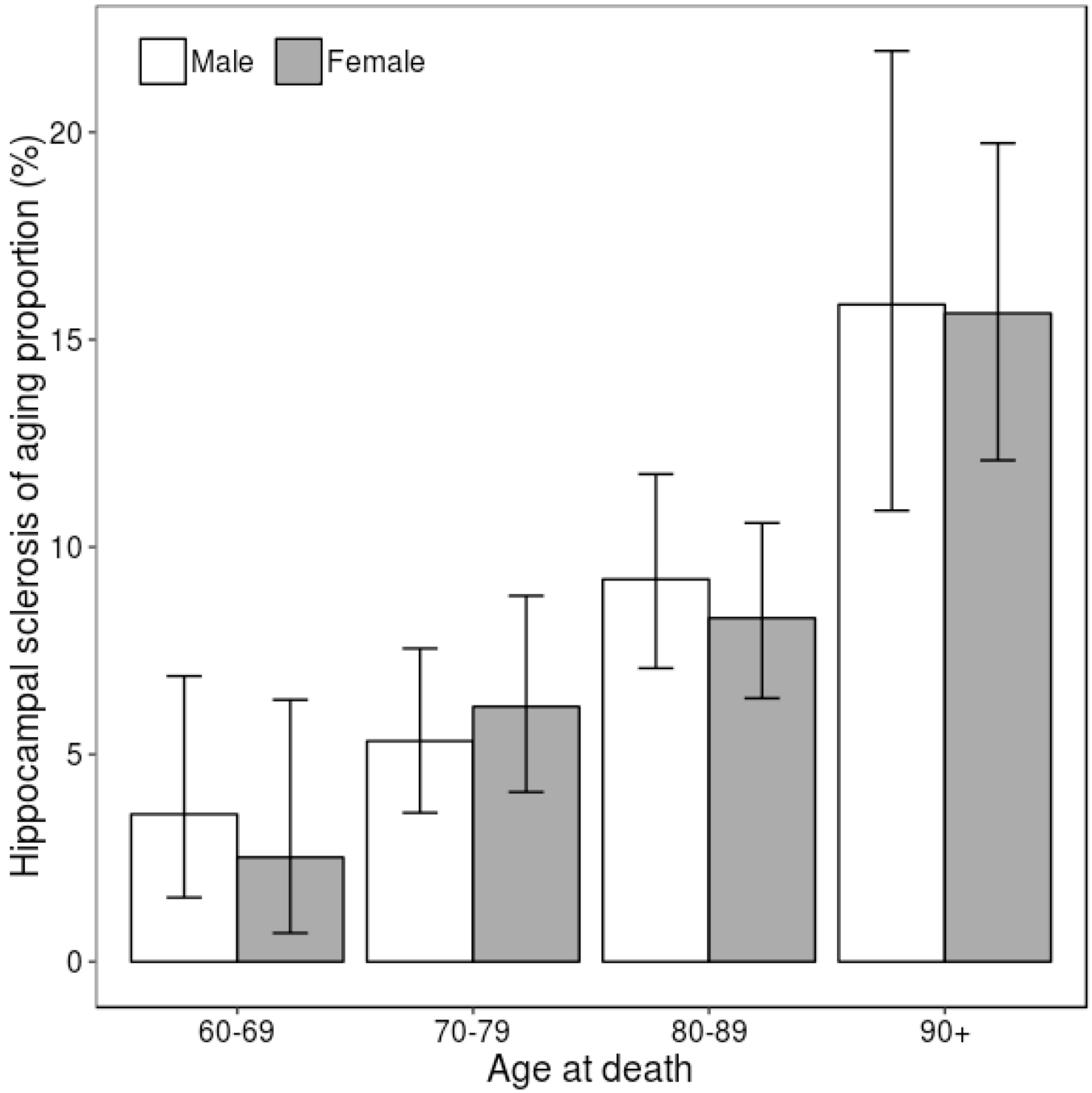
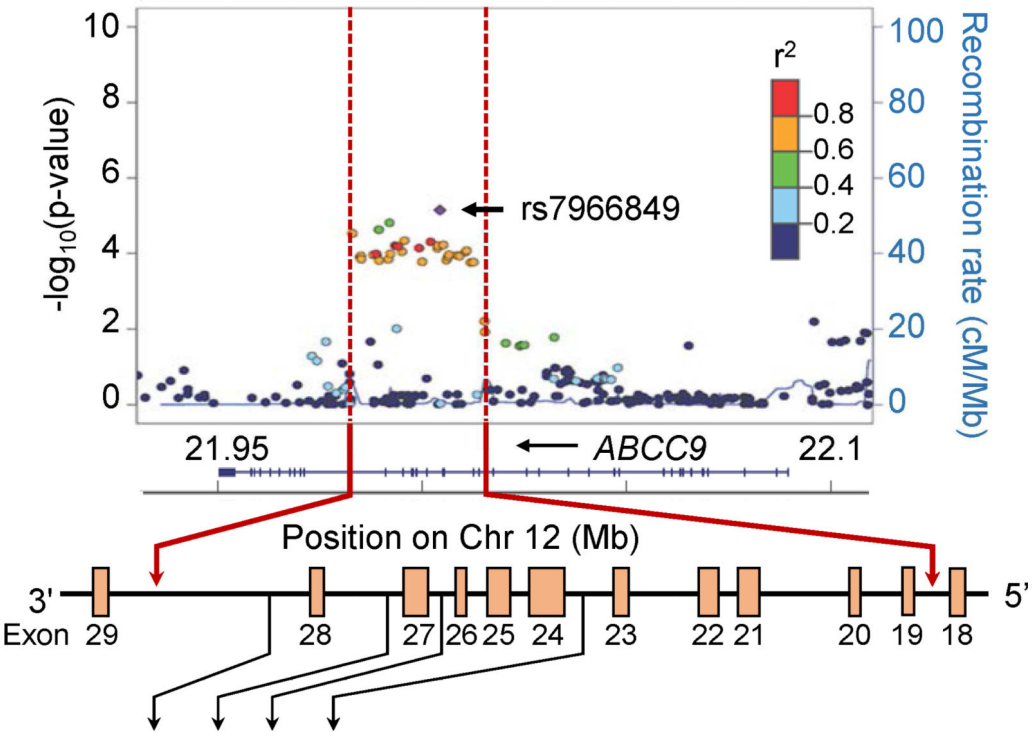


Figure 2.
Proportion and 95% confidence interval of hippocampal sclerosis of aging cases.



	rs2098574	rs704180	rs1643235	rs829080	Estimated haplotype frequency (%) Overall	Case	Control	Score statistic[a]	p-value[b]
Hap1	T	G	A	T	40.1	32.1	40.8	-2.747	0.0061
Hap2	C	A	C	C	36.8	43.7	36.2	4.277	3.3×10^{-5}
Hap3	T	A	A	C	9.9	10.5	9.8	-0.105	0.92
Hap4	T	G	A	C	8.2	9.1	8.1	-0.597	0.55
Hap5	T	A	C	C	4.7	4.4	4.7	-0.792	0.43
Global								23.267	0.0020

[a] Adjusted for age at death, sex and the top three principal components assuming recessive mode of inheritance. A positive sign indicates a risk haplotype for development of HS-Aging and vice versa.

[b] Monte-Carlo p-value from 10^7 replications

Figure 3.

Estimation of haplotype frequencies and association using four tag single nucleotide polymorphisms on the *ABCC9* gene when assuming a recessive mode of inheritance. Box indicates an exon.

Table 1

Comparison of selected characteristics between hippocampal sclerosis of aging cases and controls who died at age 60 years or older (n = 3,251)

Variable	Cases n = 271	Controls n = 2,980	p-value
Age at death, mean (SD)	84.8 (8.4)	80.5 (8.8)	<0.001
Sex, n (%)			
Male	124 (45.8)	1,458 (48.9)	0.349
Female	147 (54.2)	1,522 (51.1)	
APOE, n (%)[a]			
−/−	114 (46.0)	1,207 (44.1)	0.749
−/ε4	109 (43.9)	1,216 (44.4)	
ε4/ε4	25 (10.1)	314 (11.5)	
MAPT (rs8070723), n (%)[b]			
H1/H1	176 (66.2)	1,773 (60.1)	0.146
H1/H2	77 (28.9)	1,022 (34.7)	
H2/H2	13 (4.9)	154 (5.2)	

[a] *APOE* genotype information was available for n = 2,985.

[b] *MAPT* genotype information was available for n = 3,215.

Key: SD, standard deviation; *APOE*, apolipoprotein E; *MAPT*, microtubule-associated protein tau.

Table 2

Most associated variant with hippocampal sclerosis of aging in four genes using a logistic regression model assuming a recessive/additive/dominant mode of inheritance in people who died at age 60 years or older (n = 3,251)

Gene	MOI		Variant	Risk/protective alleles	RAF in cases	RAF in controls	OR (95% CI)[a]	p-value
GRN								
	REC		rs72824731	C/G	9.5	8.4	3.88 (1.64 – 9.22)	0.0021
	ADD	]	rs2879096	T/C	28.6	24.4	1.25 (1.02 – 1.53)	0.032
	DOM						1.38 (1.07 – 1.78)	0.014
TMEM106B								
	REC						1.53 (1.19 – 1.98)	0.0011
	ADD	]	rs3823612	G/C	64.6	56.5	1.40 (1.16 – 1.68)	3.6×10^{-4}
	DOM		rs13229988	A/G	64.0	56.4	1.67 (1.16 – 2.40)	0.0062
ABCC9								
	REC						1.84 (1.41 – 2.40)	7.1×10^{-6}
	ADD	]	rs7966849	A/G	60.3	51.2	1.46 (1.22 – 1.76)	4.4×10^{-5}
	DOM		rs829080	C/T	59.1	40.9	1.76 (1.18 – 2.62)	0.0057
KCNMB2								
	REC		rs13091964	T/C	96.1	92.9	1.84 (1.15 – 2.96)	0.011
	ADD	]	rs73183328	A/G	5.0	2.2	2.42 (1.56 – 3.76)	8.2×10^{-5}
	DOM						2.40 (1.52 – 3.78)	1.6×10^{-4}

[a] Adjusted for age at death, sex and the top three principal components

Key: MOI, mode of inheritance; RAF, risk allele frequency; OR, odds ratio; CI, confidence interval; REC, recessive; ADD, additive; DOM, dominant.

Table 3

Gene-based associations of the target four genes with hippocampal sclerosis of aging assuming a recessive/additive/dominant mode of inheritance in people who died at age 60 years or older (n = 3,251)

Gene	# of variants	Start position	End position	Gene-based p-value		
				REC	ADD	DOM
GRN	20	42,417,491	42,435,470	0.012	0.16	0.090
TMEM106B	222	12,245,848	12,281,890	0.028	0.0089	0.068
ABCC9	259	21,945,324	22,094,628	2.4×10^{-4}	0.0014	0.26
KCNMB2	939	178,249,224	178,567,217	0.57	0.0079	0.016

Key: REC, recessive; ADD, additive; DOM, dominant

Table 4

Haplotype association with *ABCC9* gene expression in human brain assuming an additive mode of inheritance

	NABEC (Frontal cortex; n = 130 brains)		UKBEC (10 brain regions; n = 134 brains)	
	Score statistic[a]	p-value	Score statistic[a]	p-value
Hap1	−2.968	0.0026	−2.250	0.024
Hap2	1.450	0.15	1.740	0.081
Hap3	1.686	0.091	−0.048	0.96
Hap4	0.214	0.83	−0.822	0.41
Hap5	0.878	0.38	1.952	0.051
Global	10.255	0.034	8.455	0.074
rs704180 only		0.010		0.011

[a] A positive sign indicates up-regression of *ABCC9* gene expression and vice versa.

Key: NABEC = North American Brain Expression Consortium (GEO accession: GSE36192); UKBEC = United Kingdom Brain Expression Consortium (http://www.braineac.org/).