Title

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

Yuriko Katsumata ^a, Peter T. Nelson ^{b,c}, Sally R. Ellingson ^d, David W. Fardo ^{a,b}

^a Department of Biostatistics, University of Kentucky, Lexington, KY 40536-0082, USA

^b Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536, USA

^c Department of Pathology, University of Kentucky, Lexington, KY 40536, USA

^d Division of Biomedical Informatics, University of Kentucky, Lexington, KY 40536, USA

E-mail

Yuriko Katsumata: katsumata.yuriko@uky.edu

Peter T. Nelson: pnels2@uky.edu

Sally R. Ellingson: sally@kcr.uky.edu

David W. Fardo: david.fardo@uky.edu

Corresponding author

David W. Fardo

205E Multidisciplinary Science Building

725 Rose Street, Lexington, KY 40536-0082

david.fardo@uky.edu

+1 859-218-2070

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 (maximum 6)

CARTS, GWAS, PGRN, KATP, rs704180

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 amnesic 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*) ϵ 4 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 FTLN with ubiquitin-positive inclusions, FTLN with no distinctive histopathology, FTLN-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 $\pm$ 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 ± 5 kb 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* $\epsilon 4$ 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 $\pm 5\text{kb}$ 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 FTLTDP has never been reported. That is, the *ABCC9* gene could potentially be a key gene on the distinction between FTLTDP 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 FTLT with TDP inclusions (FTLT-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 FTLN 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 FTLN-TDP, and rs1990622 A-allele is reported to be a risk allele of both HS-Aging and FTLN-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 $\beta 2$ subunit of the large-

conductance Ca^{2+} - and voltage-activated K^+ (BK) channel. The channel is formed by pore-forming α -subunit encoded on the *KCNMA1* gene (chromosome 10) and four β -subunits ($\beta 1$ to $\beta 4$) (Wu and Marx, 2010). The $\beta 2$ 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.

Disclosure statement

The authors declare no conflicts of interest.

Funding

This work was supported by the National Cell Repository for Alzheimer's Disease (U24 AG21886), and National Institute on Aging (K25 AG043546, UL1TR000117, and the UK-ADC P30 AG028383).

Acknowledgments

The NACC database is funded by NIA/NIH Grant U01 AG016976. NACC data are contributed by the NIA funded ADCs: P30 AG019610 (PI Eric Reiman, MD), P30 AG013846 (PI Neil Kowall, MD), P50 AG008702 (PI Scott Small, MD), P50 AG025688 (PI Allan Levey, MD, PhD), P50 AG047266 (PI Todd Golde, MD, PhD), P30 AG010133 (PI Andrew Saykin, PsyD), P50 AG005146 (PI Marilyn Albert, PhD), P50 AG005134 (PI Bradley Hyman, MD, PhD), P50 AG016574 (PI Ronald Petersen, MD, PhD), P50 AG005138 (PI Mary Sano, PhD), P30 AG008051 (PI Steven Ferris, PhD), P30 AG013854 (PI M. Marsel Mesulam, MD), P30 AG008017 (PI Jeffrey Kaye, MD), P30 AG010161 (PI David Bennett, MD), P50 AG047366 (PI Victor Henderson, MD, MS), P30 AG010129 (PI Charles DeCarli, MD), P50 AG016573 (PI Frank LaFerla, PhD), P50 AG016570 (PI Marie-Francoise Chesselet, MD, PhD), P50 AG005131 (PI Douglas Galasko, MD), P50 AG023501 (PI Bruce Miller, MD), P30 AG035982 (PI Russell Swerdlow, MD), P30 AG028383 (PI Linda Van Eldik, PhD), P30 AG010124 (PI John Trojanowski, MD, PhD), P50 AG005133 (PI Oscar Lopez, MD), P50 AG005142 (PI Helena Chui, MD), P30 AG012300 (PI Roger Rosenberg, MD), P50 AG005136 (PI Thomas Montine, MD, PhD), P50 AG033514 (PI Sanjay Asthana, MD, FRCP), P50 AG005681 (PI John Morris, MD), and P50 AG047270 (PI Stephen Strittmatter, MD, PhD). The National Institutes of Health, National Institute on Aging (NIH-NIA) supported this work through the following grants: ADGC, U01 AG032984, RC2 AG036528; NACC, U01 AG016976; NCRAD, U24 AG021886; NIA LOAD, U24 AG026395, R01AG041797; Data for this study were prepared, archived, and distributed by the National Institute on Aging Alzheimer's Disease Data Storage Site (NIAGADS) at the University of Pennsylvania (U24-AG041689-01); Banner Sun Health Research Institute P30 AG019610; Boston University, P30 AG013846, U01 AG10483, R01 CA129769, R01 MH080295, R01 AG017173, R01 AG025259, R01AG33193; Columbia University, P50 AG008702, R37 AG015473; Duke University, P30 AG028377, AG05128; Emory University, AG025688; Group Health Research Institute, U01 AG006781, U01 HG004610, U01 HG006375; Indiana University, P30 AG10133; Johns Hopkins University, P50 AG005146, R01 AG020688; Massachusetts General Hospital, P50 AG005134; Mayo Clinic, P50 AG016574; Mount Sinai School of Medicine, P50 AG005138, P01 AG002219; New

York University, P30 AG08051, UL1 RR029893, 5R01AG012101, 5R01AG022374, 5R01AG013616, 1RC2AG036502, 1R01AG035137; Northwestern University, P30 AG013854; Oregon Health & Science University, P30 AG008017, R01 AG026916; Rush University, P30 AG010161, R01 AG019085, R01 AG15819, R01 AG17917, R01 AG30146; TGen, R01 NS059873; University of Alabama at Birmingham, P50 AG016582; University of Arizona, R01 AG031581; University of California, Davis, P30 AG010129; University of California, Irvine, P50 AG016573; University of California, Los Angeles, P50 AG016570; University of California, San Diego, P50 AG005131; University of California, San Francisco, P50 AG023501, P01 AG019724; University of Kentucky, P30 AG028383, AG05144; University of Michigan, P50 AG008671; University of Pennsylvania, P30 AG010124; University of Pittsburgh, P50 AG005133, AG030653, AG041718, AG07562, AG02365; University of Southern California, P50 AG005142; University of Texas Southwestern, P30 AG012300; University of Miami, R01 AG027944, AG010491, AG027944, AG021547, AG019757; University of Washington, P50 AG005136; University of Wisconsin, P50 AG033514; Vanderbilt University, R01 AG019085; and Washington University, P50 AG005681, P01 AG03991. The Kathleen Price Bryan Brain Bank at Duke University Medical Center is funded by NINDS grant # NS39764, NIMH MH60451 and by Glaxo Smith Kline. Genotyping of the TGEN2 cohort was supported by Kronos Science. The TGen series was also funded by NIA grant AG041232 to AJM and MJH, The Banner Alzheimer's Foundation, The Johnnie B. Byrd Sr. Alzheimer's Institute, the Medical Research Council, and the state of Arizona and also includes samples from the following sites: Newcastle Brain Tissue Resource (funding via the Medical Research Council, local NHS trusts and Newcastle University), MRC London Brain Bank for Neurodegenerative Diseases (funding via the Medical Research Council), South West Dementia Brain Bank (funding via numerous sources including the Higher Education Funding Council for England (HEFCE), Alzheimer's Research Trust (ART), BRACE as well as North Bristol NHS Trust Research and Innovation Department and DeNDRoN), The Netherlands Brain Bank (funding via numerous sources including Stichting MS Research, Brain Net Europe, Hersenstichting Nederland Breinbrekend Werk, International Parkinson Fonds, Internationale Stichting Alzheimer Onderzoek), Institut de Neuropatologia, Servei Anatomia Patologica, Universitat de Barcelona. ADNI data collection and sharing was funded by the National Institutes of Health Grant U01 AG024904 and Department of Defense award number W81XWH-12-2-0012. ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease

Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California. We thank Drs. D. Stephen Snyder and Marilyn Miller from NIA who are ex-officio ADGC members. Support was also from the Alzheimer's Association (LAF, IIRG-08-89720; MP-V, IIRG-05-14147) and the US Department of Veterans Affairs Administration, Office of Research and Development, Biomedical Laboratory Research Program. P.S.G.-H. is supported by Wellcome Trust, Howard Hughes Medical Institute, and the Canadian Institute of Health Research. Funding support for the "Brain eQTL (expression data) Study" was provided through the Division of Aging Biology and the Division of Geriatrics and Clinical Gerontology, NIA. The Brain eQTL (expression data) Study includes a genome-wide association study funded as part of the Intramural Research Program, NIA.

References

- 1000 Genomes Project Consortium. 2010. A map of human genome variation from population-scale sequencing. *Nature* 467(7319), 1061-1073. doi:10.1038/nature09534.
- Amador-Ortiz, C., Lin, W.L., Ahmed, Z., Personett, D., Davies, P., Duara, R., Graff-Radford, N.R., Hutton, M.L., Dickson, D.W., 2007. TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Ann Neurol* 61(5), 435-445. doi:10.1002/ana.21154.
- Annerbo, S., Løkke, J., 2013. A clinical review of the association of thyroid stimulating hormone and cognitive impairment. *ISRN Endocrinol* 2013, 856017. doi:10.1155/2013/856017.
- Aoki, N., Murray, M.E., Ogaki, K., Fujioka, S., Rutherford, N.J., Rademakers, R., Ross, O.A., Dickson, D.W., 2015. Hippocampal sclerosis in Lewy body disease is a TDP-43 proteinopathy similar to FTLTD-TDP Type A. *Acta Neuropathol* 129(1), 53-64. doi:10.1007/s00401-014-1358-z.
- Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21(2), 263-265. doi:10.1093/bioinformatics/bth457.
- Beecham, G.W., Hamilton, K., Naj, A.C., Martin, E.R., Huentelman, M., Myers, A.J., Corneveaux, J.J., Hardy, J., Vonsattel, J.P., Younkin, S.G., Bennett, D.A., De Jager, P.L., Larson, E.B., Crane, P.K., Kamboh, M.I., Kofler, J.K., Mash, D.C., Duque, L., Gilbert, J.R., Gwirtsman, H., Buxbaum, J.D., Kramer, P., Dickson, D.W., Farrer, L.A., Frosch, M.P., Ghetti, B., Haines, J.L., Hyman, B.T., Kukull, W.A., Mayeux, R.P., Pericak-Vance, M.A., Schneider, J.A., Trojanowski, J.Q., Reiman, E.M., Alzheimer's Disease Genetics, C., Schellenberg, G.D., Montine, T.J., 2014. Genome-wide association meta-analysis of neuropathologic features of Alzheimer's disease and related dementias. *PLoS Genet* 10(9), e1004606. doi:10.1371/journal.pgen.1004606.

- Brenowitz, W.D., Monsell, S.E., Schmitt, F.A., Kukull, W.A., Nelson, P.T., 2014. Hippocampal sclerosis of aging is a key Alzheimer's disease mimic: clinical-pathologic correlations and comparisons with both alzheimer's disease and non-tauopathic frontotemporal lobar degeneration. *J Alzheimers Dis* 39(3), 691-702. doi:10.3233/JAD-131880.
- Chutkow, W.A., Pu, J., Wheeler, M.T., Wada, T., Makielski, J.C., Burant, C.F., McNally, E.M., 2002. Episodic coronary artery vasospasm and hypertension develop in the absence of Sur2 K(ATP) channels. *J Clin Invest* 110(2), 203-208. doi:10.1172/JCI15672.
- Corey-Bloom, J., Sabbagh, M.N., Bondi, M.W., Hansen, L., Alford, M.F., Masliah, E., Thal, L.J., 1997. Hippocampal sclerosis contributes to dementia in the elderly. *Neurology* 48(1), 154-160.
- Das, S., Forer, L., Schonherr, S., Sidore, C., Locke, A.E., Kwong, A., Vrieze, S.I., Chew, E.Y., Levy, S., McGue, M., Schlessinger, D., Stambolian, D., Loh, P.R., Iacono, W.G., Swaroop, A., Scott, L.J., Cucca, F., Kronenberg, F., Boehnke, M., Abecasis, G.R., Fuchsberger, C., 2016. Next-generation genotype imputation service and methods. *Nat Genet* 48(10), 1284-1287. doi:10.1038/ng.3656.
- Delitala, A.P., Orru, M., Filigheddu, F., Pilia, M.G., Delitala, G., Ganau, A., Saba, P.S., Decandia, F., Scuteri, A., Marongiu, M., Lakatta, E.G., Strait, J., Cucca, F., 2015. Serum free thyroxine levels are positively associated with arterial stiffness in the SardiNIA study. *Clin Endocrinol (Oxf)* 82(4), 592-597. doi:10.1111/cen.12532.
- Dickson, D.W., Baker, M., Rademakers, R., 2010. Common variant in GRN is a genetic risk factor for hippocampal sclerosis in the elderly. *Neurodegener Dis* 7(1-3), 170-174. doi:10.1159/000289231.

- Dickson, D.W., Davies, P., Bevona, C., Van Hoeven, K.H., Factor, S.M., Grober, E., Aronson, M.K., Crystal, H.A., 1994. Hippocampal sclerosis: a common pathological feature of dementia in very old ($> \text{ or } = 80$ years of age) humans. *Acta Neuropathol* 88(3), 212-221.
- Gao, C.X., Yang, B., Guo, Q., Wei, L.H., Tian, L.M., 2015. High thyroid-stimulating hormone level is associated with the risk of developing atherosclerosis in subclinical hypothyroidism. *Horm Metab Res* 47(3), 220-224. doi:10.1055/s-0034-1394370.
- Hernandez, D.G., Nalls, M.A., Moore, M., Chong, S., Dillman, A., Trabzuni, D., Gibbs, J.R., Ryten, M., Arepalli, S., Weale, M.E., Zonderman, A.B., Troncoso, J., O'Brien, R., Walker, R., Smith, C., Bandinelli, S., Traynor, B.J., Hardy, J., Singleton, A.B., Cookson, M.R., 2012. Integration of GWAS SNPs and tissue specific expression profiling reveal discrete eQTLs for human traits in blood and brain. *Neurobiol Dis* 47(1), 20-28. doi:10.1016/j.nbd.2012.03.020.
- Hicks, G.A., Marrion, N.V., 1998. Ca^{2+} -dependent inactivation of large conductance Ca^{2+} -activated K^{+} (BK) channels in rat hippocampal neurones produced by pore block from an associated particle. *J Physiol* 508 (Pt 3), 721-734.
- Hyman, B.T., Phelps, C.H., Beach, T.G., Bigio, E.H., Cairns, N.J., Carrillo, M.C., Dickson, D.W., Duyckaerts, C., Frosch, M.P., Masliah, E., Mirra, S.S., Nelson, P.T., Schneider, J.A., Thal, D.R., Thies, B., Trojanowski, J.Q., Vinters, H.V., Montine, T.J., 2012. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimers Dement* 8(1), 1-13. doi:10.1016/j.jalz.2011.10.007.
- Ighodaro, E.T., Jicha, G.A., Schmitt, F.A., Neltner, J.H., Abner, E.L., Kryscio, R.J., Smith, C.D., Duplessis, T., Anderson, S., Patel, E., Bachstetter, A., Van Eldik, L.J., Nelson, P.T., 2015.

- Hippocampal Sclerosis of Aging Can Be Segmental: Two Cases and Review of the Literature. *J Neuropathol Exp Neurol* 74(7), 642-652.
doi:10.1097/NEN.0000000000000204.
- Kent, W.J., Sugnet, C.W., Furey, T.S., Roskin, K.M., Pringle, T.H., Zahler, A.M., Haussler, D., 2002. The human genome browser at UCSC. *Genome Res* 12(6), 996-1006.
doi:10.1101/gr.229102. Article published online before print in May 2002.
- Leon Guerrero, C.R., Pathak, S., Grange, D.K., Singh, G.K., Nichols, C.G., Lee, J.M., Vo, K.D., 2016. Neurologic and neuroimaging manifestations of Cantu syndrome: A case series. *Neurology* 87(3), 270-276. doi:10.1212/WNL.0000000000002861.
- Leverenz, J.B., Agustin, C.M., Tsuang, D., Peskind, E.R., Edland, S.D., Nochlin, D., DiGiacomo, L., Bowen, J.D., McCormick, W.C., Teri, L., Raskind, M.A., Kukull, W.A., Larson, E.B., 2002. Clinical and neuropathological characteristics of hippocampal sclerosis: a community-based study. *Arch Neurol* 59(7), 1099-1106.
- Li, M.X., Gui, H.S., Kwan, J.S., Sham, P.C., 2011. GATES: a rapid and powerful gene-based association test using extended Simes procedure. *Am J Hum Genet* 88(3), 283-293.
doi:10.1016/j.ajhg.2011.01.019.
- Loh, P.R., Danecek, P., Palamara, P.F., Fuchsberger, C., Y, A.R., H, K.F., Schoenherr, S., Forer, L., McCarthy, S., Abecasis, G.R., Durbin, R., A, L.P., 2016. Reference-based phasing using the Haplotype Reference Consortium panel. *Nat Genet* 48(11), 1443-1448.
doi:10.1038/ng.3679.
- Miki, T., Suzuki, M., Shibasaki, T., Uemura, H., Sato, T., Yamaguchi, K., Koseki, H., Iwanaga, T., Nakaya, H., Seino, S., 2002. Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. *Nat Med* 8(5), 466-472. doi:10.1038/nm0502-466.

- Murray, M.E., Cannon, A., Graff-Radford, N.R., Liesinger, A.M., Rutherford, N.J., Ross, O.A., Duara, R., Carrasquillo, M.M., Rademakers, R., Dickson, D.W., 2014. Differential clinicopathologic and genetic features of late-onset amnesic dementias. *Acta Neuropathol* 128(3), 411-421. doi:10.1007/s00401-014-1302-2.
- Nelson, P.T., Estus, S., Abner, E.L., Parikh, I., Malik, M., Neltner, J.H., Ighodaro, E., Wang, W.X., Wilfred, B.R., Wang, L.S., Kukull, W.A., Nandakumar, K., Farman, M.L., Poon, W.W., Corrada, M.M., Kawas, C.H., Cribbs, D.H., Bennett, D.A., Schneider, J.A., Larson, E.B., Crane, P.K., Valladares, O., Schmitt, F.A., Kryscio, R.J., Jicha, G.A., Smith, C.D., Scheff, S.W., Sonnen, J.A., Haines, J.L., Pericak-Vance, M.A., Mayeux, R., Farrer, L.A., Van Eldik, L.J., Horbinski, C., Green, R.C., Gearing, M., Poon, L.W., Kramer, P.L., Woltjer, R.L., Montine, T.J., Partch, A.B., Rajic, A.J., Richmire, K., Monsell, S.E., Alzheimer' Disease Genetic, C., Schellenberg, G.D., Fardo, D.W., 2014. ABCC9 gene polymorphism is associated with hippocampal sclerosis of aging pathology. *Acta Neuropathol* 127(6), 825-843. doi:10.1007/s00401-014-1282-2.
- Nelson, P.T., Head, E., Schmitt, F.A., Davis, P.R., Neltner, J.H., Jicha, G.A., Abner, E.L., Smith, C.D., Van Eldik, L.J., Kryscio, R.J., Scheff, S.W., 2011a. Alzheimer's disease is not "brain aging": neuropathological, genetic, and epidemiological human studies. *Acta Neuropathol* 121(5), 571-587. doi:10.1007/s00401-011-0826-y.
- Nelson, P.T., Jicha, G.A., Wang, W.X., Ighodaro, E., Artiushin, S., Nichols, C.G., Fardo, D.W., 2015a. ABCC9/SUR2 in the brain: Implications for hippocampal sclerosis of aging and a potential therapeutic target. *Ageing Res Rev* 24(Pt B), 111-125. doi:10.1016/j.arr.2015.07.007.

- Nelson, P.T., Katsumata, Y., Nho, K., Artiushin, S.C., Jicha, G.A., Wang, W.X., Abner, E.L., Saykin, A.J., Kukull, W.A., Alzheimer's Disease Neuroimaging, I., Fardo, D.W., 2016a. Genomics and CSF analyses implicate thyroid hormone in hippocampal sclerosis of aging. *Acta Neuropathol* 132(6), 841-858. doi:10.1007/s00401-016-1641-2.
- Nelson, P.T., Schmitt, F.A., Lin, Y., Abner, E.L., Jicha, G.A., Patel, E., Thomason, P.C., Neltner, J.H., Smith, C.D., Santacruz, K.S., Sonnen, J.A., Poon, L.W., Gearing, M., Green, R.C., Woodard, J.L., Van Eldik, L.J., Kryscio, R.J., 2011b. Hippocampal sclerosis in advanced age: clinical and pathological features. *Brain* 134(Pt 5), 1506-1518. doi:10.1093/brain/awr053.
- Nelson, P.T., Smith, C.D., Abner, E.L., Wilfred, B.J., Wang, W.X., Neltner, J.H., Baker, M., Fardo, D.W., Kryscio, R.J., Scheff, S.W., Jicha, G.A., Jellinger, K.A., Van Eldik, L.J., Schmitt, F.A., 2013. Hippocampal sclerosis of aging, a prevalent and high-morbidity brain disease. *Acta Neuropathol* 126(2), 161-177. doi:10.1007/s00401-013-1154-1.
- Nelson, P.T., Trojanowski, J.Q., Abner, E.L., Al-Janabi, O.M., Jicha, G.A., Schmitt, F.A., Smith, C.D., Fardo, D.W., Wang, W.X., Kryscio, R.J., Neltner, J.H., Kukull, W.A., Cykowski, M.D., Van Eldik, L.J., Ighodaro, E.T., 2016b. "New Old Pathologies": AD, PART, and Cerebral Age-Related TDP-43 With Sclerosis (CARTS). *J Neuropathol Exp Neurol* 75(6), 482-498. doi:10.1093/jnen/nlw033.
- Nelson, P.T., Wang, W.X., Partch, A.B., Monsell, S.E., Valladares, O., Ellingson, S.R., Wilfred, B.R., Naj, A.C., Wang, L.S., Kukull, W.A., Fardo, D.W., 2015b. Reassessment of risk genotypes (GRN, TMEM106B, and ABCC9 variants) associated with hippocampal sclerosis of aging pathology. *J Neuropathol Exp Neurol* 74(1), 75-84. doi:10.1097/NEN.0000000000000151.

- Neltner, J.H., Abner, E.L., Baker, S., Schmitt, F.A., Kryscio, R.J., Jicha, G.A., Smith, C.D., Hammack, E., Kukull, W.A., Brenowitz, W.D., Van Eldik, L.J., Nelson, P.T., 2014. Arteriolosclerosis that affects multiple brain regions is linked to hippocampal sclerosis of ageing. *Brain* 137(Pt 1), 255-267. doi:10.1093/brain/awt318.
- Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M., McCluskey, L.F., Miller, B.L., Masliah, E., Mackenzie, I.R., Feldman, H., Feiden, W., Kretzschmar, H.A., Trojanowski, J.Q., Lee, V.M., 2006. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314(5796), 130-133. doi:10.1126/science.1134108.
- Nicholson, A.M., Finch, N.A., Wojtas, A., Baker, M.C., Perkerson, R.B., 3rd, Castanedes-Casey, M., Rousseau, L., Benussi, L., Binetti, G., Ghidoni, R., Hsiung, G.Y., Mackenzie, I.R., Finger, E., Boeve, B.F., Ertekin-Taner, N., Graff-Radford, N.R., Dickson, D.W., Rademakers, R., 2013. TMEM106B p.T185S regulates TMEM106B protein levels: implications for frontotemporal dementia. *J Neurochem* 126(6), 781-791. doi:10.1111/jnc.12329.
- Pao, W.C., Dickson, D.W., Crook, J.E., Finch, N.A., Rademakers, R., Graff-Radford, N.R., 2011. Hippocampal sclerosis in the elderly: genetic and pathologic findings, some mimicking Alzheimer disease clinically. *Alzheimer Dis Assoc Disord* 25(4), 364-368. doi:10.1097/WAD.0b013e31820f8f50.
- Pasqualetti, G., Pagano, G., Rengo, G., Ferrara, N., Monzani, F., 2015. Subclinical Hypothyroidism and Cognitive Impairment: Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab* 100(11), 4240-4248. doi:10.1210/jc.2015-2046.

- Pickering-Brown, S.M., Rollinson, S., Du Plessis, D., Morrison, K.E., Varma, A., Richardson, A.M., Neary, D., Snowden, J.S., Mann, D.M., 2008. Frequency and clinical characteristics of progranulin mutation carriers in the Manchester frontotemporal lobar degeneration cohort: comparison with patients with MAPT and no known mutations. *Brain* 131(Pt 3), 721-731. doi:10.1093/brain/awm331.
- Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A., Reich, D., 2006. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 38(8), 904-909. doi:10.1038/ng1847.
- Pruim, R.J., Welch, R.P., Sanna, S., Teslovich, T.M., Chines, P.S., Gliedt, T.P., Boehnke, M., Abecasis, G.R., Willer, C.J., 2010. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* 26(18), 2336-2337. doi:10.1093/bioinformatics/btq419.
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81(3), 559-575. doi:10.1086/519795.
- Quast, U., Stephan, D., Bieger, S., Russ, U., 2004. The impact of ATP-sensitive K⁺ channel subtype selectivity of insulin secretagogues for the coronary vasculature and the myocardium. *Diabetes* 53 Suppl 3, S156-164.
- Rademakers, R., Eriksen, J.L., Baker, M., Robinson, T., Ahmed, Z., Lincoln, S.J., Finch, N., Rutherford, N.J., Crook, R.J., Josephs, K.A., Boeve, B.F., Knopman, D.S., Petersen, R.C., Parisi, J.E., Caselli, R.J., Wszolek, Z.K., Uitti, R.J., Feldman, H., Hutton, M.L., Mackenzie, I.R., Graff-Radford, N.R., Dickson, D.W., 2008. Common variation in the

- miR-659 binding-site of GRN is a major risk factor for TDP43-positive frontotemporal dementia. *Hum Mol Genet* 17(23), 3631-3642. doi:10.1093/hmg/ddn257.
- Rieben, C., Segna, D., da Costa, B.R., Collet, T.H., Chaker, L., Aubert, C.E., Baumgartner, C., Almeida, O.P., Hogervorst, E., Trompet, S., Masaki, K., Mooijaart, S.P., Gussekloo, J., Peeters, R.P., Bauer, D.C., Aujesky, D., Rodondi, N., 2016. Subclinical Thyroid Dysfunction and the Risk of Cognitive Decline: a Meta-Analysis of Prospective Cohort Studies. *J Clin Endocrinol Metab* 101(12), 4945-4954. doi:10.1210/jc.2016-2129.
- Rutherford, N.J., Carrasquillo, M.M., Li, M., Bisceglia, G., Menke, J., Josephs, K.A., Parisi, J.E., Petersen, R.C., Graff-Radford, N.R., Younkin, S.G., Dickson, D.W., Rademakers, R., 2012. TMEM106B risk variant is implicated in the pathologic presentation of Alzheimer disease. *Neurology* 79(7), 717-718. doi:10.1212/WNL.0b013e318264e3ac.
- Sara, J.D., Zhang, M., Gharib, H., Lerman, L.O., Lerman, A., 2015. Hypothyroidism Is Associated With Coronary Endothelial Dysfunction in Women. *J Am Heart Assoc* 4(8), e002225. doi:10.1161/JAHA.115.002225.
- Schaid, D.J., Rowland, C.M., Tines, D.E., Jacobson, R.M., Poland, G.A., 2002. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 70(2), 425-434. doi:10.1086/338688.
- Sinnwell, J.P., Schaid, D.J., 2016. haplo.stats: Statistical Analysis of Haplotypes with Traits and Covariates when Linkage Phase is Ambiguous. R package version 1.7.7. <https://CRAN.R-project.org/package=haplo.stats>.
- Sun, H.S., Feng, Z.P., 2013. Neuroprotective role of ATP-sensitive potassium channels in cerebral ischemia. *Acta Pharmacol Sin* 34(1), 24-32. doi:10.1038/aps.2012.138.

- Sun, X.L., Hu, G., 2010. ATP-sensitive potassium channels: a promising target for protecting neurovascular unit function in stroke. *Clin Exp Pharmacol Physiol* 37(2), 243-252.
doi:10.1111/j.1440-1681.2009.05190.x.
- Trabzuni, D., Ryten, M., Walker, R., Smith, C., Imran, S., Ramasamy, A., Weale, M.E., Hardy, J., 2011. Quality control parameters on a large dataset of regionally dissected human control brains for whole genome expression studies. *J Neurochem* 119(2), 275-282.
doi:10.1111/j.1471-4159.2011.07432.x.
- Troncoso, J.C., Kawas, C.H., Chang, C.K., Folstein, M.F., Hedreen, J.C., 1996. Lack of association of the apoE4 allele with hippocampal sclerosis dementia. *Neurosci Lett* 204(1-2), 138-140.
- Van Deerlin, V.M., Sleiman, P.M., Martinez-Lage, M., Chen-Plotkin, A., Wang, L.S., Graff-Radford, N.R., Dickson, D.W., Rademakers, R., Boeve, B.F., Grossman, M., Arnold, S.E., Mann, D.M., Pickering-Brown, S.M., Seelaar, H., Heutink, P., van Swieten, J.C., Murrell, J.R., Ghetti, B., Spina, S., Grafman, J., Hodges, J., Spillantini, M.G., Gilman, S., Lieberman, A.P., Kaye, J.A., Woltjer, R.L., Bigio, E.H., Mesulam, M., Al-Sarraj, S., Troakes, C., Rosenberg, R.N., White, C.L., 3rd, Ferrer, I., Llado, A., Neumann, M., Kretschmar, H.A., Hulette, C.M., Welsh-Bohmer, K.A., Miller, B.L., Alzualde, A., Lopez de Munain, A., McKee, A.C., Gearing, M., Levey, A.I., Lah, J.J., Hardy, J., Rohrer, J.D., Lashley, T., Mackenzie, I.R., Feldman, H.H., Hamilton, R.L., Dekosky, S.T., van der Zee, J., Kumar-Singh, S., Van Broeckhoven, C., Mayeux, R., Vonsattel, J.P., Troncoso, J.C., Kril, J.J., Kwok, J.B., Halliday, G.M., Bird, T.D., Ince, P.G., Shaw, P.J., Cairns, N.J., Morris, J.C., McLean, C.A., DeCarli, C., Ellis, W.G., Freeman, S.H., Frosch, M.P., Growdon, J.H., Perl, D.P., Sano, M., Bennett, D.A., Schneider, J.A., Beach, T.G.,

- Reiman, E.M., Woodruff, B.K., Cummings, J., Vinters, H.V., Miller, C.A., Chui, H.C., Alafuzoff, I., Hartikainen, P., Seilhean, D., Galasko, D., Masliah, E., Cotman, C.W., Tunon, M.T., Martinez, M.C., Munoz, D.G., Carroll, S.L., Marson, D., Riederer, P.F., Bogdanovic, N., Schellenberg, G.D., Hakonarson, H., Trojanowski, J.Q., Lee, V.M., 2010. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. *Nat Genet* 42(3), 234-239. doi:10.1038/ng.536.
- Wallner, M., Meera, P., Toro, L., 1999. Molecular basis of fast inactivation in voltage and Ca²⁺-activated K⁺ channels: a transmembrane beta-subunit homolog. *Proc Natl Acad Sci U S A* 96(7), 4137-4142.
- Wickham, H. 2009. *ggplot2 : elegant graphics for data analysis*. Springer Science + Business Media, New York ; London.
- Wu, R.S., Marx, S.O., 2010. The BK potassium channel in the vascular smooth muscle and kidney: alpha- and beta-subunits. *Kidney Int* 78(10), 963-974. doi:10.1038/ki.2010.325.
- Yang, J., Lee, S.H., Goddard, M.E., Visscher, P.M., 2011. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet* 88(1), 76-82. doi:10.1016/j.ajhg.2010.11.011.
- Yu, L., De Jager, P.L., Yang, J., Trojanowski, J.Q., Bennett, D.A., Schneider, J.A., 2015. The TMEM106B locus and TDP-43 pathology in older persons without FTL. *Neurology* 84(9), 927-934. doi:10.1212/WNL.0000000000001313.
- Zarow, C., Sitzer, T.E., Chui, H.C., 2008. Understanding hippocampal sclerosis in the elderly: epidemiology, characterization, and diagnostic issues. *Curr Neurol Neurosci Rep* 8(5), 363-370.

Zarow, C., Weiner, M.W., Ellis, W.G., Chui, H.C., 2012. Prevalence, laterality, and comorbidity of hippocampal sclerosis in an autopsy sample. *Brain Behav* 2(4), 435-442.
doi:10.1002/brb3.66.

TABLES

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)	
<i>APOE</i> , n (%) ^a			
-/-	114 (46.0)	1,207 (44.1)	0.749
-/ ϵ 4	109 (43.9)	1,216 (44.4)	
ϵ 4/ ϵ 4	25 (10.1)	314 (11.5)	
<i>MAPT</i> (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
<i>GRN</i>							
	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
<i>TMEM106B</i>							
	REC	] rs3823612	G/C	64.6	56.5	1.53 (1.19 – 1.98)	0.0011
	ADD					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
<i>ABCC9</i>							
	REC	] rs7966849	A/G	60.3	51.2	1.84 (1.41 – 2.40)	7.1×10^{-6}
	ADD					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
<i>KCNMB2</i>							
	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
<i>GRN</i>	20	42,417,491	42,435,470	0.012	0.16	0.090
<i>TMEM106B</i>	222	12,245,848	12,281,890	0.028	0.0089	0.068
<i>ABCC9</i>	259	21,945,324	22,094,628	2.4×10^{-4}	0.0014	0.26
<i>KCNMB2</i>	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		UKBEC	
	(Frontal cortex; n = 130 brains)		(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/>).

FIGURES

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

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.

	rs2098574	rs704180	rs1643235	rs829080	Estimated haplotype frequency (%)			Score statistic ^a	p-value ^b
					Overall	Case	Control		
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