Reassessment of Risk Genotypes (GRN, TMEM106B, and ABCC9 Variants) Associated With Hippocampal Sclerosis of Aging Pathology

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Abstract

Hippocampal sclerosis of aging (HS-Aging) is a common high-morbidity neurodegenerative condition in elderly persons. To understand the risk factors for HS-Aging, we analyzed data from the Alzheimer’s Disease Genetics Consortium and correlated the data with clinical and pathologic information from the National Alzheimer’s Coordinating Center database. Overall, 268 research volunteers with HS-Aging and 2,957 controls were included; detailed neuropathologic data were available for all. The study focused on single-nucleotide polymorphisms previously associated with HS-Aging risk: rs5848 (GRN), rs1990622 (TMEM106B), and rs704180 (ABCC9). Analyses of a subsample that was not previously evaluated (51 HS-Aging cases and 561 controls) replicated the associations of previously identified HS-Aging risk alleles. To test for evidence of gene-gene interactions and genotype-phenotype relationships, pooled data were analyzed. The risk for HS-Aging diagnosis associated with these genetic polymorphisms was not secondary to an association with either Alzheimer disease or dementia with Lewy body neuropathologic changes. The presence of multiple risk genotypes was associated with a trend for additive risk for HS-Aging pathology. We conclude that multiple genes play important roles in HS-Aging, which is a distinctive neurodegenerative disease of aging.

Key Words: Alzheimer disease, Frontotemporal lobar degeneration (FTLD), Genome wide association study (GWAS), Hippocampal sclerosis, KATP, Progranulin, SUR2.

INTRODUCTION

Hippocampal sclerosis of aging (HS-Aging) is diagnosed neuropathologically when neuron loss and astrocitosis are observed in the hippocampal formation and are not considered attributable to Alzheimer disease (AD)—type plaques and tangles (1–3). Although there are other brain diseases with pathologic changes that are described as “hippocampal sclerosis,” HS-Aging is distinguished by the presence of TDP-43 pathology and the absence of severe symptoms or clinical signs of frontotemporal dementia (4–8). Hippocampal sclerosis of aging is a relatively common disease in elderly persons (2, 7, 9, 10) and is associated with substantial disease-specific cognitive impairment (11, 12). Even at state-of-the-art research institutions such as the US Alzheimer’s Disease Centers (ADCs), HS-Aging tends to be misdiagnosed as AD clinically because of overlapping manifestations (7, 12, 13) and, therefore, autopsy data are essential for accurate disease diagnosis.

There is no known disease-modifying treatment for HS-Aging. One factor impeding development of therapeutic strategies is that disease mechanisms are still incompletely understood. Vascular disease may contribute to HS-Aging pathology or, alternatively, pathogenetic factors may overlap with those of frontotemporal lobar degeneration (FTLD) (2, 10, 14, 15).

Genetic risk factors for HS-Aging have recently been characterized, yielding new insights into disease mechanisms. In contrast to AD, APOE gene variants are not associated with altered risk for HS-Aging (7, 9, 12, 13, 16). Here we discuss risk alleles and risk genotypes (combinations of alleles) from single-nucleotide polymorphisms (SNPs) that are associated with an altered manifestation of a phenotype. The association of an SNP with a specific phenotype (in the present study,
Neuropathology Data Set (12, 24). The 3 allele identities obtained from the Minimum Data Set, Uniform Data Set, and Human Subjects Division; protocols at individual ADCs; NACC data was approved by the University of Washington clinical data gathered through NACC (24). Research using which were analyzed together with neuropathologic and 20 ADCs) with multiple iterations of SNP data (23, 25, 26), A/G allele in near-perfect linkage disequilibrium with rs704178 and rs704180). A prior genome-wide association study (GWAS) that focused on HS-Aging pathology as an endophenotype reported that 2 SNPs in near-perfect linkage disequilibrium with each other (rs704178 and rs704180) in ABCC9 are associated with HS-Aging pathology, with an OR = 2.1 and an overall value of \( p = 1.4 \times 10^{-5} \) when all the cohorts’ data were combined (21). For practical reasons, we will refer only to rs704180 hereafter.

To learn more about genetic polymorphisms associated with HS-Aging pathology, we examined genomics data from the Alzheimer’s Disease Genetics Consortium (ADGC), correlated with clinical and pathologic data from the National Alzheimer’s Coordinating Center (NACC) database (22–24). Data were analyzed from ADC research volunteers who had been examined clinically with subsequent neuropathologic evaluation to test whether previously reported HS-Aging risk alleles (rs5848, rs1990622, and rs704180) can be replicated and to evaluate gene-gene interactions. We also tested whether the association between those alleles and HS-Aging pathology is related to AD or dementia with Lewy body (DLB) neuropathologic changes among genotyped subjects in the relatively large NACC autopsy cohort.

MATERIALS AND METHODS

Patient Subjects

The ADGC accrued genomics data from 29 different ADCs (>10 cases each from 26 ADCs, >100 cases each from 20 ADCs) with multiple iterations of SNP data (23, 25, 26), which were analyzed together with neuropathologic and clinical data gathered through NACC (24). Research using NACC data was approved by the University of Washington Human Subjects Division; protocols at individual ADCs were approved and regulated by the local institutional review boards. National Alzheimer’s Coordinating Center data were obtained from the Minimum Data Set, Uniform Data Set, and Neuropathology Data Set (12, 24). The 3 allele identities were analyzed according to ADGC SNP nomenclature and were rs5848 (A/G), rs1990622 (A/G); note that other reports have used T/C for this allele: the ‘‘A’’ allele is analogous to ‘‘T’’ allele in other reports, whereas the ‘‘G’’ allele we report is analogous to ‘‘C’’ allele), and rs704180 (this is an A/G allele in near-perfect linkage disequilibrium with rs704178, which is a G/C allele; G/C alleles are challenging because of reverse strand issues, so we recommend using rs704180).

Neuropathologic evaluations were performed according to center-specific protocols, including whether neuropathologists studied left, right, or bilateral hippocampi (12), and entered into a standardized format for NACC purposes. Only individuals who died after age 60 years were included in this study. The HS-Aging case/control operationalization in NACC was described previously (12, 21); details of the NACC parameter definitions are presented in the Supplemental Material, Supplemental Digital Content 1, http://links.lww.com/NEN/A688. Relatively few persons with FTLD-TDP, FTLD-tau, other FTLD subtypes, or spongiform encephalopathy were genotyped in our available database (Supplemental Material, Supplemental Digital Content 1, http://links.lww.com/NEN/A688). These subjects were excluded from the analyses because the subsample with FTLD and prion subtypes (together comprising 188 individuals, 24 with HS-Aging pathology) was underpowered for statistical comparisons.

After exclusions, data from a total of 2,343 NACC/ADGC research subjects with genotype and autopsy information outside of UK-ADC data were available for analyses at the time of our prior published HS-Aging GWAS (21). Some cases are included in the current study and not the HS-GWAS project because they met inclusion criteria as stated; however, these research subjects are not an independent cohort for the purpose of a replication experiment. Notably, the current study included patients who died before the year 2000. Subgroup analysis confirmed that the rate of HS diagnosis was lower before 2000, but this enabled a complete assessment of the NACC/ADGC data. Additional data from 612 research subjects with genotype and NACC autopsies were available for the present study that were not available for previous analyses and were not previously analyzed in our studies. These cases provided an independent subgroup to retest the association between putative risk alleles and HS-Aging pathology. The 612 cases comprising a replication cohort were accrued from 26 different ADCs; average number of cases per ADC was 23.5; median number of research subjects included for analyses per ADC was 21; and the maximum number of included research subjects from an ADC was 79. The main reason for these cases being now available for this study is that there was a round of database-wide quality control (QC) and imputation that was performed by the ADGC in the spring of 2014; this effort was completed after the dates the HS-Aging GWAS article was submitted and reviewed. This QC/imputation refined the availability of genotyped NACC cases and enabled inclusion of research subjects who were previously excluded because of covariate coding that was suboptimal. For example, more than 500 research subjects’ data were excluded from the NACC/ADGC analyses previously because they were classified as having an inappropriate age parameter, although those cases were within the included age range. Furthermore, and beyond the issues related to imputation and QC, the database analyzed in the current study included data from 122 research subjects whose genotyping data appeared in the database after the time stamp of the database used for the HS-Aging GWAS. Additional QC was performed on all of the cases and found that the specific targeted SNP calls for rs5848, rs19906022, and rs704180 were of adequate technical quality, but data from 6 additional
research subjects were removed because of a first-degree relationship after KING-Robust analysis.

Neuropathology

Features of AD neuropathologic changes were operationalized as diffuse plaques (most of the autopsies predated the universal application of Thal Aβ staging), neuritic plaques, and neurofibrillary tangles (NFTs). Diffuse plaques were graded according to cortical density (frequent, moderate, sparse, or none) operationalized with NACC parameter “NPDIFF”, Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) stages of neuritic plaque densities (frequent, moderate, sparse, or none) operationalized with NACC parameter “NPCERAD” (27), and NFTs according to Braak stages for neurofibrillary pathology (Stages 0–VI operationalized with NACC parameter “NPBRAAK”) (28). Dementia with Lewy body pathology was characterized according to established guidelines using NACC parameter “NPLEWY,” which indicates whether pathology “is consistent with criteria of Consortium on Dementia with Lewy body pathology, brainstem-predominant type,” “Lewy body pathology, intermediate or transitional (limbic) type,” or “Lewy body pathology, diffuse (neocortical) type” (29).

Clinical and Genomic Data

Clinical data including the Mini-Mental State Examination (MMSE) (30) were collected by trained clinicians and interviewers as previously described (12, 31). Genomic data from ADGC were transferred from PLINK format into Microsoft Excel spreadsheet for additional studies, with subsequent analyses performed using R 3.1.0 (32).

Statistical Analysis

Statistical methods included marginal logistic regression that was used to construct confidence intervals (CIs) for ORs and to test for association between candidate SNPs and HS-Aging pathology. Three modes of inheritance (MOI) were examined for each SNP using conventional genotype transformations: additive MOI (number of risk alleles, i.e. 0, 1, or 2); dominant MOI (at least 1 risk allele = 1; 0 otherwise); and recessive MOI (2 risk alleles = 1; 0 otherwise). Contingency tables were used to calculate HS-Aging pathology ORs and supporting CI for combinations of risk genotypes (using recessive for ABCC9 and dominant for TMEM106B and GRN). Chi-square and Fisher exact tests were used to compare HS-Aging pathology prevalence between individuals with both an ABCC9 A_A genotype (for rs704180) and a TMEM106B A_A genotype (rs1990622) and others for deaths occurring before 2000 and after, respectively. Similarly, MMSE between these 2 groups was examined with a 2-tailed Student t-test. Chi-square tests were used to compare differences in the prevalence of AD and DLB neuropathologic changes for each gene.

RESULTS

Patient demographic data and presence or absence of HS-Aging are summarized in Table 1. The genotyped

<table>
<thead>
<tr>
<th>TABLE 1. Demographic Data for 3,255 Subjects</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>NACC Data Outside UK-ADC Available for Use in a Prior Study* (n = 2,343)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age Group at Death, years</td>
<td>HS-</td>
<td>HS+</td>
</tr>
<tr>
<td>60s</td>
<td>120</td>
<td>6</td>
</tr>
<tr>
<td>70s</td>
<td>433</td>
<td>28</td>
</tr>
<tr>
<td>80s</td>
<td>422</td>
<td>36</td>
</tr>
<tr>
<td>&gt;90</td>
<td>72</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>1,047</td>
<td>84</td>
</tr>
<tr>
<td>NACC data unavailable for use in a prior study (n = 612)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age group at death, years</td>
<td>HS-</td>
<td>HS+</td>
</tr>
<tr>
<td>60s</td>
<td>117</td>
<td>0</td>
</tr>
<tr>
<td>70s</td>
<td>84</td>
<td>10</td>
</tr>
<tr>
<td>80s</td>
<td>65</td>
<td>10</td>
</tr>
<tr>
<td>&gt;90</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>286</td>
<td>27</td>
</tr>
<tr>
<td>Data from UK-ADC (n = 270)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age group at death, years</td>
<td>HS-</td>
<td>HS+</td>
</tr>
<tr>
<td>60s</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>70s</td>
<td>31</td>
<td>2</td>
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<tr>
<td>80s</td>
<td>46</td>
<td>3</td>
</tr>
<tr>
<td>&gt;90</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>10</td>
</tr>
</tbody>
</table>

*HS- or +, hippocampal sclerosis of aging absent or present; NACC, National Alzheimer’s Coordinating Center; UK-ADC, University of Kentucky Alzheimer Disease Center (21).
research volunteers from NACC were predominantly patients with severe AD pathology (see below). More detailed information about HS-Aging pathology cases and controls in the NACC data set without genomics data have been reported (12). Because of the more permissive inclusion criteria and the lack of GWAS-related screening exclusions, the number of subjects in the current study is larger than in the prior GWAS (21). From among the 268 pathologically verified HS-Aging cases and 2,957 controls with genotype data now available for analyses, there were 51 HS-Aging cases and 561 controls that were not available for analyses in the previous GWAS study (21); these cases were not from the UK-ADC cohort. National Alzheimer’s Coordinating Center and ADGC data also included 31 HS-Aging cases and 239 controls from the UK-ADC. DNA samples from these individuals were analyzed in the prior study using polymerase chain reaction–based SNP characterization (21).

Polymerase chain reaction data from UK-ADC cases agreed with the ADGC SNP data (not shown); therefore, ADGC-derived SNP data were used for UK-ADC data in the analyses where all the data were pooled.

A focus of the present study was genetic polymorphisms that previously were associated with the risk of developing HS-Aging pathology (Fig. 1). Of the 3,225 individuals included in the study overall, 3,210 (99.5%) had SNP calls for rs1990622 (TMEM106B), 3,213 (99.6%) had SNP calls for rs704180 (ABCC9), and 2,732 (84.7%) had SNP calls for rs5848 (GRN). The ORs with 95% CIs for the 3 putative risk alleles assuming additive, dominant, and recessive MOIs are shown in Table 2. For all 3 SNPs, the same risk alleles (i.e. rs5848 “T,” rs1990622 “A,” and rs704180 “A” alleles) showed a positive association with HS-Aging risk as in prior published studies (17–21). Data from the entire cohort showing the proportion of individuals with HS-Aging pathology.

### TABLE 2. Risk Alleles for HS-Aging Pathology, and Odds Ratios, With 95% Confidence Intervals for National Alzheimer’s Coordinating Center Data Sets

<table>
<thead>
<tr>
<th>NACC Data Available for Use in a Prior Study (n = 186 HS-Aging, n = 2,157 Controls)</th>
<th>Additive MOI</th>
<th>Dominant MOI</th>
<th>Recessive MOI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene (SNP)</td>
<td>Risk Allele*</td>
<td>Odds Ratio (95% CI)</td>
<td>Odds Ratio (95% CI)</td>
</tr>
<tr>
<td>ABCC9 (rs704180)</td>
<td>A</td>
<td>1.51 (1.22–1.89)</td>
<td>1.46 (0.99–2.17)</td>
</tr>
<tr>
<td>TMEM106B (rs1990622)</td>
<td>A</td>
<td>1.16 (0.94–1.44)</td>
<td>1.18 (0.80–1.75)</td>
</tr>
<tr>
<td>GRN (rs5848)</td>
<td>T</td>
<td>1.25 (0.99–1.59)</td>
<td>1.33 (0.97–1.82)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NACC Data Unavailable for Use in a Prior Study (replication cohort) (n = 51 HS-Aging, n = 561 controls)</th>
<th>Additive MOI</th>
<th>Dominant MOI</th>
<th>Recessive MOI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP</td>
<td>Risk allele*</td>
<td>Odds ratio (95% CI)</td>
<td>Odds ratio (95% CI)</td>
</tr>
<tr>
<td>ABCC9 (rs704180)</td>
<td>A</td>
<td>1.38 (0.93–2.05)</td>
<td>1.13 (0.59–2.18)</td>
</tr>
<tr>
<td>TMEM106B (rs1990622)</td>
<td>A</td>
<td>1.86 (1.22–2.86)</td>
<td>2.70 (1.21–3.84)</td>
</tr>
<tr>
<td>GRN (rs5848)</td>
<td>T</td>
<td>1.74 (1.12–2.69)</td>
<td>2.24 (1.16–4.33)</td>
</tr>
</tbody>
</table>

Data are for the additive, dominant, and recessive models of MOI.
*See Materials and Methods for description of rationale for risk allele identification.

HS-Aging, hippocampal sclerosis of aging; CI, confidence interval; MOI, mode of inheritance, SNP, single-nucleotide polymorphism.
pathology stratified by SNP genotype and age at death are shown in Figure 2, which conveys the descriptive trend with regard to their association with HS-Aging pathology. Note that, unlike the case for the GRN and TMEM106B SNPs, patients who are heterozygous for the putative ABCC9 risk allele show greater risk than those that are homozygous lacking the risk allele in the oldest age category, indicating a recessive MOI. The trend toward greater HS-Aging risk for individuals with both risk alleles spans a broad age range and, as expected, the overall HS-Aging prevalence is highest among the “oldest old” (7, 14, 33). Among cases that were not analyzed previously (and with the caveat that the sample size was smaller than the previous study [21]), the OR for the association between HS-Aging pathology and rs1990622 suggested a strong association (Table 2). These data were compatible with prior studies in terms of the probable MOI for these SNPs; specifically, there was an apparent recessive MOI for rs704180 but more probably an additive or dominant MOI for rs5848 and rs1990622.

All the cases were analyzed together (n = 3,225 total) to test for evidence of gene-gene interactions and genotype-phenotype effects. Individuals with more than 1 risk genotype had a risk for HS-Aging pathology that trended higher than with either gene’s risk allele(s) alone, but the data indicated an additive effect rather than a synergistic or a superadditive effect (Fig. 3 which shows results for 2,709 cases where all three SNPs were characterized). For additional statistical information, see Supplemental Material, Supplemental Digital Content 1, http://links.lww.com/NEN/A688.

A prior study showed that recognition of HS-Aging by neuropathologists at autopsy has increased during the past decade at ADCs (12). We hypothesized that, as a consequence of underdiagnosis, some patients who came to autopsy before

FIGURE 2. Proportion of cases with hippocampal sclerosis of aging (HS-Aging) pathology by age group at death and genotype. (A-C) Genotypes are shown for rs5848 (GRN [A]), rs1990622 (TMEM106B [B]), rs704180 (ABCC9 [C]). These analyses factor in the results of all the cases (n = 3,498 total), with case/control operationalizations as described in the Materials and Methods. Note that HS-Aging pathology is most common in the “oldest old,” and that the previously described risk genotypes (rs1990622 A_A, rs704180 A_A, and rs5848 T_T) have the highest risk for HS-Aging pathology across multiple age groups. For statistical analyses of relevant odds ratios, see Table 2.

FIGURE 3. Genotype combinations and the risk of being diagnosed with HS-Aging pathology. Each case (total N=2,709) included here had genotype information for each risk allele (rs5848, rs1990622, and rs704180). For these data, odds ratio (OR) was assessed in comparison to the individuals that lacked all three risk genotypes: rs5848 (T_T and T_C), rs1990622 (A_A and A_G), and rs704180 (A_A). In terms of potential confounders that might affect HS-Aging pathologic diagnosis (age and year of death), the control group (n=186) had average age at death 80.1 years and average 2001.7 year of death. The number of persons in each group (n), average age at death, OR, and 95% confidence interval (CI) are shown for each combinatorial subgroup. For p values on the statistical comparisons, see Supplemental Materials, Supplemental Digital Content 1, http://links.lww.com/NEN/A688. MOI, mode of inheritance; dom, dominant; rec, recessive.
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lower tested cognitive performance before death even without cases with the HS-Aging genetic risk alleles could have a pathologic diagnosis having been made at that time. Thus, 2000 would have the HS-Aging disease, despite no HS used. NS, not significant.

Evidence of additive effects for TMEM106B and ABCC9

A

Proportion of subjects with HS-Aging pathological diagnosis, by TMEM106B (rs1990622) and ABCC9 (rs704180) SNP genotypes

![Graph](image)

p < 0.005

NS

B

Final MMSE score of subjects without either HS-Aging or advanced Alzheimer Disease (Braak V/VI) pathologies, by TMEM106B (rs1990622) and ABCC9 (rs704180) genotypes

![Graph](image)

p = 0.03

DISCUSSION

In the large NACC autopsy data set derived from multiple research centers, the risk of developing HS-Aging pathology showed variation that was associated with specific previously described polymorphisms in TMEM106B, ABCC9, and GRN. The current study constitutes the first replication of the previously described association between the ABCC9 risk genotype and HS-Aging. Among persons with both risk-associated TMEM106B and ABCC9 genotypes, there was a higher-trending risk for being diagnosed as having HS-Aging pathology than individuals with either of these risk genotypes by themselves. The HS-Aging–associated gene polymorphisms are not associated with an increased risk for an HS diagnosis. Data presented in Figure 4 confirm that there was lower diagnostic rate of HS-Aging pathologic changes before versus after 2000 (overall proportion of cases with HS pathologic diagnosis was 1.7% before 2000 and 11.7% after 2000, p < 0.00001). The MMSE score difference between persons with versus without HS-Aging pathology among individuals who died after the year 2000 (mean final MMSE scores, 23.0 vs 18.1; p < 0.0001) is in agreement with prior studies about cognitive impairment associated with HS-Aging pathology (11, 12). Among patients lacking HS-Aging diagnosis and also without advanced AD pathology (Braak stages IV and lower) who died before 2000 (but not afterward when the pathologic diagnoses were presumably more accurate), the average final MMSE score for people with both risk genotypes (rs1990622 A_A and rs704180 A_A) was lower than that of the other genotypes (p = 0.03). Among persons with advanced AD pathology (Braak stages V and VI), the average final MMSE scores were much lower; we assume that most of the cognitive impairment in those individuals was caused by AD (not shown).

Although the gene polymorphisms we tested could cause or exacerbate HS-Aging pathology through direct mechanisms, a potential confounder is that TMEM106B, ABCC9, and/or GRN polymorphisms may be associated with altered AD or DLB pathology, which could lead to secondary brain changes that were subsequently diagnosed as hippocampal sclerosis at autopsy. Indeed, it has been hypothesized that both TMEM106B and GRN play a pathogenetic role in AD (34–36). The proportions of NACC cases with AD and DLB pathologies, stratified by risk allele genotype, are presented in Tables 3 and 4 (which essentially depict the percentages of cases in each category). The data do not support a role for these specific TMEM106B, ABCC9, or GRN alleles in AD pathogenesis because the risk SNPs are not associated with altered levels of AD-type plaques (“diffuse plaques” or “neuritic plaques” according to CERAD grading of plaque densities [27]) or NFTs (Braak staging [37]). Moreover, there is no evidence for increased risk of any DLB subtype in association with the HS-Aging risk alleles. There were insufficient numbers of genotyped FTLD cases available to test the link in those diseases; therefore, these cases were excluded (for details on exclusions, see Supplemental Material, Supplemental Digital Content 1, http://links.lww.com/NEN/A688).

FIGURE 4. Evidence of additive effects for ABCC9 and TMEM106B single-nucleotide polymorphisms (SNPs) in hippocampal sclerosis of aging (HS-Aging) pathology. In both panels, “ABCC9+” means rs704180 A_A and “ABCC9−” means rs704180 A_G or G_G; “TMEM+” means rs1990622 A_A and “TMEM−” means rs1990622 A_G or G_G. (A) The proportion of individuals with HS-Aging by pathologic diagnoses is shown, stratifying by ABCC9 and TMEM106B genotypes. Note that, as previously shown, the neuropathologic diagnosis of HS-Aging pathology was relatively unusual before 2000. In these pre-2000 cases, a subset of patients without either diagnosis of HS-Aging or AD (Braak stage <V), neuropathologic changes, cases with both TMEM106B and ABCC9 risk genotypes shows a lower global cognitive status as indicated by final Mini-Mental State Examination (MMSE) scores (p = 0.03), compatible with the hypothesis that a substantial proportion of these individuals had undiagnosed HS-Aging pathology. Statistical tests are χ² except for the pre-2000 HS-Aging pathologic evaluations for which there was a low cell count and the Fisher exact test was used. NS, not significant.

2000 would have the HS-Aging disease, despite no HS pathologic diagnosis having been made at that time. Thus, cases with the HS-Aging genetic risk alleles could have a lower tested cognitive performance before death even without...
AD or DLB pathologic changes. These observations indicate that multiple genetic influences are important in the pathogenesis of HS-Aging.

There are limitations inherent to the study design. National Alzheimer’s Coordinating Center data are derived from ADC memory disorder clinics, comprising a pooled sample that imperfectly reflects characteristics of the broader population. For example, FTLD prevalence tends to be 5% or higher at memory disorder clinics whereas, in epidemiologic samples, FTLD prevalence is usually less than 1% of demented participants (21, 38, 39). However, in the present sample, the FTLD cases were minimal because these were not yet analyzed for GWAS among the available NACC/ADGC data set. Also because of the cohort being derived from ADCs, there may be ascertainment bias toward sampling a subgroup of HS-Aging cases that clinically resemble AD (12). The paucity of HS pathologic diagnoses before 2000 indicates a change across time in diagnostic practices, and there are methodologic differences across ADCs that directly influence phenotype instantiation. For example, sampling half the brain leads to false-negatives because HS-Aging pathology is unilateral on hematoxylin and eosin stain in 40% to 55% of cases (7, 10). There was also inadequate sample size to determine conclusively the gene-gene interaction effects governing the 3 risk alleles. Despite these considerations, the NACC database represents a high-quality composite data source, extensively audited and sourced from state-of-the-art research centers, enabling the analyses of genomic determinants of human diseases.

For the understanding of genotype-phenotype correlations, this study, along with other recent scholarship (17, 20, 40), underscores the critical importance of using pathologic observations for diagnosis because clinicians may fail to detect specific underlying diseases. Here we focused on particular SNPs that were previously associated with HS-Aging pathology. From our analyses, 3 salient issues emerged: first, the strong correlation between the \textit{TMEM106B} SNP (rs1990622) and HS-Aging pathology; second, the replication of observable associations between \textit{ABCC9} SNP (rs704180) with HS-Aging pathology; and third, a reflection on how these SNPs may all contribute to a common disease characterized by TDP-43 pathology.

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There was evidence for a strong role for \textit{TMEM106B} in HS-Aging pathology in the current study. We note that, in the

<table>
<thead>
<tr>
<th>Gene (SNP)</th>
<th>No. Risk Alleles</th>
<th>n</th>
<th>Braak V or Braak VI</th>
<th>Severe CERAD Neuritic Amyloid Plaque Density</th>
<th>Severe Diffuse Amyloid Plaque Density</th>
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<tr>
<td>GRN</td>
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<td>0.79</td>
<td>0.64</td>
</tr>
<tr>
<td>(rs5848)</td>
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<td>1,041</td>
<td>0.72</td>
<td>0.80</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>228</td>
<td>0.74</td>
<td>0.82</td>
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</tr>
<tr>
<td>TMEM106B</td>
<td>0</td>
<td>638</td>
<td>0.71</td>
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<tr>
<td>(rs1990622)</td>
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<tr>
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<tr>
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</tr>
<tr>
<td></td>
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</tbody>
</table>

Neuropathologic changes are advanced Braak stages, CERAD neuritic plaque densities, or diffuse amyloid plaque densities. According to SNP genotype: GRN (rs5848 "T" is risk allele), \textit{TMEM106B} (rs1990622 "A" is risk allele), and \textit{ABCC9} (rs704180 "A" is risk allele). None of the pathologic features differ between columns at p \textless 0.05. The sample size refers to those available with Braak staging; there was some variance between pathologic variables in pathologic variable missingness.

*This sample size refers specifically to cases with Braak staging and genotype data.

CERAD, Consortium to Establish a Registry for Alzheimer’s Disease; SNP, single-nucleotide polymorphism.

<table>
<thead>
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<td>0.031</td>
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</tbody>
</table>

Single-nucleotide polymorphism (SNP) genotypes: GRN (rs5848 "T" is risk allele), \textit{TMEM106B} (rs1990622 "A" is risk allele), and \textit{ABCC9} (rs704180 "A" is risk allele). None of the pathologic features differ between columns at p \textless 0.05.
prior HS-Aging genomics study (21), we used data from fewer ADGC volunteers (included here were 2,343 research subjects plus 612 new subjects, not counting UK-ADC subjects, versus a total of 1,636 ADGC cases from the prior study). As expected, the inclusion of substantially different subject pool produced slightly different results. However, the analytic outcomes of the current study were not outside the range of expectations. Specifically, the 95% CIs of the ORs for GRN, TMEM106B, and ABC9 with regard to risk for HS-Aging pathology in the current study overlap with the 95% CI of the prior study. The TMEM106B SNP rs1990622 is a risk allele for FTLD, as determined by a GWAS (43). Subsequent studies have found that the same rs1990622 allele is associated with an altered risk for amyotrophic lateral sclerosis and for neurodegeneration linked to C9orf72 repeat expansions (44, 45). Also, the same allele is associated with HS pathology in older individuals (17, 20), and a GWAS corroborated the association (21), as does the present study. There is some evidence that rs1990622 may also change the risk for AD (34, 35), although we see no evidence for that in the NACC data set.

Unlike TMEM106B, ABC9 is not known to be associated with FTLD nor with any other neurodegenerative diseases. However, ABC9 gain-of-function mutations do cause Cantu syndrome, in which neurovascular anomalies have been observed (21, 46, 47). Blood vessel pathology is relevant because ABC9 is physiologically involved in vasoregulation (46, 48, 49), and autopsy-confirmed brain arteriolosclerosis is on average more severe in HS-Aging cases versus controls (14, 50). An ABC9 SNP within the same intron and only 1,152 base pairs away from rs704178 (which is in near-complete linkage disequilibrium with rs704180) has been associated with sleep duration and depressive symptoms (51, 52). ABC9 is a member of an evolutionarily conserved family of genes that encode large proteins with multiple membrane-spanning domains, other family members having been implicated in human diseases. For example, ABC7 (also known as CFTR) is the susceptibility gene in cystic fibrosis, and ABCC8 mutations cause congenital hyperinsulinism (53). The role of the ABC9 gene product, SUR2, is to modify KATP potassium channel function (46); therefore, it is notable that a new GWAS (published after primary review of the current study) has shown that KCNMB2, another potassium channel modulator, is also a possible risk allele for HS-Aging pathology (54).

Additional work is needed to characterize more confidently the associations between potassium channel physiology and HS-Aging pathology.

In gathering experimental evidence about disease mechanisms, a critical clue is the pathologic biomarker TDP-43 (4, 5). The border zone between HS-Aging and hippocampal TDP-43 pathology is not clearly defined either in the research community or in consensus-based neuropathologic diagnostic criteria (2). Recent studies from different centers, however, have established that HS-Aging pathology and hippocampal TDP-43 pathology both tend to occur in the same brains, and both are correlated with cognitive impairment (8, 11, 12, 20, 55–58). Hippocampal sclerosis of aging may prove to be the most prevalent TDP-43 proteinopathic condition (2). The contradistinction from AD-type pathology is supported by the lack of association between HS-Aging pathology and APOE polymorphism (7, 9, 12, 16). By contrast, if TMEM106B, ABC9, and GRN contribute to HS-Aging pathogenetically, they may do so through a mechanism related to TDP-43 pathology. This was anticipated for TMEM106B and GRN polymorphisms (17, 59) because both of those genes are directly linked to the risk for FTLD-TDP (43, 60, 61). The current study provides fresh information indicating additive risk among persons harboring a relatively common genotype that combines risk alleles. Approximately 20% of included subjects had the rs704180 “A_A” allele and at least one rs1990622 “A” allele. Interestingly, it has been reported that ABC9 protein is found in the “endosomal/lysosomal” compartment during ischemic stress conditions in cardiac myocytes (62), and this cellular compartment may be where TDP-43 pathology is intensified through TMEM106B and GRN mechanisms also (63–67).

In summary, we confirmed that specific TMEM106B, ABC9, and GRN SNPs are associated with HS-Aging pathology in the NACC cohort. None of the SNPs are exonic, and the disease usually manifests among the “oldest old” (7, 14), perhaps indicating relatively subtle dysregulation in concert with other factors that are yet to be elucidated. These results provide yet more indication that the human aged brain is an extremely complex system impacted simultaneously by diverse environmental stimuli, comorbid pathologies, and genetic vulnerabilities.

ACKNOWLEDGMENTS

We are deeply grateful to all of the study participants, clinical workers, and researchers who made this study possible. Thanks also to Dr. Gerard Schellenberg for collegial assistance on the project. Please see the Supplemental Material (Supplemental Digital Content 1, http://links.lww.com/NEN/A688) for additional acknowledgments.

REFERENCES

45. Wood HB. TMEM106B is a susceptibility locus for Ftd. Nat Rev Neurol 2010;6:184