

Interactive Effects of HLA and GM Alleles on the Development of Alzheimer Disease

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Abstract

Objective

We investigated whether particular immunoglobulin GM (γ marker) alleles—individually or epistatically with a known human leukocyte antigen (HLA) risk allele—were associated with the development of Alzheimer disease (AD).

Methods

Using a prospective cohort study design, we genotyped DNA samples from 209 African American (AA) and 638 European American (EA) participants for IgG1 (GM 3 and GM 17), IgG2 (GM 23+ and GM 23–), and *HLA-DRB1* rs9271192 (A/C) alleles by TaqMan and rhAMP genotyping assays.

Results

In EA subjects, none of the GM or HLA alleles—individually or epistatically—were associated with time to development of AD. In AA subjects, GM and HLA alleles individually were not associated with time to development of AD. However, there was a significant interaction: In the presence of GM 3 (i.e., GM 3/3 and GM 3/17 subjects), the presence of the HLA-C allele was associated with a 4-fold increase in the likelihood of developing AD compared with its absence (hazard ratio [HR] 4.17, 95% CI, 1.28–13.58). In the absence of GM 3 (GM 17/17 subjects), however, the presence of the HLA-C allele was not associated with time to development of AD (HR 1.10, 95% CI, 0.50–2.41).

Conclusions

These results show that particular GM and HLA alleles epistatically contribute to the development of AD.

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Glossary

AA = African American; AD = Alzheimer disease; EA = European American; GWAS = Genome-wide association study; HR = hazard ratio; IGHG = immunoglobulin heavy chain G.

Late-onset Alzheimer disease (AD) is a heritable, complex, and progressive brain disorder. Genome-wide association studies (GWAS) have identified numerous risk genes, but most of the heritability of AD remains unexplained, suggesting additional genes in its etiology. Many risk-conferring genes identified thus far are enriched in the immune system pathways.¹ A major gene of the immune system—*HLA-DRB1*—has been associated with AD by many studies, including the largest GWAS of AD to date.² The C allele of single-nucleotide polymorphism (SNP) rs9271192 within *HLA-DRB1* seems to be a strong risk factor for AD.³

The current GWAS of AD do not evaluate a major gene complex of the immune system—GM (γ marker) allotypes encoded by immunoglobulin heavy chain G (*IGHG*) genes on chromosome 14.^{4,5} The 3 *IGHG* genes that encode GM allotypes are highly homologous and apparently not amenable to high throughput genotyping technology used in GWAS. Therefore, a candidate gene approach is necessary to investigate the role of the immunoglobulin GM allotypes in the immunobiology of AD. There is a good rationale for the GM gene involvement in the etiopathogenesis of AD. These genes have been shown to influence the magnitude of antibody responses to various antigens.^{4,5} The presence of amyloid- β (A β) plaques is one of the hallmarks of AD. IgG heavy chains, where all GM allotypes are expressed, have inherent anti-amyloidogenic activity.⁶ Thus, polymorphic GM genes could contribute to the interindividual differences in the level of antibody responses to A β , thereby influencing the pathogenesis of the disease.

In this study, we aimed to determine the individual and/or epistatic (defined as modification of the action of a gene by an allele at another locus) contribution of GM and *HLA-DRB1* genotypes to the development of AD.

Methods

Study Design and Samples

Using a prospective cohort study design, this investigation used archived DNA specimens and data from 3 longitudinal cohorts on aging: The Minority Aging Research Study, The Rush Memory and Aging Project, and The Religious Orders Study, which have been described in detail elsewhere.^{7,8}

A stratified sampling scheme was used to select a subset of participants without dementia at baseline from each cohort. African American (AA) participants from all 3 studies were included (n = 209). A subset of European American (EA) participants was randomly selected from the 2 cohorts that are predominantly EA (N = 638).

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by Institutional Review Boards of Rush University Medical Center and Medical University of South Carolina. All participants signed an informed consent and a repository consent to allow their data to be repurposed.

Data Availability

Data can be requested at radc.rush.edu.

GM Genotyping

IgG1 markers GM 3 and 17 (arginine to lysine) and IgG2 markers GM 23- and 23+ (valine to methionine) were determined by a TaqMan genotyping assay from Applied Biosystems Inc.

HLA-DRB1 rs9271192 Genotyping

HLA-DRB1 SNP rs9271192 (A > C) was determined by a custom-designed rhAMP SNP genotyping assay from Integrated DNA Technologies Inc.

Statistical Analysis

Multivariable logistic regression was used to compare rates of AD and mortality during follow-up between EAs and AAs, while adjusting for baseline age and length of follow-up time. Associations between the candidate genes and time to development of AD were assessed using Cox proportional hazards (PH) models, which accounted for mortality and loss to follow up. Models were developed separately for EAs and AAs, given that the allelic frequencies for GM and HLA vary considerably by race. Time to development of AD was modeled as a function of covariates (baseline age, sex, years of education, and APOE-4 carrier status), the candidate genes, and gene \times gene interactions using a backwards model selection process. The covariates were forced into each model, regardless of statistical significance. For all models, the proportionality assumption was verified. No adjustment was made for multiple comparisons because this was largely a hypothesis generating exercise. Analyses were further stratified by sex to determine whether our findings were consistent for men and women. Analyses were conducted using SAS v9.4 (SAS Institute, Cary, NC).

Results

Table presents the descriptive statistics of EA and AA subjects. The proportion of subjects that developed AD during the follow-up was higher in EA than that in the AA group (37.3 vs 19.6%), although this was not significant after adjusting for baseline age and length of follow-up time, which was higher among EAs than AAs (mean [SD]: 12.5 [4.6] vs

Table Descriptive Statistics of Cohorts Stratified by Race

Variable	Statistic	European American (n = 638)	African American (n = 209)
Male		22.7%	26.3%
Alzheimer disease	% who developed AD during follow-up	37.3%	19.6%
Died	% who died during follow-up	61.4%	29.7% ^c
GM 3/17 genotypes			^d
3/3	%	44.0%	2.9%
3/17	%	45.0%	30.6%
17/17	%	10.0%	61.7%
Untypable	%	0.9%	4.8%
GM 23 genotypes			^d
+/+	%	17.4%	3.8%
+/-	%	48.9%	22.5%
-/-	%	33.1%	73.7%
Untypable	%	0.6%	0.0%
HLA-DRB1 rs9271192 genotypes			^d
AA	%	52.1%	61.4%
AC	%	40.9%	34.2%
CC	%	7.0%	4.5%
APOE 4 carrier	% Yes	23.8%	34.9% ^d
Age at baseline (y)	Mean (SD)	77.6 (6.9)	72.5 (5.8) ^d
Education (y)	Mean (SD)	15.6 (3.2)	14.9 (3.6) ^d
Follow-up time (y)	Mean (SD)	12.5 (4.6)	10.4 (3.1) ^d
Time to AD (y)^a	Mean (SD)	9.0 (5.5)	5.7 (3.8) ^d
Age at death (y)^b	Mean (SD)	91.8 (5.7)	84.7 (6.8) ^d

Abbreviations: AA = African American; AD = Alzheimer disease; EA = European American.

^a Among patients who developed AD during the study time frame (EA: n = 238, AA: 41).

^b Among patients who died during the study time frame (EA: n = 392, AA: 62).

^c $p < 0.05$ when compared with EAs by logistic regression, after adjusting for age at baseline and follow-up time.

^d $p < 0.05$ when compared with EAs, by χ^2 or Wilcoxon rank sum test, as appropriate.

10.4 [3.1], $p < 0.05$). In addition, a higher proportion of EA than AA subjects died during the follow-up (61.4 vs 30.0%). For all genes of interest, there were markedly different genotype distributions noted when comparing EAs to AAs ($p < 0.05$ for all comparisons).

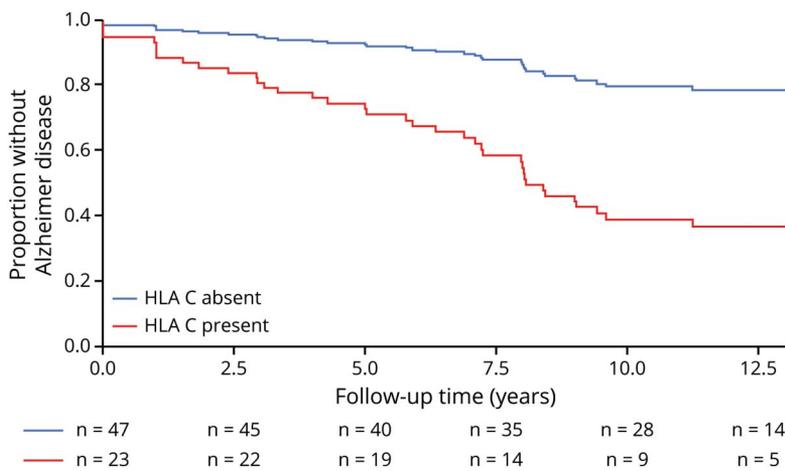
In EA subjects, none of the GM or HLA alleles—individually or epistatically—were associated with time to development of AD (all p -values > 0.10). In AA subjects, however, a different pattern emerged. When no gene by gene interactions were considered, GM and HLA alleles individually were not associated with time to development of AD. However, when we included their interaction in the model, we identified a robust interaction. In the presence of GM 3 (i.e., GM 3/3 and GM 3/17 subjects), the presence of the HLA-C allele was associated with a 4-fold increase in the likelihood of developing AD compared with its

absence (hazard ratio [HR] 4.17, 95% CI, 1.28–13.58, figure 1). In the absence of GM 3 (GM 17/17 subjects), however, the presence of the HLA-C allele was not associated with time to development of AD (HR 1.10, 95% CI, 0.50–2.41, figure 2). Because the APOE-4 allele and other variables were used as covariates in these analyses, the interactive effect of GM and HLA genotypes on the development of AD was independent of the APOE-4 allele status and other subject covariates.

Discussion

The results presented here clearly show that in AA, the C allele of the rs9271192 SNP within *HLA-DRB1* may be a strong risk factor for AD in presence of the immunoglobulin GM 3 allele. This association was not found for EA. As

Figure 1 Proportion of African American Subjects Without Alzheimer Disease Over Time Among Subjects With the GM 3 Allele, Stratified by HLA C Status



mentioned earlier, several studies have reported the association of the C allele of the *HLA-DRB1* SNP with susceptibility to AD. Because none of these studies genotyped for the GM gene complex, it is not possible to determine whether the HLA associations observed were independent of the GM genotype status of the subjects.

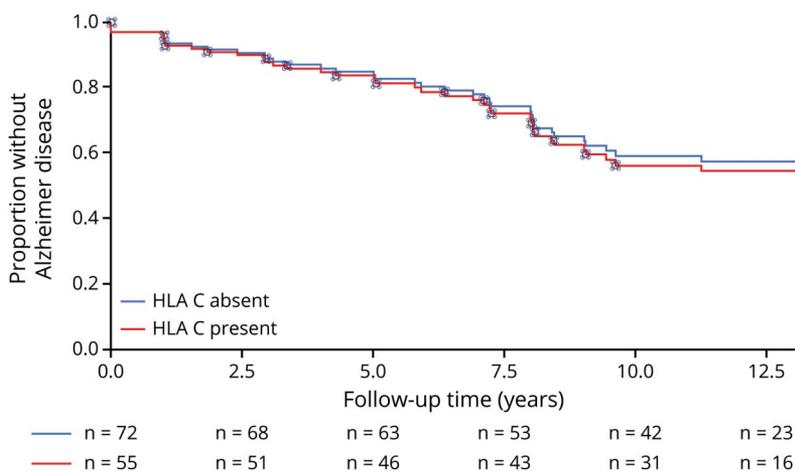
A possible mechanism of joint GM-HLA gene involvement in susceptibility to AD could be through their putative influence on antibody responses to A β via HLA-DRB1-restricted antigen processing/presentation pathway. IgG heavy chains (which express GM allotypes) have been shown to have natural antiamyloidogenic properties.⁶ It is possible that the antigen presenting B cells with the membrane-bound IgG expressing the GM 3 allotype are not effective recognition structures for the A β peptides. Furthermore, these peptides

may not fit properly in the peptide-binding groove of the at-risk *HLA-DRB1* C allele, leading to inadequate presentation to the CD4⁺ T helper cells and the consequent lack of B cell activation to generate anti-A β antibodies.

The reasons for the observed racial differences in the contribution of GM and HLA alleles in the development of AD are not clear. Both GM and HLA allele frequencies differ significantly between AA and EA populations. These differences, together with other racially associated genetic and nongenetic factors relevant to the development of AD, may have contributed to the differences observed in this investigation.

Although the phenomenon of epistasis has been known for over 100 years,⁹ there is a paucity of studies to detect possible epistatic interactions in human diseases.¹⁰ It is hoped that

Figure 2 Proportion of African American Subjects Without Alzheimer Disease Over Time Among Subjects Without the GM 3 Allele, Stratified by HLA C Status



results presented here will inspire further investigations on gene-gene interactions in AD and other complex polygenic/multifactorial diseases.

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Disclosure

Disclosures available: [Neurology.org/NG](https://www.neurology.org/NG).

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Appendix Authors

Name	Location	Contribution
Janardan P. Pandey, PhD	Medical University of South Carolina, Charleston	Design and conceptualized the study and drafted the manuscript for intellectual content
Paul J. Nietert, PhD	Medical University of South Carolina, Charleston	Analyzed the data, and revised the manuscript for intellectual content
Ronald T. Kothera, MS	Medical University of South Carolina, Charleston	Genotyped the DNA samples

Appendix (continued)

Name	Location	Contribution
Lisa L. Barnes, PhD	Rush Alzheimer's Disease Center, Chicago, IL	Design and conceptualized study and revised the manuscript for intellectual content
David A. Bennett, MD	Rush Alzheimer's Disease Center, Chicago, IL	Design and conceptualized study and revised the manuscript for intellectual content

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