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HLA class II genotypes are not associated with age related macular degeneration in a case-control, population-based study

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Abstract

Multiple lines of evidence support an immunologic basis and genetic disposition for the development of age-related macular degeneration (AMD). Comprehensive Human Leukocyte Antigens (HLA) class II typing at four loci (DRB1, DQA1, DQB1, and DPB1) was assessed using next generation sequencing methods and tested for association with Age-related Macular Degeneration (AMD) in a case-control study of 456 AMD cases and 499 controls from the population-based Study of Osteoporotic Fractures (SOF) cohort. No statistically significant associations were identified for any of the class II loci and a previously identified association between DRB1*13:01 was not replicated in this dataset. These results reported here suggest that common HLA class II genetic variation does not contribute to AMD disease risk.

Keywords

AMD; age related macular degeneration; HLA; Class II; next generation sequencing

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in persons 65 years of age and older in the developed world [1]. Multiple lines of evidence support an immunologic basis and genetic disposition for the development of AMD [2]. In this context, human leukocyte antigen (HLA) polymorphisms, encoded within the major

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histocompatibility complex (MHC) are of particular interest. The HLA system is essential for innate and adaptive immune response and has been implicated in the pathophysiology of AMD [3–9]. In AMD, for example, increased HLA class II immunoreactivity is related to drusen formation [6] and has been observed in both soft and hard drusen [7]. The HLA class II antigens associated with drusen appear to be derived from choroidal antigen-presenting cells that breach the Bruch's membrane [8].

HLA are among the most polymorphic genes within the human genome, and variation within these regions has not been comprehensively assessed as a risk factor for AMD [10]. Two small studies have reported associations between HLA genetic variation and AMD [11–13], with DRB1*1301 being the only class II allele exhibiting a significant association with disease in a single population [11]. However, this association has not been independently replicated. Because of the complexity of the HLA region (e.g., single nucleotide polymorphisms segregate for more than two alleles), standard genotyping techniques commonly used in genome-wide association studies do not properly discern the contribution of these regions to disease susceptibility. The current study leverages high throughput, massively parallel next generation sequencing methods for comprehensive HLA class II typing at four loci (HLA-DRB1, HLA-DQA1, HLA-DQB1, and HLA-DPB1). The resultant HLA genotypes were tested for associations with AMD using a nested case-control study design with the aim of identifying alleles that confer susceptibility to AMD.

2. Materials and Methods

2.1 Subjects

The Study of Osteoporotic Fractures (SOF) is a longitudinal epidemiologic study of 9,704 women aged 65–99 years (mean 71.7, SD 5.3) at baseline recruited from four study centers located in Baltimore, MD; Minneapolis, MN; the Monongahela Valley near Pittsburgh, PA; and Portland, OR. The baseline SOF exams were conducted from 1986–88. Since then, follow-up exams have taken place approximately every two years. SOF was originally designed to investigate risk factors for osteoporosis and osteoporotic fractures. An extensive eye study was performed at the year 10 and year 15 follow-up clinic visits in 1997–1998 and 2002–2004, respectively. As previously described [14, 15], forty-five degree stereoscopic fundus photographs from both eyes were graded for AMD using a modification of the Wisconsin Age-Related Maculopathy Grading System [16] used in NHANES III [17]. Early AMD was defined as the presence of soft drusen (≥ 95 microns (μm) in diameter) and 1) drusen area $<$ that of a circle with a diameter of $960 \mu\text{m}$ and retinal pigment epithelial depigmentation present; or 2) drusen area $\geq$ that of a circle with diameter $960 \mu\text{m}$ with or without pigmentary abnormalities (i.e. level 30 or 40) in at least one eye and without late AMD in either eye at year 15 in subjects with no AMD (level 10 or 20) in either eye at years 10 and/or 15. Late AMD was defined as the presence of sub-foveal geographic atrophy or choroidal neovascularization (level 50 or 60) in at least one eye at years 10 and/or 15. For AMD case-control analysis, participants who had any AMD at years 10 or 15 were categorized as AMD cases. For early and late AMD case-control analysis: participants who had early AMD at year 10 or 15 were categorized as early AMD; participants who had late AMD at year 10 or 15 were categorized as late AMD; and participants early AMD at year 10

and late AMD at year 15 were categorized as late AMD cases. A total of 955 SOF participants (456 AMD cases and 499 controls) with sufficient DNA were identified for HLA genotyping.

2.2 HLA Genotyping

All subjects were genotyped at the Children's Hospital Oakland Research Institute using the Roche 454 GS Junior (Brandford, CT) next generation sequencing system with amplicon-based HLA genotyping as previously described [18]. HLA genotypes were assigned to samples using the Conexio ASSIGN ATF™ genotyping software (Conexio Genomics, Fremantle, Western Australia) and Sequence Compilation and Rearrangement (SCORE™) software (Helmberg SCORE, Graz, Austria).

2.3 Statistical Methods and Analysis

Conformity to Hardy-Weinberg equilibrium in the control subjects was tested using PyPop following the method of Guo and Thompson [19, 20]. Alleles at each of the class II loci were in Hardy-Weinberg equilibrium (HWE) if the observed frequencies did not differ significantly from expected frequencies (p -value > 0.05). HLA genotype count data were compared between cases and controls as $2 \times N$ contingency tables using a Pearson's χ^2 test in the R statistical environment [21]. Cells with expected values less than five were binned prior to testing. Logistic regression was also employed and analyses were adjusted for age and clinic site. Odds ratios (OR) and 95% confidence intervals (CI) were calculated in analyses using the exact method in R [22]. Additional logistic regression models examined AMD risk factors as covariates, including: smoking, regular aspirin use, and hormone replacement therapy. Bonferroni corrected p -values < 0.05 were considered statistically significant. These analyses have 80% power to detect significant associations ($\alpha=0.05$) with small effect sizes (Cohen's $w=0.1$) for minor allele frequencies (MAF) $> 5\%$.

3. Results

A total of 955 female European ancestry subjects nested within the SOF study (456 AMD cases and 499 controls obtained from four clinic centers) were genotyped at four HLA class II loci (HLA-DRB1, HLA-DQA1, HLA-DQB1, and HLA-DPB1). Demographics of the cohort are described in Table 1, including age at the sixth visit within the SOF study, AMD disease stage (early, late), and clinic center. Genetic variants at all four loci did not deviate from HWE among AMD cases and controls from Portland, OR, Baltimore, MD, and Pittsburgh, PA. However, variants at the HLA-DRB1 locus deviated from HWE among controls from Minneapolis, MN ($p < 0.0003$), attributable to an overabundance of DRB1*11:01 homozygotes (data not shown). Because some statistical analyses were predicated on the assumption of HWE, all analyses were done in the presence and absence of Minneapolis, MN to ensure robust associations and all logistic regression analyses were adjusted for age and clinic site.

Genotype data were first analyzed at the overall locus level for AMD association (Table 2). No HLA class II loci produced statistically significant associations after adjustment for multiple comparisons. The frequencies of the HLA class II alleles with MAF $> 10\%$ are

presented in Table 3 at 2-field resolution (for MAF $\geq 1\%$, see Supplementary Table 1). Consistent with the locus level analysis, no single allele produced a significant association with AMD at any of the four loci tested. Nominally significant associations were not significant after correction for multiple comparisons or following testing without Minneapolis, MN.

In addition to AMD case-control comparisons, the data were grouped by disease stage, either early AMD (N=320) or late AMD (N=136). No significant associations with disease stage were observed for any of the loci tested including comparisons of early vs late and early/late vs control (data not shown). Additional adjustment for AMD risk factors including cigarette smoking (current, past, never), regular aspirin use (yes, no), and hormone replacement therapy (current, past, never) did not alter the negative results. Finally, the previously identified inverse association between DRB1*13:01 and AMD [11] was not replicated in this dataset (OR=0.78, 95% CI=0.52–1.15, P=0.19 uncorrected, Supplementary Table 1).

4. Discussion

The etiology of AMD is multifactorial. Biochemical markers of disease activity continue to be uncovered, but biomarker-based treatments are not yet available for routine clinical care [10]. Although prevention for AMD itself is not currently possible, early diagnosis and intervention can ameliorate symptoms and slow progression of AMD. Because of this, a need exists to identify biomarkers for “high risk” individuals who are likely to develop AMD or experience rapid progression of the disease.

Compelling evidence links the immune system with AMD and inflammatory processes are reported to play key roles in AMD pathogenesis [2–9]. HLA antigens are expressed both in normal and AMD affected eyes [4, 5, 9]. Increased HLA immunoreactivity has been observed in AMD patient retinas and related to the formation of drusen, the extracellular accumulations that build up between the Bruch’s membrane and the retina pigment epithelium [6–8]. Given their role in innate and adaptive immune responses, polymorphisms in HLA class I and class II and the complementary KIR systems are logical candidates for association studies. The first report by Goverdhan et al. [11] identifying an association with HLA found that HLA-C*07:01 (then Cw*0701) increased AMD risk, whereas B*40:01 and DRB1*13:01 (then B*1401 and DRB1*1301) were inversely associated with AMD risk. Goverdhan et al. [12] also investigated both HLA and KIR ligand genotype associations with disease. HLA-C*07:01, in combination with the inhibitory KIR AA genotype [23] was associated with AMD, suggesting that natural killer cells have a role in the pathogenesis of AMD, possibly by modifying the effect of HLA class I allele groups on disease. This is the only association of KIR with AMD reported to date. In addition, Goverdhan et al. [12] used immunohistochemistry to demonstrate differential HLA class I expression in choriocapillary endothelial cells, suggesting that HLA polymorphisms may possibly influence the development of AMD via modulation of choroidal immune function. Becerril et al. [13] identified an association between HLA*B27 and AMD but did not detect disease associations with HLA-A or HLA-DRB1.

In conclusion, comprehensive HLA class II assessment in a population-based study of AMD did not reveal statistically significant associations and the previously observed HLA-DRB1*13:01 association with AMD did not replicate. The current study was sufficiently powered to identify novel associations as well as replicate the HLA-DRB1*13:01 association. The extreme polymorphic nature of the HLA gene regions, as well as sampling variation between cases and controls can easily lead to false-positive or false-negative associations, particularly in studies using small sample sizes thus independent replication of associations is critical. A recent genome-wide association study of >17,000 advanced AMD cases and >60,000 controls identified 19 loci associated at $P < 5 \times 10^{-8}$, however the results did not include significant HLA region associations [24]. Future studies should be expanded to include the HLA class I and KIR loci to provide a comprehensive understanding of the immunologic basis of AMD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of genotyped AMD cases and controls from the SOF eye study.

	Cases	Controls
N	456	499
Age, mean (SD)	79.7 (3.1)	78.0 (2.7)
Age, range	74–92	74–88
AMD stage, n (%)		
Early	320 (70.2)	
Late	136 (29.8)	
Clinic site, n (%)		
Baltimore, MD	30 (7)	28 (6)
Minneapolis, MN	194 (43)	228 (46)
Pittsburgh, PA	135 (30)	165 (33)
Portland, OR	92 (21)	77 (15)

Table 2

Overall HLA class II locus level associations for AMD.

Locus	χ^2 *	DF*	P value*
DRB1	18.2 (10.9)	24 (15)	0.80 (0.76)
DQA1	3.9 (7.8)	7 (7)	0.79 (0.35)
DQB1	11.6 (10.9)	13 (13)	0.56 (0.62)
DPB1	22.2 (17.2)	13 (12)	0.05 (0.14)

* indicates values from testing without Minneapolis, MN.

Table 3

Frequencies and results for HLA class II alleles (2-field resolution) with minor allele frequencies $\geq 10\%$ for 456 AMD cases and 499 controls.

HLA	Control 2N = 998	AMD 2N = 912	Logistic regression	
			OR (95%CI)	p
DRB1*03:01	117	105	0.96 (0.71–1.29)	0.77
DRB1*07:01	130	118	1.02 (0.77–1.36)	0.88
DRB1*15:01	140	131	0.96 (0.72–1.26)	0.75
DQA1*01:01	129	134	1.19 (0.9–1.57)	0.22
DQA1*01:02	203	182	0.93 (0.73–1.18)	0.54
DQA1*02:01	130	118	1.02 (0.77–1.36)	0.88
DQA1*03:01	189	156	0.91 (0.71–1.17)	0.46
DQA1*05:01	242	231	1.05 (0.84–1.31)	0.64
DQB1*02:01	116	107	0.99 (0.73–1.34)	0.94
DQB1*03:01	187	177	1.03 (0.81–1.31)	0.8
DQB1*03:02	109	94	0.99 (0.72–1.35)	0.93
DQB1*05:01	102	108	1.24 (0.91–1.69)	0.16
DQB1*06:02	129	126	1 (0.75–1.34)	0.98
DPB1*02:01	133	106	0.89 (0.66–1.19)	0.42
DPB1*03:01	102	92	0.99 (0.72–1.37)	0.97
DPB1*04:01	433	442	1.22 (1.01–1.48)	0.04
DPB1*04:02	117	109	0.93 (0.69–1.25)	0.61