Association of HLA Class I and Class II Polymorphisms with Age-Related Macular Degeneration

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Purpose. To evaluate whether HLA genotypes are associated with age-related macular degeneration (AMD).

Methods. HLA class I-A, -B, and -Cw and class II DRB1 and DQB1 principal allele groups were genotyped in two stages: initially for principal allele groups in a cohort of 100 AMD cases and 92 control subjects, and then, in the next 100 cases and controls from the same cohort, for alleles or allele groups with $P < 0.1$ on initial typing. Genotype frequencies were compared by 2×2 contingency tables. The strongest associations for individual HLA alleles were calculated with two-locus stratification analysis and logistic regression for all possible pairwise HLA combinations. Bonferroni corrections were applied for multiple measurements (P_c). Each HLA allele was subjected to logistic regression for known AMD covariates. HLA immunohistochemistry for class I antigens was performed on elderly donor eyes.

Results. Allele Cw*0701 ($P = 0.004$, $P_c = 0.036$) correlated positively with AMD, whereas alleles B*4001 ($P = 0.003$, $P_c = 0.027$) and DRB1*1301($P = 0.001$, $P_c = 0.009$) were negatively associated. These HLA associations were independent of any linkage disequilibrium. Immunohistochemistry demonstrated differential HLA class I expression in choriocapillary endothelial cells.

Conclusions. Significant positive and negative associations exist between HLA alleles and AMD. HLA polymorphisms influence the development of AMD, possibly via modulating choroidal immune function. (*Invest Ophthalmol Vis Sci.* 2005;46: 1726–1734) DOI:10.1167/iovs.04-0928

Age related macular degeneration (AMD) is the commonest cause of blindness in the elderly, accounting for approximately 50% of all cases of registered blindness in people aged >65 years,[1] with a prevalence of 20% to 30% in the Western world.[2] AMD is at least in part genetic[3–8]; however, inflammation may also play a causal role in its development.[9–12]

Drusen, which are pathognomonic of the AMD disease process, contain proteins that modulate the body's response to inflammation, including vitronectin, complement, and immunoglobulins.[9,13] Inflammatory cells, including macrophages,[14–16] multinucleate giant cells,[11,15,17] fibroblasts, and mast cells have been observed in association with Bruch's membrane in AMD donor eyes.[18] Recently, some characteristics of AMD have also been described in mice with macrophage defects,[19] further supporting an immunologic basis for the development of AMD.

A unifying hypothesis is that immune response gene polymorphisms modulate susceptibility to AMD. This could explain the roles of inflammation and genetic predisposition reported in AMD. In this context, human leukocyte antigen (HLA) polymorphisms, encoded within the major histocompatibility complex (MHC) are of particular interest. HLA genes are the most polymorphic within the human genome, with at least 309 HLA-A, 563 HLA-B, 167 HLA-C, 447 DRB, 27 DQA1, 56 DQB1, 20 DPA1, and 107 DPB1 alleles currently recognized.[20] These molecules are critical for regulation of the immune response, through presentation of processed antigenic peptides to both CD4 helper and CD8 cytotoxic T lymphocytes.

In the human eye, HLA antigens are expressed both normally and in eyes affected with AMD. Normal expression includes retinal microglia (class I and II antigens)[16,21] and uveal pigment epithelium and vascular endothelium (class I antigens).[22] In AMD, intense HLA-DR immunoreactivity has been demonstrated in both soft and hard drusen.[13] Increased HLA class II immunoreactivity has been observed in the human retina affected with AMD and related to drusen formation.[23] Intravitreal triamcinolone acetonide has shown some promise in the treatment of choroidal neovascularization (CNV) in AMD.[24,25] The mechanism of action is unknown, but triamcinolone acetonide does decrease retinal HLA class II expression[26] in AMD.

In other eye diseases, such as birdshot chorioretinopathy, specific HLA genotypes are a significant risk factor for the development of the disease. In this study therefore, we evaluated whether specific HLA genotypes are also a risk factor for the development of AMD and in addition whether HLA class I antigens are differentially expressed in the elderly human eye.

Methods

The study was approved by the Southampton and Southwest Hants Local Research Ethics Committee (approval no. 347/02/t) and adhered to the tenets of the Declaration of Helsinki.

Patients and Control Subjects

After providing informed, written consent, white subjects older than 55 years with a diagnosis of AMD and normal white control subjects older than 55 years were recruited from ophthalmology clinics at the Southampton General Hospital. Patients for the study underwent a detailed ophthalmic examination, and fundus photographs were re-

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corded with a digital retinal camera (model TRC50IX; Topcon, Tokyo, Japan). These photographs were graded by a masked observer into four groups of increasing disease severity, as described in the Age-Related Eye Disease Study (AREDS) study.[27] Each patient provided a peripheral blood sample from which DNA was extracted by standard methods.[28]

HLA Genotyping by PCR-Sequence-Specific Primers

Genotyping was performed in two stages: initially for principal allele groups in a cohort of 100 AMD cases and 92 control subjects, and then, in the next 100 cases and control subjects from the same cohort, for alleles and allele groups that showed a trend toward significance ($P < 0.1$) on initial typing. HLA class I-A, -B, and -Cw and class II DRB1 and DQB1 genotyping for principal allele groups was performed by PCR-sequence-specific primers (SSP).[29,30] PCR amplification was performed with panels of primers specific to 92 HLA Class I A, -B, and -Cw and 30 class II DRB1 and DQB1 allele groups, plus primers specific for the HLA-DRB3, -4, and -5 genes. Primer sequences were derived from the literature[31–34] and were produced in house according to published HLA-DRB allele sequences. Primers were combined into 24 separate PCR reaction mixes to detect 21 HLA-A alleles or allele groups, 48 reactions for 46 HLA-B alleles or allele groups, and 23 reactions for 23 HLA-Cw alleles. Similarly, primers were combined into 23 reaction mixes for DRB typing, detecting 22 HLA-DRB1 alleles or allele groups, plus the presence of the HLA-DRB3, DRB4, or DRB5 genes (encoding the DR52, 53 and 51 subtypes, respectively). Eight primer mixes for DQB typing were used, detecting 8 HLA-DQB1 alleles or allele groups. Each PCR reaction mix contained separate allele or allele-group–specific PCR primers, combined with an internal control PCR primer pair to test for successful PCR amplification. Pretested PCR reaction mixes were aliquoted into 96-well plates (for single sample HLA-A, -B, and -Cw typing or three arrays per plate for HLA-DRB1/DQB1 genotyping). The total volume of each PCR reaction was 10 μL. The reaction mix for each primer pair was 1 μL of 10× buffer (200 mM NH_4SO_4, 750 mM Tris-HCl [pH 9.0], and 0.1% Tween 20; Advanced Biotechnologies Ltd., London, UK), 1 μL dNTP mix (2 mM), 0.6 μL $MgCl_2$ (25 mM), 0.05 μL *Taq* polymerase (5 U/μL), and 0.5 μL DNA template. Each PCR mix contained 5′- and 3′-allele or allele group-specific PCR primers at a final concentration of 0.5 μM. Each reaction mix also contained a second primer pair (at a final concentration of 0.05 μM) which amplified a 429-bp fragment of the human growth hormone gene and functioned as an internal positive control for each PCR reaction. PCR products were visualized by running the entire reaction mix on a 2% agarose gel (Sigma-Aldrich, Poole, UK), prestained with ethidium bromide (0.5 mg/mL gel; Sigma-Aldrich), at 100 V for 20 minutes, in 1× Tris-acetate-EDTA (TAE) buffer. Gels were photographed under ultraviolet transillumination, and images were interpreted by two independent observers (SVG, MWH). Genotyping was validated by random repeat typing in 10% of cases and control subjects, for each of the HLA genotypes that showed a significant association with AMD in the study.

HLA Class I Immunohistochemistry

Human eyes from four donors, ranging in age from 76 to 100 years, were obtained from the Iowa Lions Eye Bank (Iowa City, IA). Cryostat sections containing the retinal pigment epithelium (RPE), choroid, and sclera, were obtained from a sagittal wedge, spanning the ora serrata to the fovea, embedded in optimal cutting temperature compound (Ted Pella, Inc.; Redding, CA) without fixation. The neural retina was included in one specimen. Frozen sections were incubated with a monoclonal antibody directed against a shared epitope on expressed HLA class I-A, -B, and -C molecules (clone W6/32HL; Chemicon, Temecula, CA) at a concentration of 2 μg/mL. In some experiments, the same sections were also incubated with antibodies directed against von Willebrand factor (Chemicon) at a concentration of 5 μg/mL, to visualize the choroidal vasculature. Immunohistochemical labeling was performed as described previously,[35] with Alexa-488- and Alexa-546-conjugated secondary antibodies (Molecular Probes, Eugene, OR). Negative controls included omission of primary antibody and comparison with antibodies directed against irrelevant antigens. To compare the labeling of MHC class I and class II antigens in drusen, adjacent unfixed cryostat sections were labeled with the monoclonal antibody W6/32HL and with a monoclonal antibody directed against the MHC class II proteins HLA-DP/DQ/DR (clone CR3/43; Dako, High Wycombe, UK). Digital photomicrographs were collected with a microscope (model BX41 microscope; Olympus, Tokyo, Japan) equipped with a fluorescence attachment and a digital camera (SPOT-RT; Diagnostic Instruments, Inc., Sterling Heights, MI).

Statistical Methods and Analysis

Power Calculations and Hardy-Weinberg Equilibrium Tests. Power calculations were performed with the Epi Info statistical package (ver. 2), provided by the Center for Disease Control and Prevention (CDC). These calculations were based on the number of cases and control subjects to be genotyped and the known frequency of specific HLA alleles. Allelic distributions for each locus were tested for conformity to Hardy-Weinberg equilibrium. This was achieved using PyPop statistical software[36] from the International Histocompatibility Working Group (IHWG) based on a methodology described by Guo and Thompson.[37] Alleles at each loci were said to be in Hardy-Weinberg equilibrium if the observed homozygote and heterozygote frequencies did not differ significantly ($P > 0.05$) from expected frequencies. PyPop was also used for linkage disequilibrium (LD) analysis and haplotype estimation when complete data were available.

Two-Locus Stratification Analysis. Genotype frequencies after first- and second-stage typing were compared in cases and control subjects by 2 × 2 contingency tables and two-tailed probabilities calculated using the Fisher exact test. Adjustment for multiple comparisons was made after second-stage typing by using the Bonferroni method—that is, $P \leq 0.05$ was multiplied by the total number of study comparisons (nine in the full study group). Alleles showing significant positive or negative associations were then compared for all two-locus combinations using methodology for HLA association studies, as described by Svejgaard and Ryder,[38] involving stratification of each allele against the other, to determine whether the HLA associations were independent of each other or were reflective of LD between the HLA loci concerned. The basic data for the analyses were provided by 2 × 4 tables, giving the frequencies of the four phenotypic combinations from patients and control subjects for the two alleles being compared. These data were analyzed as 2 × 2 tables involving stratification of one of the two factors against the other. Two-tailed probabilities were calculated with the Fisher exact test in all these analyses. When looking at two-locus associations with AMD, probabilities were corrected by a multiple of the number of alleles tested for at the two loci

Table 1. Basic Demographic Characteristics of the Study Population

	AMD Cases (n = 200)	Controls (n = 192)
Age (y)		
Mean	76.66	70.77
Range	55–95	55–91
SD	7.76	10.43
Sex n (%)		
Female	119 (59.5)	116 (60.4)
Male	81 (40.5)	76 (39.6)
BMI*		
Mean	26.73	27.19
SD	4.75	5.13
Smoking status n (%)		
Current smokers	28 (14.0)	22 (11.4)
Past smokers	97 (48.5)	93 (48.4)
Nonsmokers	75 (37.5)	77 (40.1)

* AMD Cases, n = 137; Controls, n = 173.

Table 2. AREDS Subgrouping of Cases According to the Severity of AMD in Affected Eyes

AREDS Grading	Phenotypic Characteristics of AMD (Changes in Worse Affected Eye)	AMD Cases *n* (%)
Grade I	Nonextensive small drusen or no drusen in fellow eye	20 (11.5)
Grade II	Extensive small or ≥1 intermediate-size drusen; pigment abnormalities	53 (30.4)
Grade III	≥1 Large drusen-63 μm diameter or geographic atrophy (GA) <1/8 DD	39 (22.4)
Grade IV	GA ≥1/8 DD or neovascular AMD (RPE detachments and CNV)	62 (35.6)

$n = 174$. DD, disc diameter.

involved—that is, one for HLA-B, three for HLA-Cw, two for DRB1, and three for DQB1. The level of statistical significance was set at $P < 0.05$ and $P_c < 0.05$ after application of the Bonferroni correction. Odds ratios (ORs) were estimated by using the approximation of Woolf, to avoid problems when critical entries were zero.[38] Logistic regression analyses were performed for each of the significant HLA alleles, using data on age, sex, body mass index (BMI), and smoking status as covariates in the regression model, between all the AREDS groups and with the control group. All statistical analyses were performed on computer (SPSS, ver. 12.0; SPSS Science, Chicago, IL).

Results

HLA Genotyping

Baseline demographics of the unrelated white cohort recruited from a single clinic population (200 patients with AMD [cases] and 192 age-matched normal control subjects [controls]) are described in Table 1. AREDS grading data from fundus photographs were available for 174 (87%) of the 200 AMD cases (Table 2). Alleles were distributed in accordance with Hardy-Weinberg equilibrium for all loci tested. The results from genotyping repeats performed in 10% of cases and controls were found to be 100% concordant with those obtained during initial genotyping. In total, genotyping data for 92 HLA class I-A, -B, and -Cw and 30 class II DRB1 and DQB1 principal allele groups were available for analysis after the first stage of genotyping. Nine alleles revealed a trend toward significance on initial typing, based on uncorrected $P < 0.1$: B*4001 ($P = 0.001$), Cw*0302 ($P = 0.099$), Cw*0701 ($P = 0.011$), Cw*0702 ($P = 0.035$), DRB1*1301 ($P = 0.0006$), DRB1*11 ($P = 0.035$), DQB1*0301 ($P = 0.056$), DQB1*0302 ($P = 0.019$), and DQB1*0303 ($P = 0.034$). These alleles were then genotyped in the next 100 cases and 100 controls. The allele frequencies for these antigens in the control group were similar to those previously reported in white U.K. populations.[39–41] HLA genotype frequencies for both class I and II alleles in the AMD group ($n = 200$) were compared with those in the normal control subjects ($n = 192$). Data analysis (Table 3) demonstrated a positive association with class I Cw*0701 ($P = 0.004$) and class II DQB1*0303 ($P = 0.016$) alleles, whereas a negative association was found with HLA class I alleles B*4001 ($P = 0.003$) and HLA class II alleles DRB1*1301 ($P = 0.001$) and DQB1*0302 ($P = 0.009$). The probabilities for negative associations of B*4001 ($P_c = 0.027$), DRB1*1301 ($P_c = 0.009$) and the positive association of Cw*0701 ($P_c = 0.036$) remained significant after correcting for multiple comparisons. The DQB1*0302 and DQB1*0303 associations, however were not statistically significant after correction. Two-locus stratification of alleles as shown in Tables 4, 5, and 6 showed that B*4001 was negatively associated with AMD in the DRB1*1301-negative (test 4) but not in the DRB1*1301-positive subjects (test 3). Likewise, DRB1*1301 was associated with AMD in B*4001-negative but not -positive individuals (test 6). Tests 9 and 10 did not demonstrate any association (i.e., LD) between B*4001 and DRB1*1301 in patients or control subjects. The results of comparisons between DRB1*1301 and DQB1*0302, B*4001 and DQB1*0302, and Cw*0701 and DQB1*0303 were similar (Table 6). DRB1*1301 and DQB1*0302 demonstrated strong apparent negative LD in the control population (test 10). An elevated OR of 5.82 was observed only in individuals carrying both the Cw*0701 and DQB1*0303 positively associated alleles (test 8) but was not significant after a Bonferroni correction ($P = 0.025$, $P_c = 0.23$). Similarly individuals expressing both B*4001 and DQB1*0302 alleles had a reduced OR of 0.1 ($P = 0.023$, $P_c = 0.07$, OR = 0.10, 95% CI: 0.01–0.84), whereas the effect was smaller when both B*4001 and DRB1*1301 were analyzed in combination ($P = 0.045$, $P_c = 0.09$, OR = 0.21, 95% CI: 0.04–1.04). However, in each case these effects were not significant after correction for multiple comparisons.

Table 3. HLA-A, B, Cw, DRB1, and DQB1 Genotype Frequencies in AMD Patients and Age-Matched Normal Control Subjects after Final-Stage Genotyping

HLA Allele	AMD Group *n* (%)	Control Group *n* (%)	*P**	P_c†	Odds Ratio‡	95% CI
B*4001	16 (8.0)	35 (18.0)	0.003§	0.027§	0.39	0.21–0.73
Cw*0302	30 (15.0)	27 (13.9)	0.776		1.09	0.62–1.91
Cw*0701	84 (42.0)	54 (28.1)	0.004§	0.036§	1.85	1.21–2.82
Cw*0702	43 (21.5)	54 (27.8)	0.161		0.71	0.45–1.13
DRB1*1301	14 (7.0)	37 (19.7)	0.001§	0.009§	0.31	0.16–0.60
DRB1*11	33 (16.5)	25 (12.9)	0.323		1.34	0.76–2.34
DQB1*0301	73 (36.5)	68 (35.05)	0.834		1.06	0.70–1.69
DQB1*0302	27 (13.5)	46 (23.7)	0.009§	0.081	0.50	0.29–0.85
DQB1*0303	23 (11.5)	9 (4.6)	0.016§	0.144	2.67	1.20–5.93

AMD group, $n = 200$; Control Group, $n = 192$.
* Two-sided, using the Fisher exact test.
† After application of Bonferroni correction ($n = 9$).
‡ According to the approximation of Woolf.
§ Significant at $P < 0.05$.

TABLE 4. Construct of Basic Data Used for 2 × 2 Stratification Analysis

A.

HLA-A Allele	HLA-B Allele	AMD Cases (*n*)	Controls (*n*)
+	+	X1	Y1
+	−	X2	Y2
−	+	X3	Y3
−	−	X4	Y4

B.

Test	Values for 2 × 2 tables				Allele Frequencies Tested with Interpretation for Each Test
	a	b	c	d	
1	X1 + X2	X3 + X4	Y1 + Y2	Y3 + Y4	A+ vs. A−, individual A association tested
2	X1 + X3	X2 + X4	Y1 + Y3	Y2 + Y4	B+ vs. B−, individual B association tested
3	X1	X3	Y1	Y3	A+ vs. B+, association of A independent of B
4	X2	X4	Y2	Y4	A+ vs. B+, association of A independent of B
5	X1	X2	Y1	Y2	B+ vs. A+, association of B independent of A
6	X3	X4	Y3	Y4	B+ vs. A+, association of B independent of A
7	X2	X3	Y2	Y3	Difference between A and B associations tested
8	X1	X4	Y1	Y4	Combined association of A and B tested
9	X1	X2	X3	X4	LD tested between A and B in AMD cases
10	Y1	Y2	Y3	Y4	LD tested between A and B in controls

These significant HLA associations were further confirmed by logistic regression analysis. Correcting for sex, age, BMI, current smoking, or previous smoking status again produced significant HLA associations for alleles B*4001 ($P = 0.001$), Cw*0701 ($P = 0.02$), and DRB1*1301 ($P = 0.0001$; Tables 7, 8, 9). This regression model was also applied in two-locus HLA analysis, by using frequencies from all possible pair-wise combinations of the five significant alleles found in the study. In total, 10 possible pair-wise combinations were tested. None of these alleles was found to influence or predict the second allele in the combination, thus excluding confounding LD between the alleles.

Further categorization was possible in 174 subjects in the AMD group, who were divided into four groups of increasing AMD severity, according to AREDS criteria. No significant difference was found in comparison of the 4 AREDS groups and alleles B*4001, Cw*0701, DRB1*1301, DQB1*0302, or DQB1*0303. A multivariate logistic regression analysis for these five alleles in relation to age, sex, BMI, and smoking status as covariates was performed between AREDS group IV and AREDS group I (Table 8). The only positive association was with current smoking. Smoking increased the risk of development of advanced AMD fourfold, irrespective of the allele tested ($P = 0.019–0.017$, OR = 4.55–4.79). No significant associations were found between the same covariates in the other AREDS groups or compared with the control group.

HLA Class I Immunohistochemistry

Immunohistochemical detection of HLA class I antigens was performed on four human donor eyes without remarkable disease, using a pan-HLA class I monoclonal antibody. The most notable labeling was observed in endothelial cells, particularly in the choriocapillaris but also in large vessels of the choroid (Fig. 1A). This pattern was different from that of the von Willebrand factor, which was detected in capillaries but

TABLE 5. Frequencies for Individual Significant Alleles (Basic Data) Used in the 2 × 2 Stratification Analysis

B*4001	DRB1*1301	DQB1*0302	Cw*0701	DQB1*0303	AMD Patients (*n* = 200)	Controls (*n* = 192)
+	+	—	—	—	2	7
+	−	—	—	—	14	28
−	+	—	—	—	12	30
−	−	—	—	—	172	129
—	+	+	—	—	0	0
—	+	−	—	—	14	37
—	−	+	—	—	27	46
—	−	−	—	—	159	111
+	—	+	—	—	1	7
+	—	−	—	—	15	28
−	—	+	—	—	26	39
−	—	−	—	—	158	120
—	—	—	+	+	9	2
—	—	—	+	−	75	52
—	—	—	−	+	14	7
—	—	—	−	−	102	132

TABLE 6. Results for Two-Locus Stratification of Alleles Analyzed by 2 × 2 Tables

Allele Comparison		Individual Associations		Independent A Association		Independent B Association		Difference between A & B Associations	Combined Association	Association between A and B (Testing LD)	
Factor A	Factor B	(1) A	(2) B	(3) ++ vs. −+	(4) +− vs. −−	(5) ++ vs. +−	(6) +− vs. −−	(7) +− vs. −+	(8) ++ vs. −−	(9) Patients	(10) Controls
B*4001	DRB1*1301	0.004 (0.39)	0.0005 (0.31)	1.000 (0.71)	0.0047 (0.37)	0.701 (0.57)	0.0008 (0.30)	0.814 (1.25)	0.045 (0.21)	0.310 (2.04)	0.817 (1.07)
DRB1*1301	DQB1*0302	0.0005 (0.31)	0.0097 (0.50)	NP	<0.0001 (0.26)	NP	0.0009 (0.40)	0.334 (0.64)	NP	0.224 (0.20)	<0.0001 (0.03)
B*4001	DQB1*0302	0.004 (0.39)	0.0097 (0.50)	0.245 (0.21)	0.0084 (0.40)	0.409 (0.26)	0.018 (0.50)	0.687 (0.80)	0.023 (0.10)	0.701 (0.40)	0.664 (0.76)
Cw*0701	DQB1*0303	0.006 (1.83)	0.0158 (2.67)	0.441 (2.25)	0.0082 (1.83)	0.200 (3.18)	0.065 (2.58)	0.632 (0.70)	0.025 (5.82)	0.825 (0.87)	1.000 (0.71)

Data from tests 1 to 10 indicate P_c for individual two-allele comparisons, whereas those in parentheses are ORs. NP, Fisher exact test not possible, as one column of 2 × 2 table had zeros.

showed much stronger labeling in large veins and arteries of the outer choroid (Fig. 1B). Labeling of large retinal vessels was also apparent with relatively little localization to retinal capillaries. Occasional RPE cells showed minor labeling along the basal aspect (data not shown). Vessels in the sclera as well as a population of scleral cells with an elongated morphology were immunoreactive. In contrast to MHC class II antigens,[13] drusen present in these specimens did not exhibit strong immunoreactivity for HLA class I (Figs. 1C, 1D).

DISCUSSION

It is important to define associations between HLA antigens and AMD, to provide new insights into the basic pathogenesis of AMD and to help define the at-risk population in which preventive measures may be applicable. This is particularly true, because the overwhelming majority of HLA gene polymorphisms result in functional amino acid substitutions in the expressed HLA molecules, thereby modulating the immune response to a vast range of antigens, with interindividual differences in immune responses to complex or simple antigens.[42] Due to the critical role of HLA in regulating the immune response, combined with its extensive polymorphism, it is perhaps unsurprising that many HLA gene polymorphisms have been linked to susceptibility in a large number of immunologically mediated diseases, including the retina and uvea of the eye, the skin, gut, endocrine, and joint systems.[43]

Association studies for a disease like AMD, which has multiple etiological factors are difficult and require a robust study design and execution. In our study, several steps have been taken to avoid confounding variables. These included ethnically matched patients and control subjects of similar ages, with both groups having been screened for AMD; appropriate study sample sizes; and the application of stringent statistical methods to detect the strength of the associations.

Because of the extreme polymorphic nature of the HLA region, sampling variation between cases and control subjects can easily lead to both false-positive and -negative associations. Accordingly, application of a Bonferroni correction factor for the number of allelic subtypes tested is recommended and was applied in this study. A two-step genotyping procedure was followed, permitting a realistic Bonferroni correction for a small group of candidate alleles, rather than an overconservative correction for all possible HLA alleles, which may cause even true positive associations to be discarded. The two-stage method of genotyping used in our study has been modified from a previously reported similar study in which candidate alleles were identified initially by comparing frequencies in patient versus population control subjects. These short-listed candidate alleles were then genotyped and compared between the two groups.[44] This approach reduces the number of multiple comparisons made after the second stage and helps minimize the type I error rate. In addition, DNA-based typing was used, which has a better allelic resolution and accuracy compared with serologic HLA typing.

Finally, LD occurs frequently between alleles of different HLA loci, whereby an allele from one locus often occurs more frequently in combination with an allele of a second locus than is expected from their individual allele frequencies. This occurrence can complicate studies of HLA and disease predisposition. In cases in which alleles of more than one locus show positive or negative associations with disease, it is therefore important to determine whether such associations are independent or reflect LD between the alleles concerned. Methods such as the two-locus stratification analysis described by Svejgaard and Ryder[38] and the use of relevant genomic software such as PyPoP therefore assume importance in excluding LD in

TABLE 7. Multivariate Logistic Regression Analysis in AMD Cases and Controls

	HLA Allele (total n = 310)	Age (n = 310)	Sex (M/F) (n = 310)	BMI (n = 310)	Current Smokers (n = 50)	Past Smokers (n = 198)
B*4001						
Multivariate OR	0.20	1.09	1.06	1.03	1.10	0.72
AMD vs. control	(0.001)*	(0.0001)*	(0.82)	(0.34)	(0.82)	(0.27)
Cw*0701						
Multivariate OR	1.82	1.09	1.00	1.03	1.26	0.77
AMD vs. control	(0.02)*	(0.0001)*	(0.99)	(0.24)	(0.60)	(0.35)
DR*1301						
Multivariate OR	0.12	1.10	0.97	1.03	1.25	0.91
AMD vs. control	(0.0001)*	(0.0001)*	(0.91)	(0.27)	(0.62)	(0.76)
DQ*0302						
Multivariate OR	0.73	1.09	1.05	1.03	1.27	0.80
AMD vs. control	(0.33)	(0.0001)*	(0.84)	(0.26)	(0.57)	(0.44)
DQ*0303						
Multivariate OR	3.19	1.09	1.06	1.02	1.18	0.74
AMD vs. control	(0.01)*	(0.0001)*	(0.83)	(0.36)	(0.71)	(0.29)

Probabilities are shown in parenthesis below each odds ratio. AMD cases, n = 200; controls, n = 192.
* Significant.

such studies and were applied in the present investigation. PyPoP also enables haplotype estimation. Such haplotype estimation was attempted in our study, but could not be applied to the whole study group, because full genotyping data were available only in the first half of the cases and controls. However, we did obtain haplotype estimates for two- and three-locus combinations for the first 100 cases and 92 controls for which complete genotype data were available. Each two-locus haplotype estimation revealed between 85 and 161 haplotypes, but their corresponding frequencies were too small for any meaningful analysis between the groups (data not shown).

After controlling for known AMD risk factors (smoking, age, and BMI) by logistic regression analysis alleles B*4001, DRB1*1301, and Cw*0701 remained significantly associated with AMD. Allele Cw*0701 increased susceptibility to AMD, whereas HLA-B*4001 and DRB1*1301 decreased susceptibility to AMD. Two-locus stratification and logistic regression analysis indicated that these associations were independent and not reflective of LD between alleles. For example, two-locus stratification and logistic regression analysis demonstrated that the negative association of HLA-B*4001 with AMD was present in DRB1*1301-negative, but not -positive, individuals, and the negative association of DRB1*1301 with AMD was present in B*4001-negative, but not -positive, individuals (Table 6). The apparent negative LD found between DRB1*1301 and DQB1*0302 in our control but not AMD subjects is most probably a reflection of well-described strong LD between DRB1*1301 and DQB1*06 alleles in white U.K. populations.[45,46] After Bonferroni correction, alleles DQB1*0302 and DQB1*0303 were not significantly associated with AMD. However, as their uncorrected probabilities were <0.05, these alleles would merit further investigation in an independent, larger study group.

The mean age of our control group was slightly lower than that of our patient group (70.77 years vs. 76.66 years). Therefore, some of our control subjects may subsequently have AMD. However, if so, the HLA associations described would be an underestimate. HLA associations with AMD may therefore be stronger than we describe. In addition, controlling for age during regression analysis did not alter the described HLA associations.

This study had 80% power to detect significant differences in allele frequencies between patients and control subjects at $\alpha = 0.05$. For example, for an allele occurring at a frequency of 10% in the control group, detectable ORs are 2.3 for a predisposing allele and 0.5 for a protective allele, with 95% CI. We

TABLE 8. Multivariate Logistic Regression Analysis between the AREDS-1 and AREDS-4 Groups among AMD Cases

	HLA Allele (total n = 82)	Age (n = 82)	Sex (M/F) (n = 82)	BMI (n = 58)	Current Smokers (n = 13)	Past Smokers (n = 40)
B*4001						
Multivariate-OR	1.390	1.001	1.139	0.968	4.579	1.250
AREDS-1 vs. AREDS-4	(0.73)	(0.95)	(0.75)	(0.45)	(0.019)*	(0.61)
Cw*0701						
Multivariate-OR	1.505	1.003	1.081	0.968	4.654	1.245
AREDS-1 vs. AREDS-4	(0.28)	(0.89)	(0.85)	(0.45)	(0.018)*	(0.62)
DR*1301						
Multivariate-OR	0.238	1.006	1.073	0.963	4.799	1.232
AREDS-1 vs. AREDS-4	(0.22)	(0.79)	(0.86)	(0.39)	(0.017)*	(0.63)
DQ*0302						
Multivariate-OR	0.771	1.001	1.154	0.970	4.553	1.235
AREDS-1 vs. AREDS-4	(0.61)	(0.95)	(0.72)	(0.47)	(0.019)*	(0.63)
DQ*0303						
Multivariate-OR	1.448	1.003	1.136	0.966	4.676	1.206
AREDS-1 vs. AREDS-4	(0.52)	(0.88)	(0.75)	(0.42)	(0.017)*	(0.67)

Probabilities are shown in parenthesis below each odds ratio. AREDS-1, n = 20; AREDS-4, n = 62.
* Significant.

TABLE 9. Logistic Regression Analysis Adjusting for Age

Regression Variable	B*4001	Cw*0701	DRB1*1301	DQB1*0302	DQB1*0303
OR without Age	0.39 (0.003)	1.85 (0.004)	0.31 (0.001)	0.50 (0.009)	2.67 (0.016)
Age-adjusted OR	0.39 (0.005)	1.71 (0.018)	0.24 (0.0001)	0.44 (0.004)	3.22 (0.006)

Probabilities are shown in parenthesis beside each odds ratio.

may therefore not have detected smaller HLA associations with this sample size.

This study broadens the role of HLA associations in ocular disease. Previous associations have all been in uveitic diseases—for example, the strong association of HLA-B27 with anterior uveitis.[47,48] HLA-A29 confers an increased risk of birdshot chorioretinopathy—up to 224 times higher in carriers than in noncarriers.[49,50] Sympathetic ophthalmia is associated with HLA-B1*04 and intermediate uveitis with HLA-DRB1*03.[51,52] Our statistical correction for multiple comparisons is at least as conservative, and in some cases more conservative, than that used in these accepted HLA associations with uveitic eye disease.

More than one HLA locus may be involved with predisposition to disease. This multilocus association is not uncommon and has been described in other diseases, including celiac disease[53] and insulin-dependent diabetes mellitus. The HLA-DQA1 and DQB1 alleles have been associated with the former, whereas in the latter case, several predisposing and protecting alleles and allele combinations have been identified.[54] Based on our findings, multiallelic HLA associations may also be important in AMD.

Logistic regression analysis was performed to evaluate relationships between the five HLA alleles associated with AMD (Table 7) and their effect on disease severity. Smoking in association with the HLA alleles conferred a fourfold increased risk of the development of advanced AMD (AREDS group IV; Table 8). Further investigation of these five HLA alleles in a larger study would be useful in determining the amount of risk contributed by these alleles toward advanced AMD and possibly CNV. Previously, significant associations have been reported between the HLA-DRB1*15 (P_c = 0.000001) and DQB1*06 (P_c = 0.00001) alleles and the development of CNV in presumed ocular histoplasmosis syndrome.[55] These findings, along with the data from the present study, support the notion that certain HLA alleles are associated with CNV in various retinal diseases.

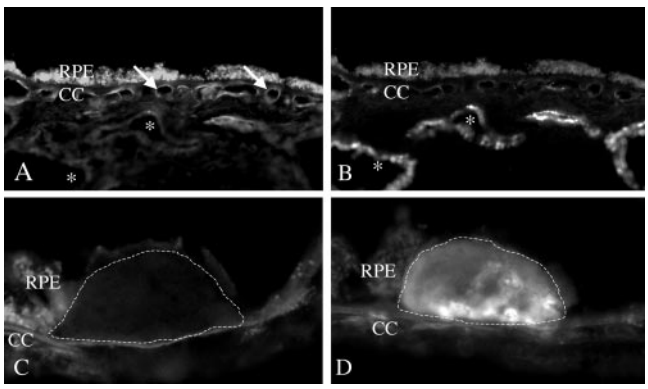

FIGURE 1. Immunolabeling of the RPE and choroid with a monoclonal antibody directed against HLA class I antigens (**A**) and polyclonal antibodies directed against von Willebrand factor (**B**) on the same section. The fluorescence of the RPE is due to lipofuscin autofluorescence. The endothelial cells with the most notable HLA labeling were cells of the choriocapillaris (CC, *arrows*), although other vessels were also weakly positive (✻). In contrast, robust labeling of large vessels was noted with antibodies directed against von Willebrand factor (**B**, ✻). Immunolocalization of HLA class I (**C**) and II (**D**) molecules in eyes with drusen revealed that, unlike MHC class II antigens,[13] MHC class I antigens are not detectable as major components of drusen or other sub-RPE deposits. *Dashed line*: drusen borders.

The localization of HLA class I antigens to choroidal endothelial cells is consistent with previous studies using tissues with longer postmortem times.[22,56] It is interesting that choriocapillary endothelial cells exhibit more robust expression than the endothelium of larger vessels, as visualized by the von Willebrand factor dual-labeling experiments. Endothelial cells in some tissues express HLA antigens constitutively[22,56] and cultured choroidal endothelial cells increase their expression of HLA class I antigens after exposure to certain cytokines. Whether these antigens are upregulated in endothelial cells or other ocular cell types in eyes with AMD remains to be determined, but this is an intriguing area of future study. It is also interesting that the expression patterns differ between HLA-I and -II antigens in the choroid and in sub-RPE deposits, suggesting possible differences in how these molecules may confer risk in macular disease. Notably, drusen contain MHC class II antigens[13] but do not appear to contain MHC class I proteins. The high level of expression of HLA class I antigens by choriocapillary endothelial cells could place the choriocapillaris at risk for T-cell-mediated attack in AMD and other inflammatory diseases, which may also be modified by specific HLA alleles.

The underlying mechanisms by which HLA genes determine susceptibility to various ocular diseases has not been fully elucidated yet, and it should be stressed that an HLA association determined by a case–control study does not necessarily implicate HLA polymorphism in causality, but may reflect LD with causal polymorphisms in other genes—for example, LD between HLA-A*03 and causal polymorphisms in the HFE gene which determine susceptibility to hereditary hemochromatosis.[57] Although we cannot exclude LD with non-HLA polymorphisms as causal in AMD, several lines of evidence, both indirect and direct, suggest a direct role for HLA in the disease process.

Firstly, a disease that is histologically similar to birdshot chorioretinopathy in humans has been shown to develop in HLA-A29 transgenic mice, confirming the functional role of the HLA-A29 molecule in the pathogenesis of this condition.[58] Thus, certain HLA alleles are known to promote the development of other inflammatory ocular diseases.

Second, and more important, accumulating evidence implicates immune as well as genetic and environmental components in the etiology of AMD. Research shows that the RPE is replete with the ability to synthesize molecules involved in the immune response.[59] Also, dendritic cells, which are potent antigen-presenting cells, have been intimately associated with drusen development, along with key complement pathways both within drusen and along the RPE-choroid interface, drusen being a constant feature and an important risk factor in all AMD phenotypes, occurring even in subclinical AMD phenotypes as typical small drusen.[9] HLA-DR and certain immune-related cluster differentiation (CD) antigens have been associated with core domains within drusen.[9] Immunohistochemical and ultrastructural studies have suggested that these cores are derived from cell processes of choroidal antigen-presenting

cells that breach the Bruch's membrane.[9] Accordingly, the HLA associations found in the present study suggest a potential direct role for HLA molecules in the cellular immune-mediated processes directed against specific local antigens or newly created antigens, particularly during drusen and AMD evolution.

Third, another mechanism that may indicate a direct role for HLA polymorphism in determining susceptibility to AMD is molecular mimicry, wherein antigenic cross-reactions occur between HLA antigens and extraneous trigger antigens, possibly from an invading organism. The latter itself may not induce a strong immune response, but instead may induce a localized autoimmune reaction responsible for the associated disease. Anti-*Chlamydia pneumoniae* antibodies have been found to be elevated in patients with AMD, suggesting an association of this pathogen with AMD.[60] The pathogenesis for this could be similar to that reported in acute anterior uveitis (AAU) in which potential Gram-negative bacterial antigens have been shown to alter the immune response of resident antigen-presenting HLA-DR(+) dendritic cells in the uvea.[61] Our findings reinforce the possible autoimmune nature of AMD as being a disordered response to inflammation or superantigens.

It is well established in genetic epidemiology research that it is important to evaluate any initial associations in at least one separate cohort, to validate initial findings. Following on from this hypothesis-generating study, further larger HLA-AMD association studies are needed in other populations to confirm these novel HLA associations. In addition, these studies, when possible, should be complemented by functional studies, to determine the contribution of these genes to the development of AMD. Dissecting the role of HLA and immune pathways in AMD may ultimately lead to opportunities to modulate these pathways by precise pharmacological means and thus improve the visual outcome in this devastating disease.

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References

1. Evans J. *Causes of Blindness and Partial Sight in England and Wales 1990–1991*: *Studies on Medical and Population Subjects*. London: Her Majesty's Stationary Office; 1995;57.
2. Royal College of Ophthalmologists. *A National Research Strategy for Ophthalmology.* London: Royal College of Ophthalmologists; 2002:5–10.
3. Heiba IM, Elston RC, Klein BE, Klein R. Sibling correlations and segregation analysis of age-related maculopathy: the Beaver Dam Eye Study. *Genet Epidemiol*. 1994;11:51–67.
4. Hammond CJ, Webster AR, Snieder H, Bird AC, Gilbert CE, Spector TD. Genetic influence on early age-related maculopathy: a twin study. *Ophthalmology*. 2002;109:730–736.
5. Meyers SM, Zachary AA. Monozygotic twins with age-related macular degeneration. *Arch Ophthalmol*. 1988;106:651–653.
6. Meyers SM, Greene T, Gutman FA. A twin study of age-related macular degeneration. *Am J Ophthalmol*. 1995;120:757–766.
7. Klein ML, Schultz DW, Edwards A, et al. Age-related macular degeneration: clinical features in a large family and linkage to chromosome 1q. *Arch Ophthalmol*. 1998;116:1082–1088.
8. Stone EM, Braun TA, Russell SR, et al. Missense variations in the fibulin 5 gene and age-related macular degeneration. *N Engl J Med*. 2004;351:346–353.
9. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retin Eye Res*. 2001;20:705–732.
10. Anderson DH, Talaga KC, Rivest AJ, Barron E, Hageman GS, Johnson LV. Characterization of beta amyloid assemblies in drusen: the deposits associated with aging and age-related macular degeneration. *Exp Eye Res*. 2004;78:243–256.
11. Penfold PL, Madigan MC, Gillies MC, Provis JM. Immunological and aetiological aspects of macular degeneration. *Prog Retin Eye Res*. 2001;20:385–414.
12. Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. *Arch Ophthalmol*. 2004;122:598–614.
13. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *FASEB J*. 2000;14:835–846.
14. Duvall J, Tso MO. Cellular mechanisms of resolution of drusen after laser coagulation: an experimental study. *Arch Ophthalmol*. 1985;103:694–703.
15. Killingsworth MC, Sarks JP, Sarks SH. Macrophages related to Bruch's membrane in age-related macular degeneration. *Eye*. 1990; 4:613–621.
16. Streilein JW, Wilbanks GA, Cousins SW. Immunoregulatory mechanisms of the eye. *J Neuroimmunol*. 1992;39:185–200.
17. Dastgheib K, Green WR. Granulomatous reaction to Bruch's membrane in age-related macular degeneration. *Arch Ophthalmol*. 1994;112:813–818.
18. Penfold PL, Killingsworth MC, Sarks SH. Senile macular degeneration: the involvement of immunocompetent cells. *Graefes Arch Clin Exp Ophthalmol*. 1985;223:69–76.
19. Ambati J, Anand A, Fernandez S, et al. An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. *Nat Med*. 2003;9:1390–1397.
20. IMGT/HLA Sequence Database, 2004. http://www.ebi.ac.uk/imgt/hla/.
21. Penfold PL, Provis JM, Liew SC. Human retinal microglia express phenotypic characteristics in common with dendritic antigen-presenting cells. *J Neuroimmunol*. 1993;45:183–191.
22. Bakker M, Grumet FC, Feltkamp TE, Kijlstra A. HLA-antigens in the human uvea. *Doc Ophthalmol*. 1986;61:271–279.
23. Penfold PL, Liew SC, Madigan MC, Provis JM. Modulation of major histocompatibility complex class II expression in retinas with age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 1997;38:2125–2133.
24. Gillies MC, Simpson JM, Luo W, et al. A randomized clinical trial of a single dose of intravitreal triamcinolone acetonide for neovascular age-related macular degeneration: one-year results. *Arch Ophthalmol*. 2003;121:667–673.
25. Jonas JB, Kreissig I, Hugger P, Sauder G, Panda-Jonas S, Degenring R. Intravitreal triamcinolone acetonide for exudative age related macular degeneration. *Br J Ophthalmol*. 2003;87:462–468.
26. Penfold PL, Wong JG, Gyory J, Billson FA. Effects of triamcinolone acetonide on microglial morphology and quantitative expression of MHC-II in exudative age-related macular degeneration. *Clin Exp Ophthalmol*. 2001;29:188–192.
27. Age-Related Eye Disease Study Research Group. Risk factors associated with age-related macular degeneration: a case-control study in the age-related eye disease study: age-related eye disease study report number 3. *Ophthalmology*. 2000;107:2224–2232.
28. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;16:1215.
29. Bunce M, O'Neill CM, Barnardo MC, et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens*. 1995;46:355–367.
30. Bateman AC, Sage DA, al Talib RK, Theaker JM, Jones DB, Howell WM. Investigation of specimen mislabelling in paraffin-embedded tissue using a rapid, allele-specific, PCR-based HLA class II typing method. *Histopathology*. 1996;28:169–174.

31. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens*. 1992;39: 225–235.
32. Schaffer M, Olerup O. HLA-AB typing by polymerase-chain reaction with sequence-specific primers: more accurate, less errors, and increased resolution compared to serological typing. *Tissue Antigens*. 2001;58:299–307.
33. Bunce M, Taylor CJ, Welsh KI. Rapid HLA-DQB typing by eight polymerase chain reaction amplifications with sequence-specific primers (PCR-SSP). *Hum Immunol*. 1993;37:201–206.
34. Olerup O, Zetterguist H. DR "low-resolution" PCR-SSP typing: a correction and an up-date. *Tissue Antigens*. 1993;41:55–56.
35. Hageman GS, Mullins RF, Russell SR, Johnson LV, Anderson DH. Vitronectin is a constituent of ocular drusen and the vitronectin gene is expressed in human retinal pigmented epithelial cells. *FASEB J*. 1999;13:477–484.
36. Lancaster A, Nelson MP, Single RM, Meyer D, Thomson G. PyPop: a software framework for population genomics: analyzing large-scale multi-locus genotype data. In: Altman RB, et al. (eds). *Pacific Symposium on Biocomputing 8*. Singapore: World Scientific; 2003:514–525.
37. Guo SW, Thompson EA. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*. 1992;48:361–372.
38. Svejgaard A, Ryder LP. HLA and disease associations: detecting the strongest association. *Tissue Antigens*. 1994;43:18–27.
39. Allele frequencies in worldwide populations; 2004. http://www.datatechniques.biz/allele/.
40. Darke C, Guttridge MG, Thompson J, McNamara S, Street J, Thomas M. HLA class I (A, B) and II (DR, DQ) gene and haplotype frequencies in blood donors from Wales. *Exp Clin Immunogenet*. 1998;15:69–83.
41. Finch T, Lawlor E, Borton M, et al. Distribution of HLA-A, B and DR genes and haplotypes in the Irish population. *Exp Clin Immunogenet*. 1997;14:250–263.
42. Howell WM, Calder PC, Grimble RF. Gene polymorphisms, inflammatory diseases and cancer. *Proc Nutr Soc*. 2002;61:447–456.
43. Thorsby E. Invited anniversary review: HLA associated diseases. *Hum Immunol*. 1997;53:1–11.
44. Follmann DA. Comparing HLA antigen frequencies between two groups of patients. *Stat Med*. 2003;22:1999–2013.
45. Thomson W, Barrett JH, Donn R, et al. Juvenile idiopathic arthritis classified by the ILAR criteria: HLA associations in UK patients. *Rheumatology* (*Oxford*). 2002;41:1183–1189.
46. Donaldson PT, Norris S. Evaluation of the role of MHC class II alleles, haplotypes and selected amino acid sequences in primary sclerosing cholangitis. *Autoimmunity*. 2002;35:555–564.
47. Derhaag PJ, Linssen A, Broekema N, de Waal LP, Feltkamp TE. A familial study of the inheritance of HLA-B27-positive acute anterior uveitis. *Am J Ophthalmol*. 1988;105:603–606.
48. Derhaag PJ, de Waal LP, Linssen A, Feltkamp TE. Acute anterior uveitis and HLA-B27 subtypes. *Invest Ophthalmol Vis Sci*. 1988; 29:1137–1140.
49. Baarsma GS, Priem HA, Kijlstra A. Association of birdshot retinochoroidopathy and HLA-A29 antigen. *Curr Eye Res*. 1990;9(suppl): 63–68.
50. Bloch-Michel E, Frau E. Birdshot retinochoroidopathy and HLA-A29+ and HLA-A29-idiopathic retinal vasculitis: comparative study of 56 cases. *Can J Ophthalmol*. 1991;26:361–366.
51. Kilmartin DJ, Wilson D, Liversidge J, et al. Immunogenetics and clinical phenotype of sympathetic ophthalmia in British and Irish patients. *Br J Ophthalmol*. 2001;85:281–286.
52. Oruc S, Duffy BF, Mohanakumar T, Kaplan HJ. The association of HLA class II with pars planitis. *Am J Ophthalmol*. 2001;131:657–659.
53. Lopez-Vazquez A, Rodrigo L, Fuentes D, et al. MHC class I chain related gene A (MICA) modulates the development of coeliac disease in patients with the high risk heterodimer DQA1*0501/DQB1*0201. *Gut*. 2002;50:336–340.
54. Cavan DA, Jacobs KH, Penny MA, et al. Both DQA1 and DQB1 genes are implicated in HLA-associated protection from type 1 (insulin-dependent) diabetes mellitus in a British Caucasian population. *Diabetologia*. 1993;36:252–257.
55. Dabil H, Kaplan HJ, Duffy BF, Phelan DL, Mohanakumar T, Jaramillo A. Association of the HLA-DR15/HLA-DQ6 haplotype with development of choroidal neovascular lesions in presumed ocular histoplasmosis syndrome. *Hum Immunol*. 2003;64:960–964.
56. Abi-Hanna D, Wakefield D, Watkins S. HLA antigens in ocular tissues. I. In vivo expression in human eyes. *Transplantation*. 1988;45:610–613.
57. Gandon G, Jouanolle AM, Chauvel B, et al. Linkage disequilibrium and extended haplotypes in the HLA-A to D6S105 region: implications for mapping the hemochromatosis gene (HFE). *Hum Genet*. 1996;97:103–113.
58. Szpak Y, Vieville JC, Tabary T, et al. Spontaneous retinopathy in HLA-A29 transgenic mice. *Proc Natl Acad Sci USA*. 2001;98:2572–2576.
59. Enzmann V, Hollborn M, Wiedemann P, Kohen L. Molecular and cellular evidence for T-cell stimulation by allogeneic retinal pigment epithelium cells in vitro. *Graefes Arch Clin Exp Ophthalmol*. 2001;239:445–451.
60. Kalayoglu MV, Galvan C, Mahdi OS, Byrne GI, Mansour S. Serological association between Chlamydia pneumoniae infection and age-related macular degeneration. *Arch Ophthalmol*. 2003;121: 478–482.
61. Chang JH, McCluskey P, Wakefield D. Expression of toll-like Receptor 4 and its associated lipopolysaccharide receptor complex by resident antigen-presenting cells in the human uvea. *Invest Ophthalmol Vis Sci*. 2004;45:1871–1878.