Article

HLA B27 as Predisposition Factor to Suffer Age Related Macular Degeneration

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To research whether specific alleles HLA class I (HLA-A and HLA-B) and class II (HLA-DR) are risk factors for the development of exudative type of Age Related Macular Degeneration (ARMD), HLA antigens are expressed both in normal and affected eyes with ARMD. We designed a prospective case-controlled study. We recruited 75 patients with choroidal neovascularization predominantly classic or occult, secondary to ARMD, and treated with photodynamic therapy. Two hundred and fifty patients over 55 years old, without ophthalmologic pathology who went to hospital for an analytical routine check were used as control. The analysis of the data shows a significant difference between two groups. Allele HLA-B27 correlated positively with ARMD ($p < 0.0113$). However, we didn't find alleles negatively associated. Thus HLA-B27 is an allele predisposed to suffer ARMD. *Cellular & Molecular Immunology*. 2009;6(4):303-307.

Key Words: HLA alleles, ARMD, HLA B27

Introduction

Age related macular degeneration (ARMD) is the most common cause of blindness in people over 60 years old, and this pathology tends to increase due to the progressive ageing of the population (1, 2).

The types of ARMD associated with visual acuity loss are divided into atrophic (dry) and exudative (choroidal neovascularization). The neovascular form can also be divided according to the angiographic pattern in classic and occult. The classic choroidal neovascularization (CNV) consists in a well defined focal area, which has an increase of fluorescence in early phases of the angiogram (3-5). The occult CNV, is not clearly identifiable in the initial or mid phases of the angiogram. Sometimes both forms can coexist. The recent development of the photodynamic therapy with verteporfin (PDT), for the treatment of CNV has created considerable interest due to its efficiency in neo-vascularization produced in ARMD, especially if it is administered in the early stages of the pathology. It is therefore important to detect the risk population in order to have an early diagnosis, and start treatment promptly.

One of the initial sign of this disease is the presence of drusen in the macular area (6). Drusen plays an important role in the injury of retinal pigment epithelium by causing inflammatory modulator effects and later the development of CNV (7). It is also known that the immune system is involved in the development of ARMD (8-10). On the other hand, it is also known that certain genetic basis exists in this pathology (11, 12).

A possible hypothesis that could unify the predisposition genetics and the participation of the immune system, would involve polymorphic genes important in the immune response that could modulate susceptibility to ARMD. In this sense, the study of the polymorphism of genes encoded within the major histocompatibility complex (HLA) could be of particular interest. HLA genes play a crucial role in regulation of the immune system and its genes are the most polymorphic within human genome (13, 14).

HLA antigens are expressed both in normal and affected eyes with ARMD (15, 16). The increase of HLA molecules has been observed in the retina of patients affected with ARMD and related to the drusen formation (17).

On the other hand, although the mechanism of action is unknown, it seems that the intravitreal triamcinolone acetonide has shown some promise in the treatment of this pathology (18, 19). After the treatment, decrease in the HLA expression was observed in the retina (20). Different molecules of HLA class I and II are significant risk for the development of other ophthalmologic pathologies such as birdshot chorioretinopathy, uveitis or disease of Behçet (21,

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Therefore, in this study we considered whether the presence of specific HLA alleles class I (HLA-A and HLA-B) and class II (HLA DR) are risk factors to exudative ARMD.

Materials and Methods

A total of 75 patients with a diagnosis of ARMD and 250 healthy subjects used as control who were older than 55, were recruited in our hospital, between May 2004 and November 2005. All the patients and controls gave their consent, under the criteria of Research and Ethics Committees.

All the patients with CNV classic or occult treated with PDT were included in this inclussion criteria: patients showed secondary subfoveal/yuxtafoveal neovascular membranes secondary to ARMD diagnosed by fluorescein angiography, predominantly classical with visual acuity between 3/60 and 20/60 and the size of CNV less than 5,400 μm of diameter. Also patients with occult CNV, with visual acuity less than 20/50 or CNV smaller than 4 disc areas with visual acuity of 20/50 or better. Patients who had less than 50% classic component CNV and those produced by other ethiologies that were not ARMD, were excluded from the study.

Genotyping HLA

DNA isolation: The DNA was isolated from samples of blood collected into ACD using a high quality DNA extraction Kit: QIAamp DNA Blood Mini Kits (QIAGEN). DNA was re-suspended in de-ionised water and stored at -20ºC until its use for the PCR.

Typing HLA: The generic typing of HLA A, B and DR was performed by a technique of PCR-SSO (Dynal RELI™ SSO HLA-A, HLA-B and HLA-DRB typing Kit of Dynal Biotech A.S.A, Oslo, Norway). The protocol provided by the manufacturer and the reagents (primer, Taq DNA polymerase and deoxynucleoside triphosphates) included in the commercial kit were used. Briefly: the test is based on three major processes: PCR target amplification, hybridisation of the amplified products to an array of immobilized sequence-specific oligonucleotide probes, and detection of the probe-bound amplified product by colour formation. The HLA alleles are assigned by reading the pattern of positive signals on the typing strip and analyzed with specific software (Dynal Biotech pattern matching program 5.41). Due to the complex nature of the HLA polymorphism an unambiguous result is possible. In those cases a second test using a higher resolution method was used. For higher resolution of genes HLA-A and HLA-B a PCR-SBT method was used and automatic genetic analyzer 3700 of Applied Biosystem (AlleleSEQR HLA-A and B PCR/sequencing Kit manufactured by Atria Genetics, Inc, San Francisco, USA). For HLA-DRB Inno-Lipa HLA- DRB of Innogenetics N.V. Gante, Belgium was used. This is also a PCR-SSO technique but with more probes and different specific amplifications. With a specific software we assigned specific HLA alleles (LIRAS software for INNO-LIPA HLA V5.00).

Table 1. Sex and age values

	Frequency	%	Max age	Min age	Average age
Female	40	53.33	89	63	76.55
Male	35	46.66	88	63	77.77
Total	75	100	89	63	77.16

Statistical analysis

A bivaried statistical analysis was made to verify the relation between the presence of alleles and the existence of ARMD, by means of EPIDAT 3.0 program (OPS). Contigency tables 2×2 were created, and the odds ratio (OR) was considered with its corresponding intervals of confidence for 95% (CI 95%). The statistic hypothesis of contrast was the Chi-Square ($p < 0.05$), or when the exact test of Fisher was not possible. To compare the averages of age according to sex, Student's *t* test of was applied or else nonparametric test (or of Mann-Whitney).

Results

The average age of the population with ARMD (75 patients) was 77.16 years old. A total number of 40 women with this disease participated in the study with an average age of 76.55 years old while the number of men was 35 with an average age of 77.77. The average age of the reference population (healthy, N = 250) was 74.65 years. In the control, the percentage of women was 57.2% (N = 143) and of men was 42.8% (N = 107) (Table 1).

The results of the typing of HLA alleles class I (A and B) and HLA class II (DRB1) were obtained from 75 patients with ARMD and 250 healthy controls were to compared with them (Table 1).

Genotyping HLA A

The patients with ARMD have most of the alleles present in the control population. HLA-A found more frequently in patients with ARMD was HLA-A2 with a frequency of 26%. In controls HLA-A2 was also the most common with a frequency of 24.8%. The frequency of different alleles HLA-A from the patients with ARMD was compared with the controls (Table 2). The analysis of the data did not demonstrate statistical significant differences between two groups. Positive association (alleles of predisposition) or alleles protectors have not been found (Table 2).

Genotyping HLA B

Most of the alleles present in control group, were also found in the patients with ARMD. HLA alleles more frequently found in patients with ARMD were HLA-B*44 and *51, with equal frequency both of 10.66%. In controls the most frequent was HLA-B*44 with a frequency of the 16.4%. The frequency of the different alleles HLA-B of the patients with ARMD, were compared with the controls (table 3). The analysis of the data demonstrated a significant association

Table 2. Frequency for the different alleles A in cases and control groups

Alleles A	Control (n = 250)		ARMD (n = 75)	
	Number	Frequency	Number	Frequency
1	52	10.4	10	6.66
2	124	24.8	39	26
3	45	9	13	8.6
11	26	5.2	9	6
23	26	5.2	3	2
24	45	9	14	9.33
25	8	1.6	4	2.6
26	37	7.4	7	4.6
29	42	8.4	13	8.6
30	32	6.4	11	7.33
31	12	2.4	3	2
32	17	3.4	9	6
33	17	3.4	4	2.6
34	1	0.2	1	0.6
28	16	3.2	10	6.66

between both groups. HLA-B27 was more frequent in the group with ARMD ($p = 0.0113$). The odds ratio obtained was of 2.9. These data suppose that HLA-B*27 increase susceptibility to ARMD. We have not found protector alleles for this pathology (Table 3).

Genotyping HLA DR

Most of the alleles present in the controls were also present in patients diagnosed ARMD. Gen HLA found more frequently in patients with ARMD was HLA-DRB1*13 with a frequency of the 13.33%. However, in the controls the alleles of HLA with greater frequency was HLA-DRB1*07 with 20.2% (Table 4). The frequency of different alleles HLA-DR from patients with ARMD were compared with the controls. Analysis of the data did not demonstrate any significant difference between the two groups; so there was no positive association (alleles of predisposition) or protective alleles (Table 4).

Discussion

In our study the 75 patients presented a very defined form of CNV and all had a very similar pathology. These patients included those with treatable-classic or occult-CNV, but excluded cases of CNV whose origin was not ARMD. We think that such a homogeneous group can avoid factors of confusion at the time of valuing the relation of HLA with this pathology.

Few works that study the relation between HLA and ARMD have been published. These works present differences with ours. Thus, Goverdham studies the existing relation between ARMD and the HLA, including 100 patients

Table 3. Frequency for the different alleles B in cases and control groups

Alelles B	Control (n = 250)		ARMD (n = 75)	
	Number	Frequency	Number	Frequency
7	40	8	7	4.6
8	18	3.6	4	2.6
13	12	2.4	5	3.33
14	40	7.8	10	6.6
15	28	5.6	3	2
18	37	7.4	18	12
27	12	2.4	10	6.6
35	43	8.6	11	7.33
37	6	1.2	3	2
38	30	6	4	2.66
39	8	1.6	6	4
40	18	3.6	10	6.66
41	9	1.8	0	0
42	3	0.6	1	0.6
44	82	16.4	16	10.6
45	11	2.2	5	3.33
47	2	0.4	1	0.6
48	1	0.2	0	0
49	19	3.8	5	3.33
50	10	2	5	3.33
51	33	6.6	16	10.66
52	8	1.6	3	2
53	6	1.2	3	2
55	6	1.2	1	0.66
56	3	0.6	1	0.66
57	12	2.4	1	0.66
58	4	0.8	1	0.66

with ARMD and 92 controls (12). In that work they established 4 groups of patients according to age-related eye disease study (AREDS study) of which only a part of the fourth group (N = 62) includes patients with choroidal neovascularization (less than us and not describe what kind of wet ARMD-treatable, non treatable) (23).

So it would be of great interest to compare the HLA obtained between each of the four groups of AREDS study, and not all together in order to study if the HLA can establish differences between different ethiologies from the CNV. In the ARMD genetic and inflammatory factors are involved as much in the beginning as in the development of this pathology (8, 11). The genetic polymorphism of the HLA and its importance in the immune response, would explain the high number of associations found between certain HLA alleles and different immune diseases (24-26).

An association study between HLA and a disease similar to CNV, which has different ethiology factors, is complicated. That is the reason why a population which is homogenous

Table 4. Frequency of the different alleles DR in ARMD and control groups

Alelles DR	Control (n = 250)		ARMD (n = 75)	
	Number	Frequency	Number	Frequency
1	60	12	17	11.33
3	57	11.4	17	11.33
4	55	11	17	11.33
7	101	20.2	21	14
8	10	2	5	3.33
9	4	0.8	4	2.66
10	12	2.4	3	2
11	49	9.8	18	12
12	2	0.4	1	0.6
13	73	14.6	20	13.33
14	17	3.4	9	6
15	54	10.8	15	10
16	6	1.2	3	2

should be chosen for this study (characteristic of our group) and it must be compared to a control of equal race, similar age and proportion of sexes. Our results suggest a positive association between HLA-B27 and ARMD. The most significant study is Goverdhan's, who finds that allele Cw*0701 implies a greater predisposition to ARMD, whereas alleles B*4001 and DRB1*1301 are protective. In our study we did not analyze HLA-Cw but nevertheless, HLA-B27 is in linkage disequilibrium in our zone with Cw 0102 and 0202 (data not shown) but this linkage disequilibrium is not described with Cw 0701. This indicates that in our population, the association is really with B27 and not by a mistake due to linkage disequilibrium with Cw0701 (27, 28). On the other hand, the mentioned study is of an English population and therefore different from the group we studied, which was a Mediterranean population of the south of Spain (29-31). However, great numbers of studies that associate HLA-B27 with different pathologies do exist (32-34), such as spondyloarthropathies (mainly the ankylosing spondylitis). A powerful association between HLA-B27 and anterior uveitis has also been well described, given the relation between this molecule and certain rheumatic diseases.

In addition to the anterior uveitis there are different ocular pathologies that are also associated to HLA (22). Different associations with molecules HLA class I (A29 and Birdshot chorioretinopathy) or class II (DR3 and likeable intermediate uveitis and DR4 and sympathetic ophthalmia) have been described. Therefore one HLA class I molecule and in concrete B27 can be a good candidate of susceptibility to an ophthalmic pathology.

We have only found one association (HLA B27). This is significant data as there can be pathologies whose association have been described with more than one allele (35), in the majority of cases there is one association that is predominant and that confers greater relative risk (DR2 and narcolepsy, B27 and EA, Celiac disease and DQ2 etc). It is the case of Celiac disease in which the association with DQ2 and DQ8 has been described although almost all the patients are DQ2 (36-38).

Our data indicate that HLA-B27 is a factor of risk of ARMD. Later studies will demonstrate that wether if this molecule participates in the pathogenesis of this disease. In this sense we know that different cellular types of the eye express HLA class I, such as endothelial cells of choroids, uvea, cells of microglía of the retina (15, 16, 39). It is also known that different factors can increase the HLA class I expression and even induce the molecule expression HLA in places where they are not expressed habitually (40, 41). The expression of these molecules in the eye could be the target of the immune attack that would finish causing the inflammatory process. This hypothesis could partly explain the pathogenesis of this disease.

Different patterns from expression of molecules of HLA class I and II in choroid is of great interest, because it would explain the association with molecules of HLA class I but not HLA class II (39). The fact that endothelium of choroids capillaries express HLA class I in a high percentage, could indicate this to be the target of the immune attack in ARMD and other inflammatory ophthalmic diseases that also are associated to certain HLA alleles.

The possible associations between certain HLA alleles and the ARMD can prove interesting to know pathogenesis of this pathology and to define a risk population who could suffer it. In this population of risk, periodic ophthalmic explorations could be initiated to screen this pathology, and it would also be recommended to broaden studies of the families with predisposition, and to start oral antioxidant treatment that is available on the market. We could approach the disease in its initial course and thus initiate its treatment precociously.

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