**Role of Major Histocompatibility Complex Genes in the Susceptibility and Protection of Primary Open Angle Glaucoma and Primary Congenital Glaucoma**

Félix Gil-Carrasco¹, Marla Alvarez-Padilla¹, Susana Hernández-Doño², José Ponce-Coria²,³, Rafael García-Silva² and Julio Granados*²

¹Glaucoma Department, Asociación para Evitar la Ceguera en México (APEC), Mexico City, Mexico
²Transplant Department, Immunogenetics Division, Instituto Nacional de Ciencias Medicas y Nutricion, Salvador Zubiran, Mexico
³Departamento de Atención a la Salud, Universidad Autónoma Metropolitana Unidad Xochimilco, Mexico City, México

**Abstract:**

**Introduction:** Glaucoma is a prevalent disease seen in the Ophthalmology department that includes a group of neurodegenerative eye pathologies associated with total loss of vision. It is known for its clinical diversity and secondary to this, it is assumed that multiple genes play a role in its pathogenesis. Among these, those that regulate the immune response which includes the HLA genes are of particular interest because they have been associated with a subgroup of glaucoma patients known as Primary Open Glaucoma.

**Methods:** In this study, we studied 3 different groups of patients with glaucoma in whom HLA alleles were determined by sequence-specific primers (SSP) technique.

**Results:** An association of HLA-DRB1*16 was found with the susceptibility to develop Primary Congenital Glaucoma. In addition, HLA-DRB1*14 was associated with glaucoma without angular dysgenesis, and HLA-DRB1*03 to glaucoma with iridocorneal dysgenesis.

**Conclusion:** In conclusion, the data obtained allow us to suggest that glaucoma is a clinical and genetically heterogeneous disease in which one of the subgroups has an autoimmune mechanism in which the Mexican mestizo population shows genetic susceptibility and it differs from POAG with angular dysgenesis and POAG without dysgenesis.

**Keywords:** Glaucoma, Heterogeneous disease, POAG, Dysgenesis, HLA, Autoimmune mechanism.

**1. INTRODUCTION**

Glaucoma is a term that describes a group of neurodegenerative eye pathologies characterized by the progressive and irreversible destruction of ganglion cells of the retina that is accompanied by morphological changes in the retina, among which the excavation of the optic nerve stands out. These neurodegenerative processes are associated with decreased visual field followed by total loss of vision [1 - 3].

Primary open-angle glaucoma (POAG) has been defined as a progressive disease of the ganglion cells of the retina characterized by structural changes in the optic disc and by slow and progressive loss of vision [4]. It is a clinically heterogeneous and also heterogeneous disease from the genetic point of view.

Genes related to glaucoma are known, but given the clinical diversity of the condition, the existence of multiple genes additional to those already described is assumed [5 - 7].

Among the relevant genes to study, are those that regulate
the immune response within the short arm of Chromosome 6, in the region of the Major Histocompatibility Complex (MHC), which includes the HLA genes, the genes of the tumor necrosis factor-alpha (TNF-ALPHA), and complement genes, among others [8, 9].

The MHC studies in Mexican patients showed an association of the allele HLA-DRB1*03-DQB1*0201 and of the allele HLA-DRB1*04 (0407) - DQB1*0302 with POAG [10, 11].

In this work, we included 3 different groups of patients with glaucoma in whom the MHC genes were studied in order to first understand the role of the genes that regulate the immune response in each of the glaucoma groups and secondly the role of ethnicity in genetic susceptibility to the development of glaucoma.

2. MATERIALS AND METHODS

2.1. Patients

A total of 45 patients from the ophthalmology department of the Association to Prevent Blindness in Mexico (APEC) were divided into 3 groups: group 1 consisted of 14 patients with Primary Congenital Glaucoma (PCG). Group 2 included 16 patients with a diagnosis of POAG with dysgenesis alterations of the iridocorneal angle (long iridian processes, irregular or abnormally high iris insertion, etc). Finally, group 3 comprised 15 patients with POAG without dysgenesis alterations.

In the three groups, the allele gene frequencies and haplotypes of HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 were determined.

2.2. Inclusion and Exclusion Criteria for each Group

A complete ophthalmological examination was performed in all patients, including visual acuity, slit-lamp examination, intraocular pressure measurement, gonioscopy, and measurements of the corneal diameter.

For group 1, the diagnosis of congenital glaucoma was made when the ophthalmological examinations were consistent with the diagnosis of congenital glaucoma, including children <3 years olds with elevated IOP (measured in the operating room with Schiotz tonometer and/or Icare Pro, established by curve adjusted to age), increased corneal diameter, corneal edema, increased axial length, and glaucomatous cupping of the optic nerve.

Almost 100% of the patients with the diagnosis of congenital glaucoma required surgical procedures for the treatment of the pathology, and most of them required conventional angle surgery such as goniomony or trabeculotomy ab externo; 65 percent of the patients with the diagnosis required the use of antiglaucoma drops, being the most frequently used beta-blockers and carbonic anhydrase inhibitors.

The inclusion criteria for group 2 were adult patients (>40 years old) who had a diagnosis of POAG with Goniodysgenesis (dysgenesis iridocorneal angle), with the following characteristics: intraocular pressure exceeding 24 mmHg, optic nerve damage, and abnormalities/malformations of the iridocorneal angle in the gonioscopic exam including at least one long iridian process, abnormally high insertion of the iris, anomalous line of Schwalbe, opaque pre-trabecular membrane, an iridocorneal sine qua, and irregular insertion of the iris.

Glaucoma was confirmed with abnormalities in visual fields and abnormalities of the nerve fiber layer and ganglion cell complex in optic coherence tomography (OCT).

All of the patients of group 2 were on antiglaucoma drops and most of them were also treated with glaucoma filtering surgery (trabeculectomy and Ahmed glaucoma valve).

For group 3, the inclusion criteria were adult patients (>40 years old) with a diagnosis of glaucoma made by intraocular pressure exceeding 24 mmHg and/or optic nerve damage, confirmed by abnormalities in visual fields and abnormalities in optic coherence tomography of the nerve fiber layer and ganglion cell complex consistent with glaucoma. In the gonioscopic exam, an open angle without angle abnormalities was required.

For this group, the most frequent treatment used was antiglaucoma drops (prostaglandin analogues and beta-blockers in 90% of them), followed by filtering surgery and SLT (selective laser trabecuoplasty).

Exclusion criteria for all groups were the presence of other ocular pathologies and other types of glaucoma, including secondary open-angle glaucomas, glaucomas associated with nonacquired ocular anomalies, and glaucoma associated with acquired conditions. Furthermore, patients with systemic pathologies were excluded.

All controls/ normals were examined in general practice (not ophthalmologic).

Inclusion criteria were no history of ophthalmologic problems, no known family members with glaucoma, and normal (corrected) vision.

2.3. HLA System Gene Typification

Genetic variants (alleles) of the loci HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 were studied using the technique of sequence-specific primers (SSP) after DNA amplification using the Polymerase Chain Reaction (PCR).

Genomic DNA was extracted from peripheral blood mononuclear cells and developed using the InVitro Gene reactants (California USA).

Gene frequencies and haplotypes of the MHC loci in the three study groups were established and were compared with those obtained in a group of 99 healthy Mexican mestizo individuals with no family history of glaucoma.

The differences between the patients with glaucoma and the control group and between each of the glaucoma groups with the control group were analyzed by nonparametric statistics that included Chi-square test and Fisher exact test, using Epi Info 11.0 statistical package, which includes STAT CALC subprogram, based on 2x2 contingency tables.
Significant statistical difference was established when $P$ values were lower than 0.05.

3. RESULTS

Table 1 shows the gene frequencies of HLA-DRB1 alleles in patients with PCG (group 1) and POAG with angular dysgenesis (group 2) compared to healthy Mexican Mestizo controls.

As can be seen, there is a significant increase in the HLA-DRB1*16 alleles in patients of groups 1 and 2 ($P$=0.05, OR=3.5, 95% CI=0.8-14.6). In the same way, a statistically significant decrease of the HLA-DR7 allele was found in these groups when compared with healthy individuals ($P$=0.03) and finally, an increased tendency of the HLA-DRB1*03 allele compared to healthy ones was observed ($P$ = 0.09).

When separating group 1 (PCG) from group 2 (POAG), it was found that the association with HLA-DR*16 is more evident in group 1, increasing Relative Risk to 4.6 (95% CI=0.8-24.2). Likewise, it should be noted that no patient with PCG was positive for HLA-DRB1*07, which when compared to controls shows a statistically significant difference ($P$ = 0.04). These data are shown in Table 2.

Table 3 compares the gene frequencies of HLA-DRB1 alleles in patients with POAG without angular dysgenesis (group 3) and healthy controls, in which a significant increase in HLA-DRB1*14 was observed ($P$ = 0.04, OR =2.5, 95% CI = 0.8-7.2).

### Table 1. Gene frequency of HLA-DRB1 alleles in Mexican mestizo patients with Primary Open Angle Glaucoma with angular dysgenesis alterations and Primary Congenital Glaucoma compared to healthy subjects.

| HLA-DRB1* | Glaucoma patients N=30 (60) | Controls N=99 (198) | $P$ | OR | 95% CI |
|-----------|-----------------------------|---------------------|-----|----|--------|
|           | n  | gene frequency | n  | gene frequency |       |      |
| DRB1*04   | 18 | 0.300         | 47 | 0.237          |       |      |
| DRB1*08   | 10 | 0.166         | 33 | 0.166          |       |      |
| DRB1*03   | 7  | 0.116         | 11 | 0.055          | 0.09  | 2.2  | 0.7-6.6 |
| DRB1*14   | 6  | 0.100         | 20 | 0.101          |       |      |
| DRB1*01   | 5  | 0.083         | 10 | 0.050          |       |      |
| DRB1*16   | 5  | 0.083         | 4  | 0.025          | 0.05  | 3.5  | 0.8-14.6 |
| DRB1*15   | 3  | 0.050         | 11 | 0.065          |       |      |
| DRB1*13   | 2  | 0.033         | 10 | 0.050          |       |      |
| DRB1*07   | 1  | 0.016         | 22 | 0.111          | 0.03  | 0.13 | 0.017-1.02 |
| DRB1*10   | 1  | 0.016         | 01 | 0.005          |       |      |
| DRB1*11   | 1  | 0.016         | 20 | 0.100          |       |      |
| DRB1*12   | 1  | 0.016         | 01 | 0.005          |       |      |

### Table 2. Gene frequency of HLA-DRB1 alleles in Mexican mestizo patients with Primary Congenital Glaucoma compared to healthy Mexican mestizo controls.

| HLA-DRB1* | PCG patients N=14 (28) | Controls N=99 (198) | $P$ | OR | 95% CI |
|-----------|------------------------|---------------------|-----|----|--------|
|           | n  | gene frequency | n  | gene frequency |       |      |
| DRB1*04   | 6  | 0.214         | 47 | 0.237          |       |      |
| DRB1*08   | 6  | 0.214         | 33 | 0.166          |       |      |
| DRB1*14   | 4  | 0.142         | 21 | 0.105          |       |      |
| DRB1*03   | 3  | 0.107         | 11 | 0.055          |       |      |
| DRB1*16   | 3  | 0.107         | 5  | 0.025          | 0.06  | 4.6  | 0.8-24.2 |
| DRB1*15   | 2  | 0.071         | 13 | 0.065          |       |      |
| DRB1*01   | 2  | 0.071         | 10 | 0.050          |       |      |
| DRB1*13   | 1  | 0.035         | 10 | 0.050          |       |      |
| DRB1*11   | 1  | 0.035         | 20 | 0.100          |       |      |
| DRB1*07   | 0  | 0.000         | 22 | 0.111          | 0.04  | .18  | 0.01-1.3 |
Table 3. Gene frequency of HLA-DRB1 alleles in Mexican mestizo patients with Primary Open Angle Glaucoma compared to healthy controls without angular dysgenesis.

| HLA-DRB1* | POAG Patients N=15 (30) | Controls N=99 (198) | P | OR | 95% CI |
|-----------|------------------------|---------------------|---|----|-------|
|           | n gene frequency        | n gene frequency    |   |    |       |
| DRB1*04   | 8 0.266                | 47 0.237            |   |    |       |
| DRB1*14   | 7 0.233                | 21 0.105            | 0.06 | 2.5 | 0.8-7.2 |
| DRB1*08   | 3 0.100                | 10 0.050            |   |    |       |
| DRB1*01   | 3 0.100                | 22 0.111            |   |    |       |
| DRB1*07   | 3 0.100                | 11 0.055            |   |    |       |
| DRB1*03   | 2 0.066                | 13 0.065            |   |    |       |
| DRB1*11   | 1 0.033                | 20 0.100            |   |    |       |
| DRB1*10   | 1 0.033                | 01 0.005            |   |    |       |

4. DISCUSSION

This study found an association of HLA-DRB1*16 with the susceptibility to the development of PCG, and a protective effect of HLA-DRB1*07 in patients with PCG and POAG with dysgenetic alterations in the iridocorneal angle. Interestingly, this protective effect has also been observed in Mexican patients with diabetic retinopathy [9]. Although the HLA-DRB1*07 protective effect was not seen in patients with POAG without angular dysgenesis, these associations became even more evident when separating group 1 from group 2.

If we compare the groups of POAG with angular dysgenesis alterations (group 1) and PCG (group 2) against the group of POAG without dysgenetic alterations (group 3), we observe that the collection of immunogenetic markers is different. For instance, HLA-DRB1*07 seems to act as a protective factor, whereas HLA-DRB1*03 may play a role as a risk factor during the onset of the disease with angular dysgenesis. In addition, HLA DRB1*14 seems to be a risk factor in the appearance of POAG without angular dysgenesis.

Findings of association between HLA-DRB1*16 and PCG development are interesting since it is a frequent allele in the Mexican population (mestizo and indigenous). Based on the fact that HLA-DRB1*07 exists in the Mexican population through miscegenation with Europeans, it is likely that that PCG in Mexico may have an indigenous genetic background.

When analyzing exclusively the groups of POAG without dysgenesis against the group of healthy individuals, an association with HLA-DRB1*14 in the group of POAG without dysgenesis was observed.

This work confirms data gathered previously in which, although not statistically significant, a high gene frequency of HLA-DRB1*14 and HLA-DRB1*03 alleles was found in patients with POAG [12]. In this study, an association of HLA-DRB1*14 was found in patients with glaucoma without angular dysgenesis and HLA-DRB1*03 in patients with glaucoma with iridocorneal dysgenesis, with a statistically significant difference for each of them when compared with controls (Tables 1 and 3).

In 1998, Wax et al. first described the involvement of the immune system in glaucoma after the detection of antibodies against the toxic shock protein (HSP) in serum of patients with normal-tension glaucoma [13]. In the following years, different antibodies have been described in patients with glaucoma, such as anti-gamma-enolase, neuron-specific, anti-glycosaminoglycans, and anti-beta 2 adrenergic receptor [14, 15].

Although the exact disease mechanism has not been established, the presence of diverse autoantibodies against ocular proteins and the presence of HLA-DRB1*03 supports the hypothesis that the etiopathogenesis of glaucoma has an autoimmune origin.

CONCLUSION

Data obtained in this study allow us to suggest a possibly different etiopathogenesis between the groups of POAG with angular dysgenesis and POAG without dysgenesis. Studies with a larger number of patients will be needed to corroborate these results.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Ethics Committee from the Glaucoma Department, Association to prevent blindness in Mexico, Mexico under ethical approval no. Reference number GL-14-05.

HUMAN AND ANIMAL RIGHTS

No Animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was taken from all the participants when they were enrolled.

AVAILABILITY OF DATA AND MATERIALS

The datasets presented in this study can be found in the online repository: Allele frequency net database (AFND) with...
Role of Major Histocompatibility Complex Genes

The accession Mexico Mexico City Mestizo population (n=45) at http://www.allelefrequencies.net/.

FUNDING
None.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS
Declared none.

REFERENCES

[1] Rieck J. The pathogenesis of glaucoma in the interplay with the immune system. Invest Ophthalmol Vis Sci 2013; 54(3): 2393-409. [http://dx.doi.org/10.1167/iovs.12-9781] [PMID: 23539162]

[2] Quigley HA. Glaucoma. Lancet 2011; 377(9774): 1367-77. [http://dx.doi.org/10.1016/S0140-6736(10)61423-7] [PMID: 23539162]

[3] Evangelho K, Mogilevskaya M, Losada-Barragan M, Vargas-Sanchez JK. Pathophysiology of primary open-angle glaucoma from a neuroinflammatory and neurotoxicity perspective: A review of the literature. Int Ophthalmal 2017; 37: 1-14. [http://dx.doi.org/10.1007/s10792-017-0795-9] [PMID: 27400652]

[4] Junemann A, Hohberger B, Rech J, et al. Agonistic autoantibodies to the β2-Adrenergic receptor involved in the pathogenesis of open-angle glaucoma. Front Immunol 2018; 9: 145. [http://dx.doi.org/10.3389/fimmu.2018.00145] [PMID: 29483090]

[5] Liu Y, Allingham RR. Molecular genetics in glaucoma. Exp Eye Res 2011; 93(4): 331-9. [http://dx.doi.org/10.1016/j.exer.2011.08.007] [PMID: 21871452]

[6] Carbone MA, Chen Y, Hughes GA, et al. Genes of the unfolded protein response pathway harbor risk alleles for primary open angle glaucoma. PLoS One 2011; 6(5)e20649 [http://dx.doi.org/10.1371/journal.pone.0020649] [PMID: 21655191]

[7] Khor CC, Do T, Jia H, et al. Genome-wide association study identifies five new susceptibility loci for primary angle closure glaucoma. Nat Genet 2016; 48(5): 556-62. [http://dx.doi.org/10.1038/ng.3540] [PMID: 27064256]

[8] Trowsdale J, Knight JC. Major histocompatibility complex genomics and human disease. Annu Rev Genomics Hum Genet 2013; 14: 301-23. [http://dx.doi.org/10.1146/annurev-genom-091212-153455] [PMID: 23875801]

[9] Zúñiga J, Yu N, Barquera R, et al. HLA class I and class II conserved extended haplotypes and their fragments or blocks in Mexicans: Implications for the study of genetic diversity in admixed populations. PLoS One 2013; 8(9):e74442 [http://dx.doi.org/10.1371/journal.pone.0074442] [PMID: 24086347]

[10] Zu N, et al. Susceptibility to Develop Glaucoma. 1999; pp. 297-300.

[11] Quiroz-Mercado H, Suárez-Licona A, Fromow-Guerra J, et al. Human lymphocyte antigen DR7 protects against proliferative retinopathy with type II diabetes mellitus. Arch Med Res 2002; 33(2): 123-7. [http://dx.doi.org/10.1016/S0188-4409(01)00378-2] [PMID: 11886709]

[12] Gil-Carrasco F, Granados J, Barajas-Weber E, Gilbert-Lucido ME, Vargas-Alarcén G. Immunogenetic aspects in primary open-angle glaucoma in family members of Mexican mestizo glaucomatous patients. Am J Ophthalmol 1994; 118(6): 744-8. [http://dx.doi.org/10.1016/S0002-9394(94)72553-X] [PMID: 7977600]

[13] Wax MB, Tezel G, Saito I, et al. Anti-Ro/SS-A positivity and heat shock protein antibodies in patients with normal-pressure glaucoma. Am J Ophthalmol 1998; 125(2): 145-57. [http://dx.doi.org/10.1016/S0002-9394(98)00084-1] [PMID: 9467439]

[14] Grus FH, Joachim SC, Wünschig D, Rieck J, Pfeiffer N. Autoimmunity and glaucoma. J Glaucoma 2008; 17(1): 79-84. [http://dx.doi.org/10.1097/IJG.0b013e318156a592] [PMID: 18303391]

[15] Wenzel K, Schulze-Rothe S, Haberland A, Müller J, Wallukat G, Davidite H. Performance and in-house validation of a bioassay for the determination of beta1-autoantibodies found in patients with cardiomyopathy. Heliyon 2017; 3(7):e00362 [http://dx.doi.org/10.1016/j.heliyon.2017.e00362] [PMID: 28795160]

© 2021 Gil-Carrasco et al.
This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International Public License (CC-BY 4.0), a copy of which is available at: (https://creativecommons.org/licenses/by/4.0/legalcode). This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.