The genetic contribution of HLA-E*01:03 and HLA-E*01:03-G*01:01 to Posner-Schlossman syndrome in southern Chinese

Xiaosheng Huang1,2#, Yunping Xu3#, Wenchieh Chen2, Tianhui Zhu1,2, Liumei He3, Songxing Wang3, Shiming Peng1,2, Shaoyi Mei1,2, Yan Wang2, Jun Zhao1,2

1School of Ophthalmology & Optometry Affiliated to Shenzhen University, Shenzhen 518040, China; 2Shenzhen Eye Institute, Shenzhen Eye Hospital Affiliated to Jinan University, Shenzhen 518040, China; 3Institution of Transfusion Medicine, Shenzhen Blood Center, Shenzhen 518035, China

Contributions: (I) Conception and design: J Zhao; (II) Administrative support: J Zhao; (III) Provision of study materials or patients: J Zhao, Y Xu; (IV) Collection and assembly of data: X Huang, W Chen, T Zhu, L He, S Wang, S Peng, S Mei, Y Wang; (V) Data analysis and interpretation: J Zhao, X Huang, Y Xu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

#These authors contributed equally to this work.

Background: The polymorphisms of classical HLA-Ia and HLA-II loci have been associated with Posner-Schlossman syndrome (PSS) in the southern Chinese population. However, the associations of non-classical HLA-Ib (e.g., HLA-E and HLA-G) loci with PSS have not been reported in the southern Chinese population. This study aimed to evaluate the associations of the HLA-E and HLA-G loci with PSS in a southern Chinese Han population group.

Methods: Ninety-seven unrelated patients with PSS and 90 ethnically matched control subjects were recruited from the Shenzhen Eye Hospital in China. The full-length sequences of HLA-E and HLA-G genes were amplified by long-range high-fidelity PCR, and the third exon of the HLA-E gene and the coding region of the HLA-G gene were sequenced.

Results: The allele frequency of HLA-E*01:03 in patients with PSS was significantly higher than that in the control group (P=0.017, corrected P=0.034, OR =1.66). The genotype frequencies of HLA-E*01:01/01:03 and HLA-E*01:03/01:03 in the PSS group were significantly higher than that in the control group (P=0.027, OR =2.62; P=0.011, OR =3.05; respectively). There were no significant differences in the frequency of HLA-G alleles and genotypes between the two groups (all P>0.05). The haplotype frequency of HLA-E*01:03-G*01:01 in the PSS group was significantly higher than that in the control group (P=0.019, OR =1.63), although this association did not survive the Bonferroni correction (corrected P=0.13).

Conclusions: This study proved for the first time that HLA-E*01:03 and HLA-E*01:03-G*01:01 might be risk factors for PSS.

Keywords: Posner-Schlossman syndrome (PSS); HLA-E; HLA-G; genetic association; Chinese

Submitted Jul 21, 2019. Accepted for publication Nov 12, 2019.
doi: 10.21037/atm.2019.11.70
View this article at: http://dx.doi.org/10.21037/atm.2019.11.70

Introduction

Posner-Schlossman syndrome (PSS), also known as a glaucomatocyclitic crisis, was first reported by Posner and Schlossman in 1948 (1). PSS is a secondary glaucoma, which occurs in young adults. PSS is a self-limiting and recurrent eye disorder, and 24.3% of patients have more than 2 episodes per year, up to 12 episodes in some cases (2). The main clinical manifestations of PSS are the elevated intraocular pressure (IOP) in one eye with keratic precipitates...
(KPs). Normally, symptoms in most patients can be relieved by using IOP-reducing and anti-inflammatory drugs, but many studies have shown that PSS is not a completely benign disease (3). Long-term recurrent PSS can lead to optic nerve damage and a reduced number of corneal endothelial cells. Permanent visual impairment can develop in severe cases. Anti-glaucoma surgery is needed in some patients with PSS because of inadequate disease control (3,4).

The human leukocyte antigen (HLA) gene is located in the short arm of chromosome 6 (6p21.31), with a total length of about 3.6 Mb. It is rich in polymorphism and takes part in immune response and the regulation of immune function (5). HLA gene polymorphisms are associated with a variety of eye diseases, such as uveitis, glaucoma, Graves’ ophthalmopathy, and the transplant rejection after corneal transplantation (6-10). The polymorphisms of the classical HLA-Ia and HLA-II genes have been associated with PSS (11-13). The study of Hirose et al. [1985] first reported that the HLA-Bw54 and HLA-Bw54-Cw1 haplotype had a significantly higher frequency in Japanese patients with PSS than in normal controls (11). In two recent studies of the southern Chinese population, Zhao et al. found that HLA-C*14:02, HLA-A*11:01-C*14:02 and HLA-B*51:01-C*14:02 were associated with increased risk of PSS, while HLA-B*13:01, HLA-DPA1*02:01, HLA-DPB1*14:01, HLA-DPB1*17:01, HLA-B*13:01-C*03:04 and HLA-DPB1*14:01-DPA1*02:01 might be protective factors for PSS (12,13).

Non-classical HLA-Ib (HLA-E and HLA-G) molecules play an essential inhibitory role in innate and adaptive immunity (14,15). The receptors can recognize HLA-E molecules on natural killer (NK) cells and some cytotoxic lymphocytes (CTL) (CD94/NKG2 receptor) to regulate cytotoxic activity (15). HLA-G molecules can directly inhibit the function of immune cells (NK cells, CTLs, B cells, and dendritic cells) (14,15). HLA-E and HLA-G gene polymorphisms are closely related to viral infection, rejection after organ transplantation, tumor surveillance, and autoimmune system abnormalities (16-25). However, the correlation between HLA-E and HLA-G gene polymorphisms and the pathogenesis of PSS is still unclear. In this study, we evaluated the association between HLA-E and HLA-G gene polymorphisms and PSS in a southern Chinese Han population.

Methods

Patients and controls

Between December 2015 and December 2018, a total of 97 unrelated PSS patients were recruited from patients attending the Shenzhen Eye Hospital Clinic. The diagnosis of PSS was based on the following criteria (1-4,12,13): (I) single-eye onset in young adults, mild discomfort in the eye, and no significant decrease or slight decrease in visual acuity; (II) elevated IOP with recurrent episodes and mutton-fat KPs; (III) open iridocorneal angle under high IOP without peripheral anterior synechia; (IV) no visual field loss and optic nerve damage in patients with shorter course of disease; and (V) no history of other eye diseases except for refractive error. Ninety unrelated subjects were recruited at the Shenzhen Blood Center from healthy volunteer blood donors with normal IOP and optic discs. Patients and controls were all southern Han Chinese and matched on age, sex, and ethnicity. The study protocol was approved by the Ethics Committee of Shenzhen Eye Hospital and was in accordance with the tenets of the Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from all study participants.

DNA extraction

DNA was extracted from peripheral blood samples of all participants using the MagCore nucleic acid extraction kit according to the instructions of the manufacturer (Promega Corporation, Madison, WI, USA).

Polymerase chain reaction (PCR) amplification

The full-length sequences of HLA-E and -G were amplified by long-range high-fidelity PCR. PCR amplification was performed in a 20 μL reaction system, including 0.5 μL pfuUltraTM Fusion HS DNA polymerase, 2 μL of 10× pfuUltraTM Rxn Buffer, 1 μL of dNTP (2.5 mmol/L) mixture, 0.5 μL of each PCR primer (10 μmol/L, Table 1), 14.5 μL ddH2O, and 1 μL genomic DNA.

The PCR products were purified using a magnetic bead reagent before the sequencing reaction to remove non-specific products generated during PCR amplification. Eighteen μL Mag-Bind EZ Pure magnetic beads were added to each well and mixed thoroughly. After standing for 10 min, the plate was placed on a magnetic stand. After adsorbing on the magnetic beads for 10 min, the supernatant was discarded. 200 μL of 70% ethanol was used to wash twice for 1 min. After standing at room temperature for 20 min for the ethanol completely evaporated, 30 μL of ddH2O was added to each well to
collect the purified products.

**Selection of polymorphisms and genotyping**

Because the *HLA-E*^*E*01:01 allele and the *HLA-E*^*E*01:03 allele are different at the rs1264457 site of the third exon, exon 3 of the *HLA-E* gene was selected for sequencing (26,27). Since there are 43 single nucleotide polymorphism sites in the *HLA-G* gene according to the IMGT/HLA database (http://www.ebi.ac.uk/ipd/imgt/hla/, Release 3.36.0, 2019 April), the coding region of the *HLA-G* gene was sequenced. The sequencing primers were listed in Table 2.

The sequencing reaction products (10 μL) were purified by adding 2.5 μL NaOAc/EDTA and 50 μL of 80% ethanol to each well, mixed well, and centrifuged at 3,000 g for 30 min. Two hundreds μL of 80% ethanol was used to wash again, centrifuged at 3,000 g for 5 min. After standing at room temperature for 20 min for the ethanol completely evaporated, a 15 μL formamide solution was added to dissolve the products and denatured at 95 °C for 2 min. Electrophoresis of the purified sequencing reaction products was done in an ABI 3730 sequencer (Applied Biosystem, Foster City, CA, USA).

The sequencing reaction products were electrophoresed in an ABI 3730 sequencer (Applied Biosystem, Foster City, CA, USA). The sequence data was imported into the Assign 3.5 genotype calling. HLA genotypes were assigned at the four-digit level. HLA haplotypes were analyzed using the Arlequin 3.5.1 software.

---

**Table 1** PCR primers for amplification of *HLA-E* and *HLA-G* genes

<table>
<thead>
<tr>
<th>PCR primer</th>
<th>Nucleotide position</th>
<th>Primer sequence (5’→3’)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-E-PCR-F</td>
<td>−75</td>
<td>CAGCGTGGCCACGACTCCCAG</td>
<td>3,600</td>
</tr>
<tr>
<td>HLA-E-PCR-R</td>
<td>3502</td>
<td>CCTAAGTGCTGGATTACAG</td>
<td></td>
</tr>
<tr>
<td>HLA-G-PCR-F</td>
<td>−294</td>
<td>AGAACGCTTTGGCACAAGAGTA</td>
<td>3,100</td>
</tr>
<tr>
<td>HLA-G-PCR-R</td>
<td>2776</td>
<td>CCTCAACACCCCCACACACAT</td>
<td></td>
</tr>
</tbody>
</table>

F, forward; R, reverse.

**Table 2** Sequencing primers for *HLA-E* and *HLA-G* genes

<table>
<thead>
<tr>
<th>Sequencing primer</th>
<th>Nucleotide position</th>
<th>Primer sequence (5’→3’)</th>
<th>Target exon</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-E-SEQ-3F</td>
<td>549</td>
<td>AGATTCACCCCCAAGGCTG</td>
<td>3</td>
</tr>
<tr>
<td>HLA-E-SEQ-3R</td>
<td>1061</td>
<td>TCCTGTTTTTCTCTAC</td>
<td></td>
</tr>
<tr>
<td>HLA-G-SEQ-1F</td>
<td>282</td>
<td>ACAAAGATGAGGGGGTCAG</td>
<td>1</td>
</tr>
<tr>
<td>HLA-G-SEQ-1R</td>
<td>250</td>
<td>GATGAAAGCGGGGCTCCCCCGG</td>
<td></td>
</tr>
<tr>
<td>HLA-G-SEQ-2F</td>
<td>201</td>
<td>CTCCCACTCATGAGGTAT</td>
<td>2</td>
</tr>
<tr>
<td>HLA-G-SEQ-2R</td>
<td>780</td>
<td>CGAGGTAATCCTTGGCATTGT</td>
<td></td>
</tr>
<tr>
<td>HLA-G-SEQ-3F</td>
<td>706</td>
<td>ACCCTCCAGTTGATGATTG</td>
<td>3</td>
</tr>
<tr>
<td>HLA-G-SEQ-3R</td>
<td>1276</td>
<td>GAGGCAGAGAACAAGCCTG</td>
<td></td>
</tr>
<tr>
<td>HLA-G-SEQ-4F</td>
<td>1252</td>
<td>CTTGCGACGGAGCTTTTC</td>
<td>4</td>
</tr>
<tr>
<td>HLA-G-SEQ-4R</td>
<td>1771</td>
<td>TGCTCCTCTCGAGAAGGCAC</td>
<td></td>
</tr>
<tr>
<td>HLA-G-SEQ-5F</td>
<td>1778</td>
<td>CTGGGAGGAGCGAGGATAT</td>
<td>5</td>
</tr>
<tr>
<td>HLA-G-SEQ-5R</td>
<td>2281</td>
<td>CCATCATACACTACATCAAG</td>
<td></td>
</tr>
<tr>
<td>HLA-G-SEQ-6F</td>
<td>2291</td>
<td>GTACGTGATGGGGACCTG</td>
<td>6</td>
</tr>
<tr>
<td>HLA-G-SEQ-6R</td>
<td>2776</td>
<td>CTCAACACCCCCCAACAT</td>
<td></td>
</tr>
</tbody>
</table>

F, forward; R, reverse.
Statistical analysis

Statistical analysis was performed using SPSS (version 20.0, SPSS Inc., Chicago, IL, USA). Age and IOP were compared between patients with PSS and controls using independent-samples t-test. Hardy-Weinberg equilibrium (HWE) was evaluated using the chi-squared test. The difference in sex, allele frequency, genotype frequency, and haplotype frequency between cases and controls were evaluated using the chi-squared test or Fisher’s exact test. The maximum expectation algorithm determined haplotype frequency in the Arlequin 3.5.1 software. Multiple testing was corrected using the Bonferroni method. P<0.05 was considered statistically significant. Odds ratio (OR) and 95% confidence interval (CI) were calculated whenever applicable.

Results

Demographic and clinical features of participants

There were 46 (47.4%) males and 51 (52.6%) females in the PSS group, with an average age of 41.5±12.9 years. In the control group, there were 45 (50.0%) males and 45 (50.0%) females, with an average age of 40.4±12.0 years. No significant difference in sex and age was found between cases and controls (P=0.73 and 0.55, respectively, Table 3). The mean IOP was 44.5±7.9 mmHg in patients with PSS while 15.3±3.5 mmHg in controls. The IOP in cases was significantly higher than that in controls (P<0.001, Table 3).

HLA-E allele and genotype frequencies

Two alleles (E*01:01 and E*01:03) and three genotypes (E*01:01/01:01, E*01:01/01:03 and E*01:03/01:03) at the HLA-E locus were detected in both PSS and control groups (Table 4). The genotype distributions of HLA-E in both groups were following HWE (P>0.11; data not shown). The allele frequency of HLA-E*01:03 in patients with PSS was significantly higher than that in the control group (P=0.017, OR =1.66, 95% CI: 1.09–2.53, Table 4), which survived the Bonferroni correction (corrected P=0.034). The genotype frequencies of HLA-E*01:01/01:03 and HLA-E*01:03/01:03 in the PSS group were significantly higher than that in the control group (P=0.027, OR =2.62, 95% CI: 1.10–6.22; P=0.011, OR =3.05, 95% CI: 1.27–7.35, respectively, Table 4).

HLA-G allele and genotype frequencies

Four alleles at the HLA-G locus were detected in the PSS group (G*01:01, G*01:04, G*01:05N and G*01:06) and control group (G*01:01, G*01:03, G*01:04 and G*01:06) respectively (Table 5). Five genotypes (G*01:01/01:01, G*01:01/01:04, G*01:04/01:04, G*01:01/01:05N and G*01:01/01:06) were detected in the PSS group, while 6

Table 3 The demographic and clinical features of the PSS cases and controls

<table>
<thead>
<tr>
<th>Feature</th>
<th>PSS (n=97)</th>
<th>Controls (n=90)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>46/51</td>
<td>45/45</td>
<td>0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (year, mean ± SD)</td>
<td>41.5±12.9</td>
<td>40.4±12.0</td>
<td>0.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IOP (mmHg, mean ± SD)</td>
<td>44.5±7.9</td>
<td>15.3±3.5</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>KPs (Y/N)</td>
<td>Y</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>, Chi-squared test; <sup>b</sup>, independent-samples t-test. PSS, Posner-Schlossman syndrome; M, male; F, female; IOP, intraocular pressure; KPs, keratic precipitates; Y, yes; N, no.

Table 4 HLA-E allele and genotype frequencies of the PSS cases and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Count (%)</th>
<th>Count (%)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele</td>
<td>PSS (2n=194)</td>
<td>Controls (2n=180)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E*01:01</td>
<td>64 (33.0)</td>
<td>81 (45.0)</td>
<td>1.00 (ref.)</td>
<td></td>
</tr>
<tr>
<td>E*01:03</td>
<td>130 (67.0)</td>
<td>99 (55.0)</td>
<td>0.017</td>
<td>1.66 (1.09–2.53)</td>
</tr>
<tr>
<td>Genotype</td>
<td>PSS (n=97)</td>
<td>Controls (n=90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E*01:01/01:01</td>
<td>10 (10.3)</td>
<td>22 (24.4)</td>
<td>1.00 (ref.)</td>
<td></td>
</tr>
<tr>
<td>E*01:01/01:03</td>
<td>44 (45.4)</td>
<td>37 (41.1)</td>
<td>0.027</td>
<td>2.62 (1.10–6.22)</td>
</tr>
<tr>
<td>E*01:03/01:03</td>
<td>43 (44.3)</td>
<td>31 (34.4)</td>
<td>0.011</td>
<td>3.05 (1.27–7.35)</td>
</tr>
</tbody>
</table>

P was assessed using a Chi-squared test. PSS, Posner-Schlossman syndrome; OR, odds ratio; CI, confidence interval; ref., reference.
genotypes (G*01:01/01:01, G*01:01/01:04, G*01:04/01:04, G*01:01/01:03, G*01:03/01:04 and G*01:04/01:06) were detected in the control group (Table 5). The top three HLA-G genotypes were G*01:01/01:01, G*01:01/01:04, and G*01:04/01:04. The genotype distributions of HLA-G polymorphisms in both PSS and control groups were in accordance with HWE (P>0.11; data not shown). There was no significant difference in the frequency of HLA-G alleles and genotypes between the two groups (P>0.05, Table 5).

**Discussion**

HLA-E and HLA-G gene polymorphisms affect their expression levels and immune function (26-28). The HLA-E and HLA-G alleles exhibit limited polymorphisms, with only ten 4-digit HLA-E alleles and twenty-two 4-digit HLA-G alleles (IMGT/HLA, http://www.ebi.ac.uk/ipd/imgt/hla/, Release 3.36.0, 2019 April). In this study, the distributions of HLA-E and HLA-G alleles in the control group are similar to those in the normal Chinese Han population (Table 4) (21,24,29).

The frequency of the HLA-E*01:03 allele in the PSS group was significantly higher than that in the control group (Table 4), suggesting that the HLA-E*01:03 allele is a susceptibility gene for PSS. The HLA-E*01:03 allele has been associated with cytomegalovirus (CMV) infection in patients after kidney transplantation, chronic hepatitis B, rheumatoid arthritis, pemphigus vulgaris, nasopharyngeal carcinoma and ovarian cancer, while the HLA-E*01:01 allele is a protective gene for diseases such as hepatitis C virus infection, Behcet’s disease and Hodgkin’s lymphoma (16-23,25). The difference between the HLA-E*01:01 allele-encoded protein (HLA-E^8) and the HLA-E*01:03 allele-encoded protein (HLA-E^8) is...
encoded protein (HLA-E\textsuperscript{G}) is caused by the nucleotide change from adenine to guanine (A → G) at the rs1264457 site (16,27). Compared with HLA-E\textsuperscript{R}, HLA-E\textsuperscript{G} is characterized by the higher expression on the surface of NK cells, higher affinity for binding to receptors, and higher stability of binding to signal peptides (26,27). Besides, several studies have shown that CMV infection may be the main pathogenic factor of PSS (3), which might inhibit the immune surveillance function through HLA-E molecules. For example, the CMV UL40 protein has a similar structure to the HLA-E signal peptide, which can up-regulate the expression of HLA-E molecules on the cell surface and enhance the binding of HLA-E molecules to inhibitory receptors (CD94/NKG2A), inhibiting the immune function of NK cells or some CTLs (30).

Polymorphisms in the coding region of the HLA-G gene can affect the coding of nucleotides. For example, the HLA-G*01:13N allele changes the first base of codon 54 (α1 domain) from cytosine to thymine (C → T), ending the coding early, resulting in the inability to synthesize the HLA-G protein (28). The HLA-G*01:05N allele deletes the last nucleotide of codon 129 or in the first nucleotide of codon 130 (exon 3), causing a stop codon in advance at the codon 189, which encodes an incomplete HLA-G protein, affecting its function (28). The HLA-G*01:04 and HLA-G*01:05N alleles are susceptibility genes for habitual abortion (23). The HLA-G*01:05N allele is also a protective gene for HIV infection, while the HLA-G*01:01:08 allele is a susceptibility gene for HIV infection (21). In this study, we did not find a significant difference in the HLA-G allele and genotype frequency between patients with PSS and controls (Table 5), indicating that the HLA-G gene polymorphisms might not be related to PSS in the southern Chinese Han population.

The HLA-E and HLA-G loci are adjacent loci at 660 kb. Studies have reported that HLA-E and HLA-G polymorphisms are simultaneously related to a variety of diseases. For instance, both the HLA-E*01:03 allele and the HLA-G 14bp INS/DEL polymorphism are associated with CMV infection (31,32). Both HLA-E*01:03 and HLA-G*01:05N alleles are protective factors for HIV-1 infection (33). The HLA-E*01:01 allele and the HLA-G*01:01:01 allele are both protective factors for Behcet’s disease (34). We found that the haplotype frequency of HLA-E*01:03-G*01:01 was higher in the PSS group than in the control group (Table 6), despite this association did not survive the Bonferroni correction. We hypothesized that the HLA-G*01:01 encoded protein as a signal peptide can enhance the stability of the HLA-E*01:03 encoded HLA-E peptide complex and inhibited the function of NK cells or some CTLs so that the HLA-E*01:03-G*01:01 haplotype may be more likely to promote the pathogenesis of PSS. Further research must reveal the underlying mechanisms. We reported for first time that polymorphisms of non-classical HLA-I\textsubscript{b} genes (i.e., HLA-E and HLA-G) were associated with PSS in the southern Chinese Han population. We found that the HLA-E*01:03 allele was a susceptibility gene for PSS, and the HLA-E*01:03-G*01:01 haplotype might be a risk factor for PSS. Further investigation into the expression of HLA-E and HLA-G molecules at the transcriptional and protein levels is required to must evaluate their role in the pathogenesis of PSS.

Acknowledgments

We are indebted to the participants for their excellent co-
operation and support. We want to thank the volunteer blood donors for generously supplying blood samples for this study. We also thank Dr. Baojian Fan for his insightful comments on this manuscript.

Funding: This study was supported by the Science, Technology, and Innovation Commission of Shenzhen Municipality under Grant (number JCYJ20180228164400218 and GJHZ20180420180937076), Health and Family Planning Commission of Shenzhen Municipality under Grant (number SZGW2017005), and Sanming Project of Medicine in Shenzhen Grant (number SZSM201812090 and SZSM201811092).

Footnote
Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were following the ethical standards of the local Ethics Committee of Shenzhen Eye Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all study participants.

References


