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Human leukocyte antigen-G 3' untranslated region polymorphisms are associated with asthma severity

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ABSTRACT

Asthma is a genetically complex chronic inflammatory airway disorder, and according to disease pathogenesis, clinical manifestations may vary according to asthma severity. A gene region close to the human leukocyte antigen-G (*HLA-G*) gene was identified as an independent susceptibility marker for asthma. Considering that the HLA-G immune checkpoint molecule may modulate inflammation, we evaluated the diversity of the *HLA-G* 3′ untranslated region (3′UTR) in asthmatic patients stratified according to disease severity. We evaluate the entire *HLA-G* 3′UTR segment in 115 Brazilian patients stratified into mild ($n=29$), moderate ($n=21$) and severe asthmatics ($n=65$), and in 116 healthy individuals. *HLA-G* 3′UTR typing was performed using Sanger sequencing. The multiple comparisons among patients stratified according to disease severity revealed several associations; however, after Bonferroni's correction, the following results remained significant: i) the +3010C and +3142G alleles were overrepresented in mild asthma patients when compared to controls; ii) the +3010G and +3142C alleles were overrepresented in severe asthma patients in comparison to patients with mild asthma. In conclusion, the +3010C/G and +3142C/G *HLA-G* 3′UTR variation sites were differentially associated according to asthma severity.

1. Introduction

Asthma is a heterogeneous chronic inflammatory disease, exhibiting various phenotypes (Gauthier et al., 2015). Asthma prevalence is increasing worldwide, and it has been predicted that by the year 2025, 400 million people will develop asthma worldwide (Global Initiative for Asthma, 2017). According to the 2017 Update of the Global Initiative for Asthma (GINA), asthma severity can be classified into mild, moderate and severe (Global Initiative for Asthma, 2017).

Asthma severity is retrospectively evaluated based on the treatment necessary to control symptoms and according to exacerbations after several months of follow-up, which may change along time. According to GINA steps 1 and 2 guidelines, mild asthma is well controlled requiring the need of short acting bronchodilators alone (step 1) or low intensity daily treatment, including low dose inhaled corticosteroid (ICS) (step 2). Moderate asthma is asthma well controlled with step 3 treatment, requiring low dose of ICS plus long-acting beta2-agonist (ICS + LABA) as first line of therapy. Most patients with severe asthma needs high-dose ICS-LABA (Step 4) to avoid that disease becomes uncontrolled, and some patients require add-on therapies including anti-leukotrienes, anti-cholinergic agents, and therapy with anti-IgE (omalizumab) or anti-IL-5 (mepolizumab, reslizumab) monoclonal antibodies (Step 5) (Global Initiative for Asthma, 2017).

Mild asthma patients may present allergic response to allergens and an eosinophilic inflammation that is usually associated with a Th2 polarization profile (Holgate, 2012). Severe asthma patients may exhibit increased number of exacerbations, decreased response to corticosteroid therapy, neutrophilia with predominance of a Th17 cytokine profile (Bell et al., 2011) and a Th1 polarization, involving IFN-γ production accompanied with a reduced Th2 and Th17 response (Gauthier

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Table 1
Clinical and laboratory features of the asthmatic patients, stratified according to disease severity.

	Whole Group	Mild	Moderate	Severe	*P-value*
Age, mean (± SD)	45.8 (17.01)	40.2 (18.17)	42.9 (19.09)	49.2 (15.09)	NS[a]
Gender (%)					NS[b]
Female	82 (71.30%)	22 (75.86%))	17 (80.95%)	43 (66.15%)	
Male	33 (28.70%)%)	07 (24.14%)	04 (19.05%)	22 (33.85%)	
Skin Color					NS[b]
White	91 (79.13%)	24 (82.76%)	17 (80.95%)	50 (79.13%)	
Mulatto	13 (11.30%)	04 (13.79%)	02 (9.52%)	07 (11.30%)	
Black	11 (9.57%)	01 (3.45%)	02 (9.52%)	08 (9.57%)	
Atopy					NS[b]
Yes	83 (72.17%)	19 (65.52%)	16 (76.19%)	48 (73.85%)	
No	16 (13.91%)	01 (3.45%)	03 (14.29%)	12 (18.46%)	
N/A	16 (13.91%)	09 (31.03%)	02 (9.52%)	05 (7.69%)	
Skin Prick Test					NS[b]
Positive	74 (64.35%)	18 (62.07%)	16 (76.19%)	40 (61.54%)	
Negative	19 (16.52%)	01 (3.45%)	03 (14.29%)	15 (23.08%)	
N/A	22 (19.13%)	10 (34.48%)	02 (9.52%)	10 (15.38%)	
Total IgE, geometric mean (range)*	182.4 (7.06-6340.00)	324.3 (27.9-4396.0)	139.3 (7.06-3010.00)	179.1 (9.66-6340.00)	NS[a]
Spirometry values					
FVC (% predicted), mean (± SD)**	85.3 (19.70)	101.2 (13.40)	92.0 (13.70)	76.9 (18.90)	< 0.0001[a]
FEV1 (% predicted), mean (± SD)**	65.4 (22.70)	88.1 (12.90)	74.0 (16.30)	53.6(19.30)	< 0.0001[a]
FEF 25-75 (% predicted), mean (± SD)**	37.8 (25.90)	60.0 (20.90)	44.5 (20.80)	27.0 (22.90)	< 0.0001[a]
FVC/FEV1 (% observed), mean (± SD)**	61.2 (17.80)	74.2 (11.00)	67.2 (15.80)	53.9 (17.00)	< 0.0001[a]
Smoking habits					NS[b]
Yes	10 (8.70%)	02 (6.90%)	02 (9.52%)	06 (9.23%)	
No	75 (65.22%)	20 (68.97%)	17 (80.95%)	38 (58.46%)	
Ex-smoker	25 (27.69%)	05 (17.24%)	02 (9.52%)	18 (27.69%)	
N/A	05 (4.35%)	02 (6.90%)	0 (0.00%)	93 (4.62%)	

Abbreviations: SD: Standard deviation; FVC: Forced Vital Capacity; FEV1: Forced Expiratory Volume in the First Second; FEF 25–75%: Forced Expiratory Flow between 25% and 75% of the FVC; N/A: missing information in the medical records; NS: Non-significan.
Differences between groups were analyzed using the [a]Kruskal–Wallis test, followed by Dunn *post hoc* test, and the [b]chi-square test.
*This information was available for 64 patients in the medical records.
**This information was not available for 6 patients in the medical records.

et al., 2015; Raundhal et al., 2015).

As a heterogeneous genetically complex disorder, many immune and non-immune genes have been involved on asthma susceptibility, and the interaction of genetic predisposition with environment factors may account for the asthma phenotype (Kuhlen et al., 2014; Wenzel, 2012). Genome-wide association studies have disclosed several susceptibility genes associated with asthma, including *ADAM33* (Disintegrin and metalloproteinase domain-containing protein-33), *DPP10* (Inactive dipeptidyl peptidase-10), *PTGDR* (prostaglandin D receptor), *CTNNA* (Catenin alpha-3), *IL1RL1* (Interleukin 1 receptor-like 1), *PDE4D* (cAMP-specific 3′,5′-cyclic phosphodiesterase 4D), *TLE4* (Transducin-like enhancer protein 4), *DENND1B* (DENN domain-containing protein 1B), and the genes of the Major Histocompatibility Complex (MHC), including *HLA-DQ* and *HLA-DP* alleles (Lee et al., 2015). Besides the association with MHC class II genes, a study conducted on a large number of asthmatic patient together with their families identified the *HLA-G* gene region (6p21) as an independent susceptibility factor (Nicolae et al., 2005).

HLA-G is a non-classical histocompatibility class I molecule that plays a pivotal role on immune system regulation, acting as a checkpoint molecule. It is mainly expressed in the first and second trimester placental tissues, facilitating the maternal-fetal tolerance, inhibiting the cytotoxicity effects of T CD8+ and natural killer (NK) cells. However, HLA-G can expressed in several pathological conditions, such as cancer and viral infection, leading to the impairment of the immune response against tumor cells or pathogens (Morandi et al., 2016). Alternative splicing of the primary transcript may produce at least four membrane-bound (HLA-G1 to HLA-G4) and three soluble (HLA-G5 to HLA-G7) isoforms that modulate the immune system by interaction with inhibitory receptors (ILT-2 and ILT-4) present on the surface of several immune cells, including macrophages, monocytes, dendritic cells, T cells and NK cells (Donadi et al., 2011).

The *HLA-G* gene may be regulated by several transcriptional and post-transcriptional elements. Particularly, the 3′ untranslated region (3′UTR) exhibits several polymorphic sites that may influence the *HLA-G* expression profile by modifying mRNA stability and/or influencing the microRNAs (miRNA) binding to the mature mRNA (Rousseau et al., 2003; Tan et al., 2008; Yie et al., 2008). Individual patterns of HLA-G expression have been associated with *HLA-G* 3′UTR variation sites (Martelli-Palomino et al., 2013) and these variation sites have associated with many pathological situations, including autoimmune, chronic inflammatory and infectious disorders (Ciliao Alves et al., 2012; Dias et al., 2015; Donadi et al., 2011; Silva et al., 2013).

Considering that: i) HLA-G may modulate the severity of chronic inflammatory disorders (Morandi et al., 2016), ii) the inflammatory profile of asthmatic patients may be different according to asthma severity (Gauthier et al., 2015), and iii) a region close the *HLA-G* gene has been associated with susceptibility to asthma (Nicolae et al., 2005), we studied the role of *HLA-G* 3′UTR polymorphic sites and haplotypes, and their relationship with asthma severity.

2. Material and methods

2.1. Subjects

We studied 115 asthmatic patients (82 women), aged 9 to 82 years old (mean: 45.8 years, SD ± 17.0), selected from the Allergy Clinics of the University Hospital of the Ribeirão Preto Medical School, University of São Paulo, Brazil. Patients were stratified according to disease severity into mild ($n=29$), moderate ($n=21$) and severe asthmatics ($n=65$), according to the 2017 GINA guidelines (GINA, 2017).

Patients with severe asthma presented values of Forced Expiratory