Human Immunology 77 (2016) 325–329

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Nonclassical human leukocyte antigen (HLA-G, HLA-E, and HLA-F) in coronary artery disease

Ines Zidi [a,*,1], Najla Kharrat [b,1], Rania Abdelhedi [b], Amna Ben Hassine [a], Ahmed Baligh Laaribi [a], Hamza Ben Yahia [a], Nouha Bouayed Abdelmoula [c], Leila Abid [d], Ahmed Rebai [b], Roberta Rizzo [e]

[a] Laboratory Microorganismes et Biomolécules Actives, Sciences Faculty of Tunis, University of Tunis El Manar, Tunis, Tunisia
[b] Centre de Biotechnologie de Sfax, Molecular and Cellular Screening Process, route Sidi Mansour, BP1177, 3018 Sfax, Tunisia
[c] Faculté de Médecine de Sfax, Laboratory of Histology, Avenue Majida Boulila, 3029 Sfax, Tunisia
[d] Hospital of Hedi Chaker (CHU), Service de cardiologie, Route el Ain Km 0.5, 3000 Sfax, Tunisia
[e] Department of Experimental and Diagnostic Medicine, Section Microbiology, University of Ferrara, Ferrara, Italy

ARTICLE INFO

Article history:
Received 1 June 2015
Revised 13 November 2015
Accepted 7 January 2016
Available online 11 January 2016

Keywords:
Coronary artery disease
HLA-G
HLA-E
HLA-F
Polymorphism

ABSTRACT

Aims: Several evidences suggest the association between the evolution of coronary artery disease (CAD) and the development of coronary syndrome that is often associated with disrupted plaque and partial or complete thrombosis of the related artery. Because of the inflammatory nature of CAD, we investigated the human leukocyte antigen (HLA)-G, HLA-E, and HLA-F genetic polymorphisms within CAD patients and evaluated their potential association with this disease in Tunisian population.

Methods: Different polymorphisms in HLA-G (14-bp Insertion/Deletion, +3142C/G), HLA-E (HLA-E*01:01/01:03 A/G), HLA-F (HLA-F*01:02 T/C, 01:03 C/T, 01:04 A/C) genes were typed using different laboratory techniques in a cohort of 89 CAD patients and 84 controls.

Results: A significant association was reported between the HLA-G +3142 G allele (OR = 1.64, 95% CI = 1.05–2.56, $p = 0.02$) and increased risk of CAD. No association was found for the other studied polymorphisms. When we considered the haplotypes, we found TDELCA and TDELGG haplotypes associated to CAD with $p = 0.008$ and $p = 0.030$, respectively, suggesting the potential interaction between HLA-G and HLA-E genes.

Conclusions: Our findings indicated that the HLA-G +3142C/G polymorphism and TDELCA and TDELGG haplotypes can harbour a reliable diagnosis value for the risk of CAD development suggesting that HLA-G, -E and -F molecules might be involved in the pathogenesis of the disease. However, further studies are necessary to confirm our results.

1. Introduction

A considerable amount of data has shown that coronary artery disease (CAD) could be in the origin of coronary syndrome development characterized by a disrupted plaque and partial or complete thrombosis of the related artery [1].

As inflammatory pathways have a biological role in the process of plaque destabilization [2,3], many researchers focused on screening inflammatory biomarkers in CAD [2]. Such markers could be considered as potential candidates for the enhancement of cardiovascular risk, clinical diagnosis, prognosis of patients with CAD [4].

Clinical and experimental studies have shown the implication of nonclassical human leukocyte antigen (HLA) class I (HLA-G, -E and-F) in several diseases such as inflammatory disease, autoimmune disease, cancer and viral infections. The HLA-G molecule was reported as a biomarker in liver, lung, kidney and heart transplantation with reduced acute rejection [5–9]. HLA-G molecule originally found in trophoblasts, is implicated in immune tolerance during pregnancy. In pathological conditions: it could be protective in inflammatory and autoimmune diseases or it could be associated to harmful effects like in cancer or infectious diseases [10]. Differently, HLA-E molecule is expressed in different human tissues [11]. It is characterized by low polymorphism in comparison with classical HLA class I antigens. Indeed, thirteen alleles are

* Corresponding author.
E-mail address: ines.zidi@techemail.com (I. Zidi).
[1] I. Zidi and N. Kharrat participated equally to this work.

http://dx.doi.org/10.1016/j.humimm.2016.01.008
0198-8859/

described for HLA-E encoding for only five HLA-E proteins [12]. The most important proteins are HLA-E*01:01 and HLA-E*01:03 that differ only by one amino acid in the α2 heavy chain domain at position 107 (respectively, Arg and Gly) [13]. This molecule can modulate the function of Natural killer (NK) and CD8+ T cells [11,14].

HLA-F molecule function is poorly understood. Noteworthy, HLA-F has been considered as a potential regulator of immune responses interacting with ILT2 and ILT4 NK cell inhibitory receptors [15]. Like HLA-E, HLA-F has low polymorphism with 22 alleles encoding for four proteins (HLA-F*01:01, HLA-F*01:02, HLA-F*01:03 and HLA-F*01:04) [16].

Taken together, nonclassic HLA class-I molecules seem to play an important role in immunotolerance, as in organ transplantation [17]; however, more studies are required to predict the implication of the genetic polymorphisms.

Because inflammatory pathways are considered as an integral part in the initiation and progression of CAD [18], the prognostic value of inflammatory biomarkers in this disease may help to reduce the risk of adverse cardiovascular events.

The aim of this study was to investigate, for the first time, the potential association of HLA-G, HLA-E and HLA-F genetic polymorphisms with CAD.

2. Materials and methods

2.1. Patient population

Eighty-nine patients were enrolled in the study. All patients were recruited from the Department of Cardiology of Hedi Chaker University Hospital of Sfax, Tunisia (from April 2010 to December 2012). Included patients have at least one of the following criteria: unstable angina (ECG changes without evidence of myocardial necrosis and clinical symptoms), acute myocardial infarction (positive markers of myocardial necrosis) including ST-segment elevation myocardial infarction (STEMI). We identified in our sample 10 artery bypass graft and 79 percutaneous coronary stenting (PCI) with stent implantation. Patients with congenital heart disease, multiple failure syndromes, malignancy were excluded.

Eighty-four control unrelated subjects from southern Tunisia (Sfax region) participated to this study. Some of the controls have hypertension ($n = 25$) and diabetes mellitus while the results of coronary angiogram are normal, which means that the control groups are exposed to the same risk factors of cardio vascular disease. The summary of the clinical features of the Tunisian sample is given in the Table 1. All participants have given informed consent.

Table 1
Clinical features of the Tunisian samples.

Characteristics	All patients ($N = 89$)	Controls ($N = 84$)
Mean (age)	56.88 ± 11.48[a]	55.65 ± 12.04[a]
Male	76 (85.4%)	71(84.5%)
Female	13 (14.6%)	13(15.5%)
BMI	25 ± 4.53[a]	
Blood pressure		
Systolic	128.03 ± 22.65[a]	
Diastolic	77.31 ± 12.96[a]	
Dyslipidemia	51 (57.3%)	
Alcohol	3 (3.3%)	
Smoking	64(71.9%)	
Hypertension	31(34.8)	
Diabetes	52(58.4%)	
Medications		
Statines	56 (62.9%)	
Inhibitor of angiotensine converting enzyme IEC	38 (42.7%)	
β-Blockers	52 (58.4%)	
Pump Proton inhibitors	60 (67.4%)	
Diagnosis		
Accurate syndrome coronary ST+	63 (70.8%)	
Accurate syndrome coronary ST−	18 (20.2%)	
Unstable angina	8 (9%)	
LVEF		
LVEF >50%	31(34.8%)	
LVEF 45–55%	30 (33.7%)	
LVEF 35–45%	11 (12.53%)	
LVEF <35%	8 (8.98%)	

N = number of samples. BMI: Body Mass Index; LVEF: Left Ventricular Ejection Fraction.
[a] Standard deviation.

2.2. Genotyping

Genetic analysis was made on genomic DNA extracted from peripheral blood by phenol–chloroform extraction [19].

2.3. Genetic variations

Polymerase chain reaction-sequence-specific priming (PCR-SSP) was used to detect HLA-E*01:01/01:03 A/G [20] and HLA-F*01:02 T/C, 01:03 C/T, 01:04 A/C [16] alleles. The sets of amplification primers were designed based on the published sequences [21]. Each PCR included specific primers and internal control primers pair (Hormone Growth Factor gene, amplification fragment length 439 bp) [20].

The 14-bp Insertion/Deletion (Ins/Del) polymorphism genotyping was performed by PCR with the primers referenced in Shankarkumar et al. [22]. Two PCR products were generated: 224-bp and 210-bp bands corresponding respectively to Insertion and Deletion alleles.

The polymorphism HLA-G +3142C/G performed by RFLP-PCR according to Graebin et al. [23].

Each gel was checked by two observers. In case of discrepancy, an independent PCR-SSP assay was performed.

2.4. Statistical analysis

The estimation of allelic, genotypic and haplotypic frequencies, and also the Hardy–Weinberg equilibrium test were carried out using Plink-1.07, a free open-source whole genome association analysis toolset [24]. The Armitage's trend test was used in order to explore genotypic associations. Also, a standard chi-square test implemented in Plink-1.07 was used to test the allelic and haplotypic association. A binary regression test was conducted using SPSS Inc, release 17.0 (Chicago, IL, US). The empirical power of the study was calculate as described by Kammoun-Krichen et al. (2009) [25].

3. Results

All studied genetic polymorphisms were in Hardy–Weinberg equilibrium ($p > 0.05$) except for the two markers HLA-G +3142C/G and HLA-F*01:03 C/T (respectively: $p = 0.012$; $p = 0.032$).

The allelic distribution of the Tunisian samples was shown in the Table 2. No association was reported concerning the polymorphisms of HLA-F gene. In fact, both HLA-F*01:02 and HLA-F*01:04 alleles have the same frequencies in control and patients groups.

The frequency of HLA-F*01:03 allele was similar in control participants (0.13) and patient groups for the frequency of the C allele (0.16) (Table 2). Similarly, no difference was reported in both groups concerning the frequency of HLA-G Del allele (−14 bp; Table 2). No association was retained ($p = 0.3$). These frequencies were analogous to African populations [26].