IL-10R1 (Ser138Gly) functional polymorphism is associated with acute myocardial infarction in Tunisian patients

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Coronary atherosclerosis is a chronic disease that can be considered as a form of chronic inflammation with stable and unstable phases corresponding to activated inflammation in the vascular wall (1). Evidence has been shown that cytokines, which are immunoregulatory proteins, play a complex role in the development of atherosclerotic lesions (1).

In addition, a variety of studies showed that interleukin-10 (IL-10) is a potent anti-inflammatory cytokine (2). Indeed, IL-10-deficient mice exhibited an increased lipid accumulation, whilst increased IL-10 serum level is a beneficial prognostic determinant in patients with acute coronary syndromes (3). Moreover, there is evidence that IL-10 may have protective effects against plaque rupture and thrombus formation by modulating several cellular processes involved in the development and stability of the atherosclerotic plaque (4). The main function of IL-10 seems to limit and ultimately terminate the inflammatory signal in inflammatory cells (5). That plays a major role in suppressing immune and inflammatory responses which down regulates the production of pro-inflammatory cytokines and chemokines (6).

The biological function of IL-10 is mediated through specific cell surface receptors. The functional receptor complex of IL-10 consists of two subunits, IL-10 receptor 1 (IL-10R1) and IL-10R2 (7).

The human *IL-10R* gene has been mapped to chromosome 11q23.3 and shown to be highly polymorphic. Nearly 335 single-nucleotide polymorphisms (SNPs) have been identified in *IL10R1* gene. Amongst these, SNP3 (rs3135932, c.536A/G, p.S138G) is of particular importance since it maps to exon 4 encoding IL-10R1 receptor domain (7) and has been associated with various diseases (e.g. schizophrenia, progression of fibrosis in chronic hepatitis C...) (8-10).

The aim of the present study was to evaluate the association of SNP3 (rs3135932) polymorphism with the risk of developing myocardial infarction (MI) in the Tunisian patients.

We designed a case-control study involving 120 adult Tunisian patients (64 males and 56 females, mean age 63.5±10 years) who presented a MI followed in the department of cardiology of the University Hospital Frahat Hached (Sousse, Tunisia). 100 healthy individuals (57 males and 43 females, mean age 52.5±6 years) were included as controls. These had no symptoms of coronary syndrome after assessment by a cardiologist.

Genomic DNA was isolated from peripheral white blood cells using standard procedures (Wizard Genomic DNA Purification Kit, Promega, USA). Genotyping of IL-10RA SNP3 polymorphism in the study population (220 individuals) was performed using a bidirectional PCR amplification of specific alleles (BiPASA) according to Gasche et al. (7).

The genotype distributions and allele frequencies for the SNP3 polymorphism investigated in healthy volunteers and MI patients were compared by chi-square (χ^2) test. Independent sample *t*-test was used for comparison of continuous variables. Threshold for the statistical significance (p) was set at 0.05. Hardy-Weinberg equilibrium was assessed in both of the study groups. Statistics were performed using SPSS18.0.0 and Epi info 6.0 softwares.

The epidemiological and biological characteristics are compiled in Table 1 and are expressed as means±standard deviations (SD) for quantitative variables and as percentage for qualitative ones (Table 1). The two groups are matched for gender (p>0.5). The conventional cardiovascular risk factors showed significant association with disease status.

Interestingly, in our study, the distribution of SNP3 genotypes and allele frequencies were significantly different between MI patients and controls (p=0.01) with the G allele conferring a higher risk for MI (OR (AG and GG)=2.73; 95% CI= [1.16-6.4]); (Table 2). Further more, given the role of cytokines in the pathogenesis of atherosclerosis, no association was found between

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Table 1. Demographic and clinical data by diagnostic groups

Variables	Patients (n=120)	Controls (n=100)	*P*
Women /Men	56/64	43/57	NS
Age, years	63.5±10	52.5±6	p<0.001
Total Cholesterol, mmol/L	5.08±1.33	4.54±1.22	
HDL, mmol/L	1.18±0.45	1.33±0.29	
LDL, mmol/L	2.6±1.25	1.76±0.83	
Triglycerides, mmol/L	2.18±1.46	1.56±1.39	
Glucose, mmol/L	9.62±4.38	5.87±1.52	
CRP, mg/L	11.94±15.33 (M=6)	7.91±4.7 (M=6)	
Homocystein, µmol/L	19.45±10.51	12.22±4.02	
Hypertension, %	70.8%	33%	
BMI, kg/m^2	29±4.16	24±3.29	
Smokers	27.5%	20%	

BMI - body mass index; CRP - C-reactive protein; HDL - high density lipoprotein; LDL - low density lipoprotein; M - median, NS - not significant

SNP3 genotypes and the conventional risk factors for CHD under different models.

The response of cells to IL-10 depends on the activation of the IL-10 receptor (IL-10R) complex (3). We assume that SNP3 polymorphism may generate some conformational rearrangements of the IL-10R1 receptor domain which, subsequently, affects IL-10 complex binding and alters its downstream signal (7). This can lead to an excessive inflammatory reaction promoting atherosclerosis and subsequent complications. This is illustrated by the significantly higher serum levels of inflammatory proteins (i.e.: CRP and homocystein) in our group of patients compared to controls.

In conclusion, IL-10 is known to play key roles in immune regulation; therefore a deficiency in IL-10 appears to be a good biomarker for CHD studies. Our findings gave unprecedented evidence that the SNP3 polymorphism is associated with MI. Further functional studies need to be performed to investigate thoroughly the underlying cellular mechanisms. This signaling pathway will provide practitioners with important targets for therapeutic strategies aimed at limiting the inflammatory response involved in the development of CHD and other inflammatory disorders.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval: The authors declare that the experiments comply with the current laws of Tunisia and with the ethical principles of the WMA Declaration of Helsinki.

Table 2. Distribution of the *SNP3* (rs3135932) alleles and genotypes in patients and control groups

IL-10R1 (536A/G)	Controls, n (%) (n=100)	Patients (n=120)	*P*	OR	95% CI
AA	92 (92%)	97 (81%)		1	
AG	8 (8%)	21 (17.5%)	0.045	2.49	1.05-5.9
GG	0	2 (1.5%)		-	-
Allele G	8 (4%)	25 (10.5%)	0.01		

CI - confidence interval; OR - odds ratio

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