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Rapid Communication

Interleukin-10 gene promoter polymorphisms in celiac patients from north-eastern Italy

Luisa Zupin [a], Vania Polesello [a], Eulalia Catamo [b], Sergio Crovella [a,b], Ludovica Segat [a,*]

[a] Institute for Maternal and Child Health, IRCCS "Burlo Garofolo", Via dell'Istria 65/1, 34137 Trieste, Italy
[b] University of Trieste, Piazzale Europa 1, 34127 Trieste, Italy

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ABSTRACT

Celiac disease is a complex chronic intestinal disorder driven by an immune response against the gliadin fraction of gluten: many factors are involved in the pathogenesis of the disease, and among these Interleukin-10 could play an important role. In the present study, the −1082A>G, −819T>C and −592A>C *IL10* functional polymorphisms were analyzed in 565 celiac patients and 576 healthy controls from north-eastern Italy, stratified for *HLA class II* celiac disease risk haplotypes. No significant differences were observed for the three *IL10* polymorphisms distribution between celiac patients and controls with the exception of a slightly increased risk for the −1082A allele in *HLA-DQ8* male individuals. Although our findings suggest that the *IL10* genetic variants analyzed do not have a major role in the susceptibility to the development of celiac disease in north-eastern Italian patients, we think that the possible involvement of IL10 gene in CD should deserve further investigation and that large-scale studies are recommended to confirm our findings.

1. Introduction

Celiac disease (CD) is the most common enteropathy induced by gluten intake in humans. The disease is influenced by the interplay between immune, genetic and environmental factors [1]. Specific *MHC II* alleles that map to the *HLA-DQ* locus are known to be the major genetic factors involved in CD [2].

Most of CD patients (near 90%) carry the *HLA-DQ2.5* haplotype (encoded by *DQA1*05, DQB1*02* and *DRB1*03* alleles), whereas a smaller percent of subjects carry the HLA-DQ8 haplotype (encoded by *DQA1*03*, *DQB1*0302* and *DRB1*04* alleles). A smaller percent of CD patients may present the *HLA-DQ2.2* haplotype (*HLA DQA1*0201*, *DQB1*02*, *DRB1*07*) known to confer only a minor risk for CD development with respect to *HLA-DQ2.5*. Indeed, this haplotype is able to present only few different gluten peptides, while the more predisposing haplotype (*HLA-DQ2.5*) can expose a greater variety of them [3].

In CD patients the ingestion of gluten promotes immune responses with inflammatory reaction in the upper small intestine, characterized by infiltration of inflammatory cells within the lamina propria and the epithelium with villous atrophy [4]. An important regulatory cytokine in the intestinal mucosa is Interleukin 10 (IL-10): it is an inhibitor of Th1 cell development and so underproduction of IL-10 may contribute to an increased Th1-driven inflammation, responsible for the intestinal lesions typical of CD [5]. Low levels of IL-10 have been associated with anti-tissue transglutaminase (anti tTG) antibodies in CD patients [6] and Salvati et al. [7] have also observed a suppression of gliadin-specific T cell activation using recombinant human IL-10 (rhIL-10).

The *IL10* gene maps on the long arm of chromosome 1 (1q31–q32) a locus previously identified by different genome wide association studies as possibly involved in CD [8–10].

Three single nucleotide polymorphisms (SNPs) at positions −1082 (A>G), −819 (T>C) and −592 (A>C) in the promoter region of the gene may affect the *IL10* gene expression [11–14]; previous studies have analyzed the influence of the three *IL10* SNPs on CD susceptibility with discordant results [15–17].

The aim of this study is to evaluate the possible association between *IL10* and CD, by analyzing the −1082A>G, −819T>C and −592A>C promoter polymorphisms in CD patients and healthy controls from north-eastern Italy.

* Corresponding author. Fax: +39 040 3785540.
E-mail address: ludovica.segat@burlo.trieste.it (L. Segat).

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2. Methods

2.1. Patients and controls

The 565 CD Italian patients (European–Caucasian; mean age = 21.75, standard deviation = 15.70, range = 4–85; sex, 338 females and 227 males) analyzed in this study, represented a selected subset of a bigger historical cohort (around 2500 samples) of celiac individuals that were recruited at the Gastroenterology Service of IRCCS Burlo Garofolo (Trieste, Italy) from July 2000 to December 2009.

The whole cohort was initially genotyped for *HLA class II* (*DQ*) haplotypes and patients were classified as presenting, in homozygosis or heterozygosis, *HLA* CD high risk haplotypes (*HLA-DQ2.5* and /or *HLA-DQ8*) (*HR HLA*) or the low risk *HLA-DQ2.2* haplotype; then we have randomly selected from the these groups 304 *HLA-DQ2.5*, 93 *HLA-DQ8* and 168 *HLA-DQ2.2* patients to be further analyzed for *IL10* polymorphisms.

CD diagnosis was made according to the European Society for Pediatric Gastroenterology, Hepatology and Nutrition guidelines [18].

As healthy controls, we enrolled a total of 576 European–Caucasian individuals (European–Caucasian, mean age = 40.56, standard deviation = 10.30, range = 18–78, sex, 319 females and 257 males), 213 carrying in homozygosis or heterozygosis high risk haplotypes (148 *HLA-DQ2.5* and 66 *HLA-DQ8*), 74 *HLA-DQ2.2*, 292 not carrying any of the *HLA* risk haplotype (nor, *HLA-DQ2.5*, nor *HLA-DQ2.2*, nor *HLA-DQ8*) (*NR HLA*). All had no clinical signs related to the disease and no familiar history of CD. CD was excluded by testing the subjects for the presence of anti-tTG antibodies.

A written free and informed consent was obtained from all subjects (or their parents in case of minor age).

The study was approved by the local ethical committee (Burlo Garofolo protocol no. CE/V-71).

2.2. HLA and IL10 genotyping

Genomic DNAs were extracted using the Wizard Genomic Purification kit (Promega, Madison, WI, USA). Patients and controls were screened for the presence of *HLA class II* risk haplotypes by using the Eu-Gen Risk kit (Eurospital).

IL10–1082A>G (rs1800896), −819C>T (rs1800871) and −592C>A (rs1800872) polymorphisms were detected using, respectively, C_1747360_10, C_1747362_10 and C_1747363_10 fluorogenic TaqMan SNP Genotyping Assay (Applied Biosystem – Life Technologies, Carlsbad, California, USA) on the 7500 Fast DX Real Time PCR Instrument (Applied Biosystem).

2.3. Statistical analysis

IL10 gene polymorphisms, allele and genotype frequencies were calculated by direct counting, while haplotype frequencies and linkage disequilibrium were computed using the Arlequin software (version 3.1) (http://cmpg.unibe.ch/software/arlequin3/). The Fisher's exact test was used for pair wise comparison of allele, genotype and haplotype frequencies using contingency tables as appropriate and only p-values < 0.05 were considered to be significant. All the statistical analyses were carried out using the open-source R package, available at the http://www.r-project.org site.

Table 1
IL10–1082A>G, −819T>C and −592A>C polymorphisms allele, genotype and haplotype frequencies (and counts) in all celiac disease (CD) patients and totality of healthy controls (HC). Hardy–Weinberg χ^2 and p values are also reported.

IL10 SNPs	Celiac patients			Healthy controls			CD vs. HC
	Total n = 565	Females n = 338	Males n = 227	Total n = 576	Females n = 319	Males n = 257	
−1082A>G							
A	0.60 (682)	0.59 (402)	0.62 (280)	0.61 (699)	0.63 (400)	0.58 (299)	p-Value = 0.90
G	0.40 (448)	0.41 (274)	0.38 (174)	0.39 (453)	0.37 (238)	0.42 (215)	
A/A	0.36 (202)	0.35 (118)	0.37 (84)	0.36 (205)	0.37 (119)	0.33 (86)	p-Value = 0.92
G/A	0.49 (278)	0.49 (166)	0.49 (112)	0.50 (289)	0.51 (162)	0.49 (127)	
G/G	0.15 (85)	0.16 (54)	0.14 (31)	0.14 (82)	0.12 (38)	0.17 (44)	
	χ^2 = 0.45	*χ^2 = 0.12*	*χ^2 = 0.43*	*χ^2 = 1.52*	*χ^2 = 2.34*	*χ^2 = 0.06*	
	p = 0.50	*p = 0.73*	*p = 0.51*	*p = 0.22*	*p = 0.13*	*p = 0.80*	
−819C>T							
C	0.73 (823)	0.73 (493)	0.73 (330)	0.72 (834)	0.73 (466)	0.72 (368)	p-Value = 0.85
T	0.27 (307)	0.27 (183)	0.27 (124)	0.28 (318)	0.27 (172)	0.28 (146)	
C/C	0.53 (299)	0.54 (183)	0.51 (116)	0.52 (302)	0.53 (170)	0.51 (132)	p-Value = 0.97
T/C	0.40 (225)	0.38 (127)	0.43 (98)	0.40 (230)	0.39 (126)	0.40 (104)	
T/T	0.07 (41)	0.08 (28)	0.06 (13)	0.08 (44)	0.07 (23)	0.08 (21)	
	χ^2 = 0.02	*χ^2 = 0.79*	*χ^2 = 1.73*	*χ^2 = 0.001*	*χ^2 = 0.003*	*χ^2 = 0.01*	
	p = 0.88	*p = 0.37*	*p = 0.19*	*p = 0.98*	*p = 0.96*	*p = 0.94*	
−592C>A							
C	0.73 (821)	0.72 (487)	0.74 (334)	0.72 (832)	0.72 (462)	0.72 (370)	p-Value = 0.85
A	0.27 (309)	0.28 (189)	0.26 (120)	0.28 (320)	0.28 (176)	0.28 (144)	
C/C	0.53 (300)	0.53 (180)	0.53 (120)	0.52 (302)	0.53 (168)	0.52 (134)	p-Value = 0.97
C/A	0.39 (221)	0.38 (127)	0.41 (94)	0.40 (228)	0.39 (126)	0.40 (102)	
A/A	0.08 (44)	0.09 (31)	0.06 (13)	0.08 (46)	0.08 (25)	0.08 (21)	
	χ^2 = 0.14	*χ^2 = 1.53*	*χ^2 = 0.95*	*χ^2 = 0.10*	*χ^2 = 0.04*	*χ^2 = 0.07*	
	p = 0.71	*p = 0.22*	*p = 0.33*	*p = 0.75*	*p = 0.84*	*p = 0.80*	
Haplotypes							
GCC	0.39 (443)	0.40 (270)	0.38 (173)	0.39 (451)	0.37 (238)	0.41 (213)	p-Value = 0.42
ACC	0.51 (370)	0.32 (216)	0.34 (154)	0.33 (376)	0.35 (223)	0.30 (153)	
ATA	0.26 (296)	0.26 (179)	0.26 (117)	0.27 (313)	0.27 (171)	0.28 (142)	
Others	0.02 (21)	0.02 (11)	0.02 (10)	0.01 (12)	0.01 (6)	0.01 (6)	