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IL-10 gene promoter region polymorphisms and their association with celiac disease

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ABSTRACT

Introduction: in celiac disease (CD), immune response activation results in local cytokine network impairment. Interleukin 10 (IL-10) is a key anti-inflammatory cytokine in the prevention of inflammatory conditions.

Objective: to analyze the association of single nucleotide polymorphisms in the IL-10 gene promoter region with CD in a population of Misiones Province, Argentina.

Patients and methods: DNA from whole blood was extracted from 40 patients with CD and 80 controls and the IL-10 gene promoter region containing polymorphisms

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rs1800896A/G, rs1800871T/C and rs1800872A/C was amplified. Risk was established by calculating odds ratios (OR) and statistical significance was considered as $p < 0.05$.

Results: there were no significant differences in rs1800896 genotype distribution between celiac patients and controls. The frequency of the CC genotype for rs1800871T/C and rs1800872A/C was lower among celiac patients (35 % *vs* 65 %; p = 0.002). CD risk was associated with carriers of the more uncommon T allele of rs1800871T/C and the more uncommon A allele of rs1800872A/C, with a dominant model (OR = 2.79; 95 % CI: 1.27-6.09; p = 0.01). A risk effect was found for haplotype ATA (OR = 3.05; 95 % CI: 1.25-7.46; p = 0.01).

Conclusion: carriers of the less common T allele of rs1800871T/C and the less common A allele of rs1800872A/C in the IL-10 gene promoter are at high risk of CD with a dominant model. There was no risk for rs1800896A/G. The ATA haplotype showed an association with CD development.

Keywords: Celiac disease. Interleukin 10. Gene polymorphisms.

INTRODUCTION

Celiac disease (CD) is a chronic, immune-mediated enteropathy that is triggered by gluten ingestion in genetically predisposed individuals (1,2).

CD may develop in both children and adults, with an incidence approaching 1.0 % of the population (3) and a wide spectrum of clinical manifestations, from severe malabsorption to minimally symptomatic or asymptomatic presentation. A diagnosis requires the presence of duodenal villous atrophy and circulating antibodies against tissue transglutaminase (2,4,5).

The pathogenesis of the disease depends upon the interaction of predisposing genes with gluten and environmental influences. CD is strongly associated with human leukocyte antigen (HLA) genes (chromosome 6p21). Most patients have the genotype variant HLA-DQ2 (alleles DQA1*05 and DQB1*02) and some have the variant HLA-DQ8 (DQA1*03, DQB1*0302). The expression of these molecules is necessary, but insufficient for disease development. Even though 30-40 % of the population are DQ2 carriers, only 1 % will develop CD. The absence of HLA-DQ2 or HLA-DQ8 has a negative

predictive value nearing 100 % to exclude a CD diagnosis (5-7). Other non-HLA regions have been identified that include susceptibility genes, which could also be shared by other immune-based chronic disorders.

In CD, innate and adaptive immune responses become activated, which alters the local cytokine network and leads to the development of intestinal inflammation and damage. Interleukin 10 (IL-10), whose gene is located in chromosome 1q31-32, is a powerful anti-inflammatory cytokine that often plays a key role in the prevention of inflammatory and autoimmune conditions. Primary sources for IL-10 include T-helper (Th) cells, monocytes, macrophages and dendritic cells (8-11). Cytokine production may be modulated by both genetic and environmental factors. The presence of polymorphisms in the coding or non-coding regions of cytokine genes may alter the efficiency of gene transcription and therefore, cytokine production. Various studies have reported that three single nucleotide polymorphism (SNPs) play a significant causal role in the regulation of IL-10 promoter activity. These are located at positions -1082 (rs1800896A/G), -819 (rs1800871T/C) and -592 (rs1800872A/C) with respect to the transcription start site. Together they represent three haplotype blocks that code for high (GCC), intermediate (ACC) or low (ATA) IL-10 expression (12-15). The predisposition to express high or low levels of a given cytokine may be related to the presence of polymorphisms in the region coding for its gene expression.

The hypothesis is that CD is an autoimmune disorder where inflammatory response is associated with the presence of the gene polymorphisms -1082 G/A (rs1800896), -819 C/T (rs1800871) and -592 C/A (rs1800872) in the IL-10 promoter.

The goal of this study was to analyze the association of single nucleotide polymorphisms in the IL-10 gene promoter region with celiac disease in a population living in Misiones Province.

PATIENTS AND METHODS

Type of study and population assessed

A case-control design was used. Blood samples were collected from 40 cases (mean age: 19, range: 1 to 56 years; 29 females and 11 males) and 80 controls (mean age: 27; range: 1 to 79 years; 47 women and 33 men) once they had given their informed

consent.

Cases included patients diagnosed with celiac disease according to positive serum anti-transglutaminase IgA (a-Ttg-IgA) antibodies and abnormal intestinal villi (according to the Marsh classification) in duodenal tissue samples obtained by endoscopic biopsy (2,16). The control population included asymptomatic, healthy volunteers with negative a-Ttg-IgA antibodies.

Blood samples were collected at the laboratory of Hospital Provincial de Pediatría Dr. F. Barreyro in Posadas, Misiones, from 2015 to 2017. The study was approved by the Ethics Committee at the Hospital Provincial de Pediatría Dr. Fernando Barreyro.

DNA extraction

Genomic DNA was extracted from whole blood samples using the modified salting-out method (17). The primers used to amplify the regions containing polymorphisms -1082 G/A (rs1800896), -819 C/T (rs1800871) and -592 C/A (rs1800872) within the IL-10 gene promoter were designed with the Primer3Plus software (available online), using reference sequences from the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/).

Amplification of study regions

All three polymorphisms were detected by the polymerase chain reaction (PCR) method using common primers (sense (5`-3`): CACACACACAAATCCAAGACAA; antisense (5`-3`) CCTGGGATGAATACCCAAGAC). These primers produced a 695-bp amplicon containing the regions encompassing the polymorphisms of interest.

Amplification was performed in a final reaction volume of 20 µl containing the following: 1x buffer, 3.5 mM magnesium chloride ($MgCl_2$), 200 µM triphosphate deoxyribonucleotide (dNTPs), 0.5 pM sense and antisense primer, 1 U thermus aquaticus polymerase (Taq polymerase) and 1 µl matrix DNA (dilution, 1:14). Cycling conditions were: pre-denaturation at 94 °C for 3 min, then 25 denaturation cycles at 94 °C for 30 sec, hybridization at 55 °C for 30 sec, elongation at 72 °C for 40 sec and a final extension at 72 °C for 5 min. The 695-bp amplicons obtained were verified by 2 % (p/v) agarose gel electrophoresis (p/v) in 0.5x tris-borate-EDTA (TBE buffer), stained with

GelRed™ and sequenced using Macrogen's automated sequencing service (http://dna.macrogen.com) (Fig. 1).

Statistical analysis and genetic testing

The comparison of genotype frequencies between cases and controls was performed with the Chi-squared test using the Epidat v. 4.2 software package. Control samples were tested for Hardy-Weinberg equilibrium and an association with the risk factor was established (presence of the lower frequency allele for each SNP) (18). Odds ratios (OR) were used with 95 % confidence intervals. ORs were estimated using the Epiinfo v. 7.2.1.0 software program. $p < 0.05$ was considered to be statistically significant. The "snpstats" program (https://www.snpstats.net/start.htm?) was used to calculate haplotype frequencies and associations for each inheritance model, namely dominant (Do), codominant (Co), overdominante (Ov), additive (Ad) and recessive (Re). The Akaike information criterion (AIC) was used to identify the model that best accounted for independent data, according to which the model with a lower AIC value was selected from among candidate models.

RESULTS

The genotype distributions did not deviate from the Hardy-Weinberg equilibrium. Table 1 lists the genotype frequencies obtained from both cases and controls. Genotypes TC in polymorphism rs1800871 and AC in polymorphism rs1800872 were more frequently found in celiac patients. Genotype CC for both polymorphisms was more frequently found in control subjects. The lower allele frequency distribution of polymorphisms in celiac patients and controls was rs1800896G/A: G: 0.33 *vs* 0.35 (p = 0.81); rs1800871C/T: T: 0.37 *vs* 0.21 (p = 0.01); and rs1800872C/A: A: 0.37 *vs* 0.21 (p = 0.01). The frequency of alleles T and A was higher in celiac patients.

Table 2 shows the association of each polymorphism with an inheritance model. A significant risk value was obtained for IL-10 polymorphisms rs1800871 and rs1800872 in the Do, Co, Ov and Ad models. For these polymorphisms, the AIC was significant in every model: Do = 150, Ov = 151, Co = 152, Ad = 152 and therefore, the Do model was selected.

Table 3 shows the haplotype frequencies and the association of each haplotype with response. A mild protective effect was found for haplotype ACC at positions -1082, -819 and -592 when compared with other haplotypes, and a risk effect of haplotype ATA (OR = 3.05; 95 % CI 1.25-7.46; p = 0.01).

DISCUSSION

In celiac disease, the interaction between genetic and environmental factors results in a loss of tolerance to gluten. This promotes T-cell activation, differentiation and proliferation, and the induction of cytokine expression programs. Cytokine IL-10 plays a role in both the susceptibility to and course of several disorders. In fact, polymorphisms in the IL-10 gene promoter have been associated with disease prevalence and severity (11). Fonseca-Camarillo et al. (19) showed that IL-10 gene and protein expression is increased in the mucosa of patients with chronic idiopathic ulcerative colitis (CIUC) in remission, as compared to patients with active CIUC and control subjects without inflammation.

Individual susceptibility or resistance to different diseases is primarily dependent on single nucleotide polymorphisms (SNPs) (14). In a study of patients with irritable bowel syndrome with diarrhea (IBS-D), Schmulson et al. (13) reported a low frequency (8.9 %) of the IL-10 -1082GG genotype and a high frequency (14 %) of the -1082AA genotype. This suggests a genetic predisposition to impaired immune regulation with a lower anti-inflammatory component. The same polymorphism was studied by Gonsalkorale et al. (20), who found a low frequency (21 %) of the GG genotype in patients with irritable bowel syndrome (IBS) as compared to controls (32 %; $p < 0.05$). The allele frequency for polymorphism rs1800896 obtained in our study was similar to that identified in various populations such as Americans (A, 70 %; G, 30 %), Africans (A, 69 %; G, 31 %), South Asians (A, 76 %; G, 24 %) and Europeans (A, 55 %; G, 45 %) (21). The frequency of genotype -1082GG was low, and the frequency of the low-producer polymorphism (-1082AA) was high, both in celiac patients and control subjects, suggesting that this variant does not contribute to CD susceptibility in our population.

The allele frequency for polymorphism rs1800871(-819) obtained in our study was similar to that of American (C, 67 %; T, 33 %) and European (C, 76 %; T, 24 %)

populations, with a higher frequency of allele C. The frequencies are inverted in East Asia (C, 32 %; T, 68 %) (21).

With regard to the rs1800871 polymorphism, carriers of the less frequent T allele are at higher risk of CD when this allele was inherited in a dominant manner (TC and TT). SNP rs1800872 exhibited a similar risk for allele A carriers. Until now, there are no reports according to the inheritance model. Núñez et al. (22) studied patients in the Autonomous Region of Madrid (Spain) and reported that IL-10 polymorphisms rs1800896 and rs1800872 do not seem to play a role in CD predisposition. Zupin et al. (23) observed no significant differences in the distribution of all three IL-10 polymorphisms between celiac cases and controls in patients from north-eastern Italy. Except for a slightly higher risk for allele A of SNP rs1800896 in HLA-DQ8 male individuals.

IL-10 promoter haplotypes may contribute to the impaired cytokine expression and systemic inflammation seen in celiac disease. Studies to measure the relationship between SNP frequency, disease incidence and specific haplotype penetrance in various populations report conflicting results. The frequency of GCC at positions -1082, -819 and -592 is above 50 % among Caucasians but below 5 % among populations of Asian descent (11). Garrote et al. (24) found an association with haplotype ATA in celiac cases (OR = 0.47; 95 % CI: 0.23-0.96; p = 0.025) in patients from Valladolid and Barcelona (Spain). As previously stated, Núñez et al. (22) studied patients from Madrid (Spain) and reported that IL-10 polymorphisms do not seem to play a role in celiac disease predisposition. Whereas Zupin et al. (23) found no significant differences in the distribution of IL-10 polymorphisms between celiac patients and controls in patients from north-eastern Italy, except for a slightly higher risk for allele -1082A among HLA-DQ8 male individuals. The higher prevalence of the IL-10 promoter haplotype ATA and reduced expression of IL-10 may contribute to the development of CD, together with genetic predisposition. In our study, haplotypes ATA coding for reduced IL-10 expression were more common in CD patients than in controls. This is consistent with the findings of Hofmann et al. (15), who reported that ATA haplotypes were more common in patients with CD (p = 0.01), whereas GCC haplotypes were more frequent among controls (p = 0.03). The association study suggested a protective effect for

haplotype ACC at positions -1082, -819 and -592, and a risk effect for haplotype ATA. These polymorphisms may be used as predictors of inflammatory response in CD.

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Table 1. Genotype frequencies of SNPs rs1800896, rs1800872 and rs1800871 at the I-10 gene promoter in celiac patients and controls

Polymorphism	*Genotypes*	*Celiac patients-cases*		*Controls*		*p*
		n	%	n	%	
rs1800896A/G	GG	5	12.5	13	16	0.588
	AG	16	40	30	38	0.791
	AA	19	47.5	37	46	0.897
rs1800871T/C	TT	4	10	6	7.5	0.640
	TC	22	55	22	27.5	0.003
	CC	14	35	52	65	0.002
rs1800872A/C	AA	4	10	6	7.5	0.640
	AC	22	55	22	27.5	0.003
	CC	14	35	52	65	0.002

n: number of patients or controls; p: statistical significance.

Table 2. Risk analysis of polymorphisms rs1800896, rs1800871 and rs1800872 at the IL-10 gene promoter according to inheritance model

Model of inheritance	*Genotype*	*Cases*		*Controls*		*OR*	*95 % CI*	*p*
		n	%	n	%			
rs1800896A/G								
Do	AG-GG	21	53	43	54			0.89
	AA	19	47	37	46	0.95	0.44-2.02	
Re	GG	5	13	13	16			0.10
	AG-AA	35	87	67	84	0.29	0.06-1.38	
Co	AA	19	47	37	46	1.0	-----	0.86
	AG	16	40	30	38	1.03	0.46-2.31	
	GG	5	13	13	16	0.75	0.23-2.41	
Ad	---	---	---	---	---	1.10	0.65-1.88	0.72
Ov	AG	16	40	30	32	1.11	0.51-2.42	0.79
rs1800871T/C								
Do	TC-TT	24	60	28	35	2.79	1.27-6.09	0.01
	CC	16	40	52	65			
Re	TT	4	10	6	8	1.37	0.36-5.16	0.64
	TC-CC	36	90	74	92			
Co	CC	16	40	52	65	1.0	-----	0.03
	TC	20	50	22	27	2.95	1.29-6.74	
	TT	4	10	6	8	2.16	0.54-8.64	
Ad	---	---	---	---	---	2.16	1.19-3.93	0.01
Ov	TC	20	50	22	27	2.64	1.19-5.81	0.01
	CC-TT	20	50	58	73			
rs1800872A/C								
Do	AC-AA	24	60	28	35	2.79	1.27-6.09	0.01
	CC	16	40	52	65			
Re	AA	4	10	6	8	1.37	0.36-5.16	0.64

	AC-CC	36	90	74	92			
Co	CC	16	40	52	65	1.0	-----	0.03
	AC	20	50	22	27	2.95	1.29-6.74	
	AA	4	10	6	8	2.16	0.54-8.64	
Ad		---	---	---	---	2.16	1.19-3.93	0.01
Ov	AC	20	50	22	27	2.64	1.19-5.81	0.01

Co: codominant; Do: dominant; Re: recessive; Ad: additive; Ov: overdominant; p: statistical significance.

Table 3. Analysis of polymorphism haplotypes at the IL-10 gene promoter

Polymorphism	*rs1800 896*	*rs1800 871*	*rs1800 872*	*Cases*		*Controls*		*p*	*OR (95 % CI)*	*p*
				n	%	n	%			
Haplotype	A	C	C	13	32	39	47	0.13	0.37 (0.17-0.79)	0.001
	G	C	C	12	31	26	33	0.76	0.76 (0.33-1.74)	---
	A	T	A	14	35	12	17	0.02	3.05 (1.25-7.46)	0.01
	G	T	A	1	2	1	1.0	0.80	---	---
	G	T	C	0	0	1	1.0	0.80	---	--
	A	C	A	0	0	1	1.0	--	---	--
	A	T	C	0	0	0	0	--	---	--

n: number of patients; %: percentage; OR: odds ratio; CI: confidence interval; p: statistical significance.

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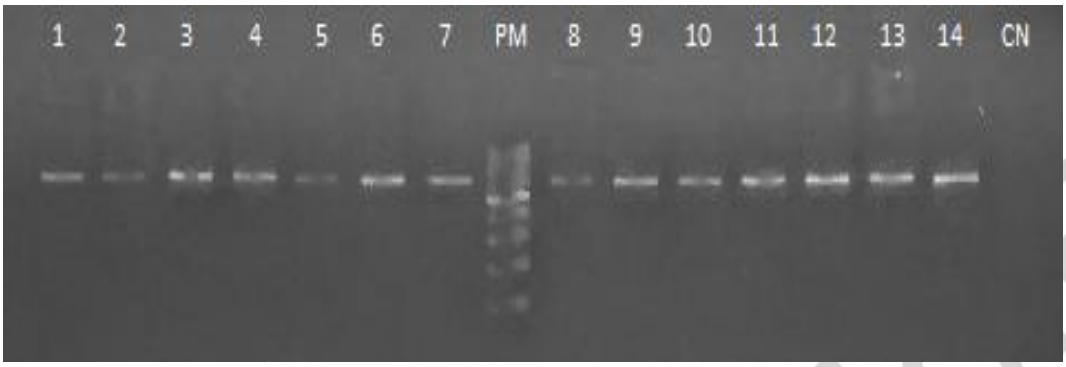

Fig. 1. Control amplification of the IL-10 gene promoter region containing the three polymorphisms of interest. A. 2 % agarose gel containing the 695 bp amplicons (PM: molecular weight marker; CN: negative control). B. Genotypes of SNPs -1082 G/A, -819 C/T and -592 C/A via sequencing of each fragment.

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