

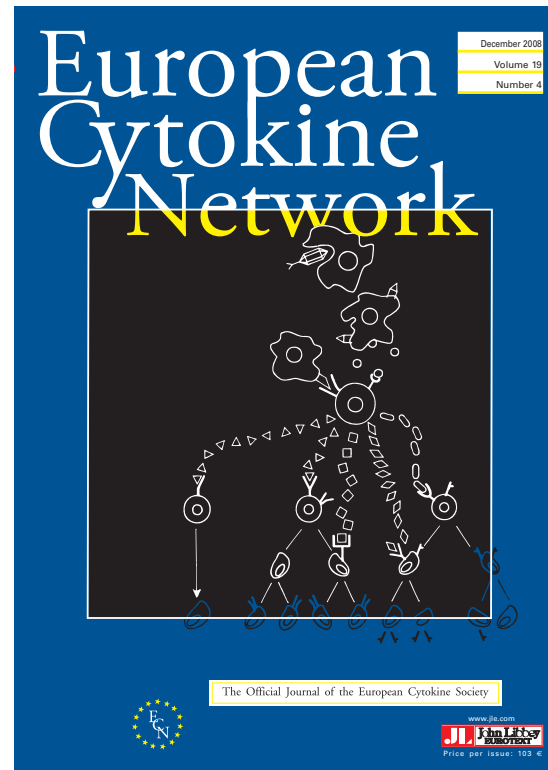


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**paru dans**

European Cytokine Network, 2008, Volume 19, Numéro 4

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## IL-23R Arg381Gln polymorphism in Chilean patients with inflammatory bowel disease

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Accepted for publication September 24, 2008

**ABSTRACT.** Crohn's disease (CD) and ulcerative colitis (UC) are multifactorial diseases with a genetic background. Recent results have shown that a non-synonymous, single nucleotide polymorphism (rs11209026, c.1142G>A, p.Arg381Gln) located in the IL-23R gene is associated with inflammatory bowel disease (IBD). The prevalence of IBD is rapidly rising in Chile and there is no information about the frequency of this polymorphism in the Chilean population. **Aim.** To assess the distribution of DNA variants in the IL-23R gene in Chilean patients with IBD. **Methods.** We studied 100 IBD patients (38 CD and 62 UC) and 59 healthy controls. IL-23R Arg381Gln (G1142A) was genotyped by the polymerase chain reaction and restriction fragment length polymorphism assay. Clinical and demographic features were characterized. **Results.** The IL-23R genetic variant did not have an association with IBD in Chilean patients. This polymorphism was present in 5.2% of the control group and 5% of IBD patients (7.9% for CD and 3.2% for UC) ( $p > 0.05$ ). **Conclusions.** These results suggest that the IL-23R Arg381Gln seems not to be involved in the genetic predisposition to IBD in a Chilean population, and confirms that there are ethnic differences in the genetic background of IBD. Replication studies by independent groups are necessary to elucidate the contribution of susceptibility genes to IBD in different ethnic populations.

**Keywords:** Chilean, inflammatory bowel disease, IL-23R

Inflammatory bowel disease (IBD) includes ulcerative colitis (UC), Crohn's disease (CD) and non-classifiable IBD, which are characterized by a chronic illness of unknown etiology; however, its development is influenced by genetic, environmental and immunological factors [1].

In recent years, there has been dramatic progress in the understanding of the genetics of IBD, however the impact on diagnosis and treatment remains limited [2, 3]. In 2001, the *NOD2/CARD15* gene, located on chromosome 16, was identified as the first susceptible gene for IBD, specifically in CD [4, 5]. A meta-analysis of 37 studies of *NOD2/CARD15* variants estimated that homozygous carriage of *NOD2* mutant alleles confers a 17.1-fold increased risk of developing CD, whereas heterozygous carriage increases the risk of CD 2.4-fold [6]. Although several studies have confirmed this link, *NOD2/CARD15* accounts for less than 20% of all CD cases [6]. A recent Chilean study has confirmed that inflammatory bowel diseases are polygenic disorders [7].

In the last two years, new genetic susceptibility factors for IBD have been identified [1, 2]. Recently, by means of genome-wide association (GWA) studies, the non-synonymous, single nucleotide polymorphism (rs11209026, c.1142G>A p.Arg381Gln) located in the *IL-23R* gene has been identified as a protective variant for inflammatory bowel disease (IBD) in North American and European adult populations [8-13]. Studies have also confirmed this genetic association in others ethnic populations [14, 15], as well as pediatric IBD patients [16-18]. Interestingly, no association was observed in a well-powered, Japanese CD cohort, indicating alternative disease mechanisms in different population groups [19].

The *IL-23R* gene on chromosome 1p31 encodes a subunit of the receptor for IL-23 and associates with IL-12RB1 to form the IL-23 receptor. IL-23 promotes, along with TGF- $\beta$ 1 and IL-6, the expansion of the development of a new lineage of CD4 T cells, the proinflammatory IL17-secreting cells [20]. Th17 cells have been linked to the pathogenesis of IBD [21, 22].

To date, there have been no studies evaluating the frequency of the *IL-23R* Arg381Gln polymorphism in Chilean patients. Only one study in South-America has replicated the associations between *IL-23R* and IBD [15],

however, this study was performed only in Brazilian patients with CD. We undertook this study therefore, to determine the presence and prevalence of this gene mutation in Chilean IBD patients.

## DONORS AND METHODS

### *Patients and controls*

We carried out a case-controlled, matched cohort, comprising one hundred IBD patients (38 CD and 62 UC) and 59 healthy individuals. All individuals included in this study came from families that had been born in Chile over at least two consecutive generations. The 80% of individuals included were of a low-medium socioeconomic status.

The case group included IBD patients recruited from the Section of Gastroenterology of the Hospital Clínico Universidad de Chile, which belongs to the Health Service and serves mainly low-medium socioeconomic classes. Patients with the diagnosis of CD or UC were included in the study based upon clinical endoscopic, radiological and histological findings, according to standardized criteria and had been followed for at least one year. Clinical data of IBD patients were obtained through retrospective analysis of the patients' clinical charts prior to genotyping. The diagnosis of UC and CD were determined according to the Montreal classification system [23]. Here, those with non-classifiable IBD or indeterminate colitis were excluded.

The following data for the IBD patients were obtained: age, age at diagnosis, gender, familial or spontaneous diseases (familial disease was considered when one first- or second-degree relative had also been diagnosed with IBD), smoking habits (current smoking, history of smoking, or never smoked), disease localization, disease pattern, extra-intestinal manifestations (articular, ophthalmological, dermatological and hepatic manifestations), occurrence of colorectal cancer, perianal disease and surgery.

The control group comprised healthy individuals who had no history of immune-mediated diseases. The study was approved by the appropriate ethics committee and written informed consent was obtained from all participants involved in the study.

### *Genotyping of the Arg381Gln IL-23R variant*

Blood samples were collected in tubes containing EDTA, and genomic DNA was prepared from peripheral blood lymphocytes using the Wizard<sup>®</sup> Genomic DNA purification kit (Promega, Madison WI, USA). The Arg381Gln (G1142A) polymorphism was genotyped using the polymerase chain reaction followed by restriction fragment length polymorphism assay (PCR-RFLP). For the PCR, oligonucleotides primers were synthesized based on GenBank NW 921351. Primer sequences were: 5' – CTTTTCTGGCAGGGTCATTTTG-3' (sense) and 5' – AAGTTGTTTCTGGGGTAGTTGTG – 3' (antisense). We performed PCR using 1.25 U GoTaq Flexi DNA Polymerase (Promega, Madison WI, USA) and a primer annealing temperature of 55°C. The 508-bp PCR product was run on a 2% agarosa gel (Promega, Madison WI,

USA) and stained with ethidium bromide for visualization on a UV transilluminator. For the RFLP, 20  $\mu$ L of amplicons were digested with 5 U of *Hpy 188I* restriction endonuclease (New England Biolabs, MA, USA) over two hour at 37°C, and the digestion products were run on a 3% agarose gel and stained with ethidium bromide for visualization on a UV transilluminator. *Hpy 188I* digestion of wild-type DNA yields fragments of 288, 103, 82 and 35 base pairs, whereas DNA containing the G1142A polymorphism yields fragments of 323, 103 and 82 base pairs.

### *Statistical analysis*

Comparison of genotypes and allelic frequencies of Arg381Gln (G1142A) between patients (IBD, CD and UC) and controls was performed using the Chi-square test, and odds ratio was calculated with a confidence interval of 95%. A p value less than 0.05 was considered statistically significant.

## RESULTS

Demographic and clinical characteristics of CD and UC patients are shown in *tables 1* and *2*, respectively. In the CD group, the mean age at diagnosis was 45.4 yr (range 15-68), 24 patients were female. Of these patients, based on the Montreal Classification (23), 44.8% of patients had disease location L2 + L4 (colonic with or without upper tract disease), 26.3% had L3 + L4 (ileocolonic with or without upper tract disease) and 28.9% had L1 + L4 (ileum with or without upper tract disease). The distribution of disease behavior at diagnosis was: 63.2%

**Table 1**  
Clinical characteristics of patients with Crohn's disease

<b>Patients</b>	n = 38
Male/female	14/24
Age (years)	45.4 (15-68)
Age at diagnosis (years)	43.7 (14-68)
<b>Disease duration before diagnosis (months)</b>	11.7
Disease localization n (%)	
Ileum	11 (28.9)
Colon	17 (44.8)
Ileocolon	10 (26.3)
<b>Perianal disease n (%)</b>	5 (13.2)
<b>Upper gastrointestinal tract n (%)</b>	1 (2.6)
<b>Behavior n (%)</b>	
Inflammatory	24 (63.2)
Stricturing	10 (26.3)
Penetrating	4 (10.5)
<b>Extra-intestinal manifestations n (%)</b>	
Articular	14 (36.8)
Skin	3 (7.9)
Oral ulcers	4 (10.5)
<b>Surgery n (%)</b>	11 (28.9)
<b>Familial IBD n (%)</b>	2 (5.3)
<b>Smoking habits n (%)</b>	
Never	21 (55.3)
Current	7 (18.4)
Ex-smoker	10 (26.3)

**Table 2**  
Clinical characteristics of patients with ulcerative colitis

<b>Patients</b>	n = 62
Male/female	22/40
Age (years)	38.5 (17-68)
Age at diagnosis (years)	33.3 (11-68)
Disease duration before diagnosis (months)	14.0
<b>Disease localization n (%)</b>	
Proctitis	5 (8.1)
Rectosigmoiditis	9 (14.5)
Left-sided colitis	14 (22.6)
Pancolitis	34 (54.8)
<b>Extra-intestinal manifestations n (%)</b>	
Articular	20 (32.3)
Skin	2 (3.2)
Ocular	1 (1.6)
Oral ulcers	3 (4.8)
<b>Surgery n (%)</b>	2 (3.2)
<b>Familial IBD n (%)</b>	2 (3.2)
<b>Smoking habits n (%)</b>	
Never	36 (58.1)
Current	10 (16.1)
Ex-smoker	16 (25.8)

of patients had B1 + P (non-penetrating, non-perforating with or without perianal disease), 26.3% had B2 + P (stricturing with or without perianal disease), and 10.5% had B3 + P (penetrating). Fourteen (36.8%) patients had arthralgias. Eleven (28.9%) patients needed surgery. In the UC group, the mean age at diagnosis was 38.5 yr (range 15-61), 40 patients were female. Fifty five percent of patients had pancolitis, 22.6% had left-sided colitis, 14.5% had rectosigmoiditis and 8.1% had proctitis. Twenty (32.3%) patients had arthralgias. Two patients needed surgery.

Genotypes and allelic frequencies of *IL-23R* rs11209026G/A (Arg381Gln) for the IBD and control cohorts are shown in table 3. No significant differences were found in the presence of this genetic variant in IBD patients and healthy controls, 5% and 5.2% respectively ( $p = 0.96$ ); odds ratio: 0.96; IC95% (0.18-6.45). The allelic frequency 1142A was present in the same proportion in patients with IBD as in the control group, 2.5% and 2.6% respectively ( $p = 0.96$ ). In CD and CU, there were no significant differences in genotype (7.9% CD and 3.2% UC) ( $p = 0.29$ ) or allelic frequency (3.9% CD and 1.6% UC) compared with controls when analyzed separately ( $p = 0.47$ ).

The genetic analysis performed by PCR-RFLP showed the presence of the G1142A polymorphism, but only

with a heterozygous pattern. The PCR product and RFLP assay are shown in figure 1.

## DISCUSSION

*IL-23R* Arg381Gln polymorphism has recently been identified as a potential genetic susceptibility factor for IBD. In spite of the possible association between *IL-23R* Arg381Gln and IBD, the functional consequences of this polymorphism are still unknown [2, 3].

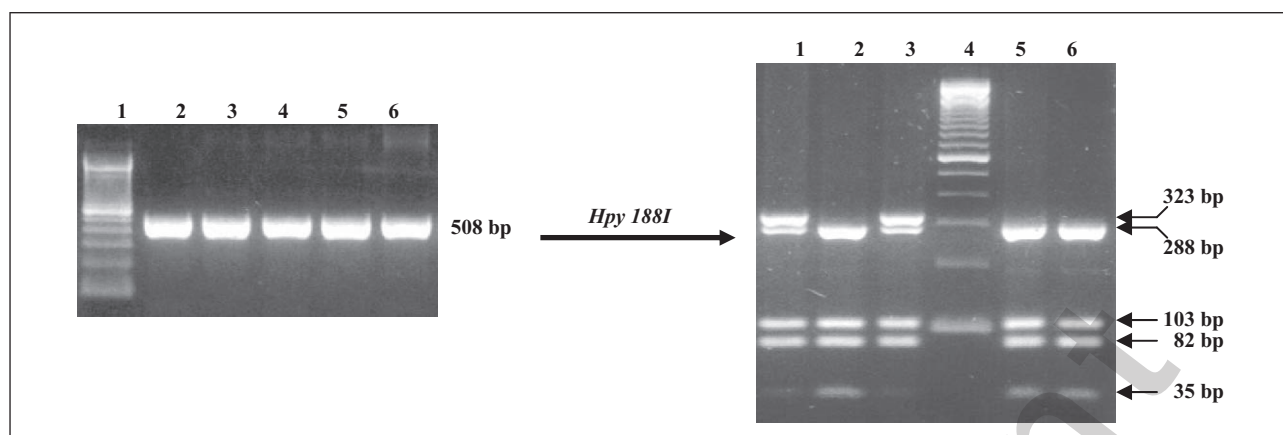
This is the first study that has sought to determine the prevalence of the *IL-23R* Arg381Gln polymorphism in Chilean patients with IBD (CD and UC). Although the number of patients included in the present study is small, our results have failed to demonstrate any positive association between this *IL-23R* variant and IBD in Chilean patients. Even though the results are in disagreement with North American and European studies [8-13], our experience is in accordance with data recently obtained from Japanese CD patients [19].

Genetic research represents a powerful and direct approach to determine the basis of human diseases. As regards IBD, the goals of genetic studies are the identification of mechanisms contributing to susceptibility to, and expression of clinical disease, and the development of predictive risk models. However, in many cases, reported associations are not consistently reproducible due to the diverse ethnic background between the IBD populations studied and differences in the environmental factors of each region. In Chile, allele frequencies for genetic markers are known to vary, according to socioeconomic strata from those observed in European Caucasians and Amerindians (of Mongoloid origin), the native Amerindian alleles being more prevalent in the lower socioeconomic stratum [24]. Eighty percent of individuals included in this study were from a low-medium socioeconomic stratum. Our results confirm that, as in the Japanese population [19], associations between genetic variants and IBD are not consistently reproducible in different ethnic populations. We were not able to find any obvious positive associations between the *IL-23R* Arg381Gln polymorphism and Chilean IBD patients from the low-medium socioeconomic stratum. The rs11209026 polymorphism was absent both in Japanese CD patients and healthy controls [19]. Therefore, independent result replication in different ethnic populations should be a key feature of genetic studies to elucidate and validate accurately disease-gene associations [25].

**Table 3**  
Distribution of the Arg381Gln variant within *IL-23R* in inflammatory bowel disease patients and controls

Arg381Gln <i>IL-23R</i>	Control	IBD	p value	CD	p value	UC	p value
GG	55/58 (94.8%)	95/100 (95%)	0.96	35/38 (92.1%)	0.29	60/62 (96.8%)	0.29
GA	3/58 (5.2%)	5/100 (5%)	0.96	3/38 (7.9%)	0.29	2/62 (3.2%)	0.29
AA	0	0		0		0	
Allele A	3/116 (2.6%)	5/200 (2.5%)	0.96	3/76 (3.9%)	0.47	2/124 (1.6%)	0.29

IBD: inflammatory bowel disease; UC: ulcerative colitis; CD: Crohn's disease.



**Figure 1**

PCR-RFLP Assay for rs11209026 IL-23R (G1142A). Left: Agarose gel electrophoresis showing the 508 bp PCR amplicons of IL-23R gen. Lane 1, 100 bp DNA molecular weight marker; lane 2, 3, 4, 5 and 6, samples. Right: Electrophoretic pattern of RFLP on digestion with *Hpy 188I*. Lanes 2, 5, and 6, samples with wild-type pattern. Lane 4, 100 bp DNA molecular weight marker. bp: base pairs.

The first genetic study in South America linking *IL-23R* and IBD was reported in Brazilian individuals [15], in which the generic variant was present in 3.8% of CD patients compared to 11.2% in controls. In contrast to this study, our findings did not lead to any positive association between the *IL-23R* polymorphism and CD. It is important to highlight that our study is the first one in South America to include UC patients, and in which no positive association was observed. These differing results confirm the strong, regional, ethnic heterogeneity in the Latinoamerican population.

Although the etiology of IBD remains unresolved, disease initiation may be related to an abnormal inflammatory response to enteric commensal microflora, in genetically predisposed individuals. Clinical and epidemiological studies do not support a simple Mendelian model of inheritance for CD and UC. IBD is considered to be a complex polygenic disease and this genetic susceptibility is a major factor contributing to the disease. Several novel IBD candidate genes have recently been identified, some of them with genetically associated functional polymorphisms. However, in many cases, reported polymorphic genetic associations have not been consistently reproducible. While mutations of the *NOD2/CARD15* gene are more frequent in Caucasian patients with CD than in Caucasian control subjects [6], they have not been observed [26], or are very rare [27] in Asian, Arabic [28], and African patients [29]. Our previous findings showed that the frequency of three *NOD2/CARD15* mutations identified in a European cohort population was not applicable to Chilean CD patients. In this study, 13.6% of CD patients had one of the three *NOD2/CARD15* polymorphic variants [7]. Overall, our results that relate to the *NOD2/CARD15* and *IL-23R* polymorphisms in Chilean IBD patients, confirm that the complex genetic background that may allow the development of IBD is not yet fully understood.

Many studies have evaluated variations in phenotypic expression and genetic polymorphisms. *NOD2/CARD15* has consistently been shown to be a susceptibility factor and a predictor of the CD phenotype [5, 6, 30, 31].

In epidemiological studies, *NOD2/CARD15* variants are significantly overrepresented in patients with early-onset disease, ileal involvement, fibrostenosing disease behavior, non-perianal fistulizing phenotypes and those pediatric CD patients at increased risk of surgery [5, 6, 30, 31]. Because of the low frequency of *NOD2/CARD15*, we could not demonstrate an association between this genetic variant and its phenotypic expression in Chilean CD patients [7]. In contrast to *NOD2/CARD15*, the results currently available have suggested that *IL-23R* variants do not determine disease phenotype [10, 11].

Importantly, no obvious association has been observed between *IL-23R* Arg381Gln and any other systemic inflammatory disease, such as systemic lupus erythematosus [32], rheumatoid arthritis [33] or syndromes with local tissue inflammation, such as coeliac disease [9]. However, the *IL-23R* Arg381Gln polymorphism has been associated with other autoimmune diseases: focusing on the psoriatic population, Capon *et al.* studied 519 patients and 528 controls, and reported a protective influence ( $p = 0.00014$ ) [34]. Similarly, a positive association between eight single nucleotide polymorphisms spanning the *IL-23R* gene in a Spanish cohort with ankylosing spondylitis, using the case-controlled design, has been reported [35]. For our study, IBD patients with a history of any other immune-mediated diseases were excluded.

So what are the exciting discoveries awaiting us in IBD genetics over the next few years? The power to ultimately determine which variants are truly responsible for genetic susceptibility to IBD will require testing DNA samples from large numbers (*i.e.*, several thousands) of IBD cases and ethnically-matched control. Moreover, replication studies in different ethnic populations will have to be developed as well as evaluation of associations in more phenotypically homogenous subsets, such as inflammatory, penetrating or stricturing CD patients to maximize the analysis. Finally, when our understanding of the specific implications of IBD susceptibility gene variants becomes established, we will likely see huge impacts on diagnosis, prediction of disease evolution, medical therapies, and potentially preventive treatments, and all

implemented on the basis of a patient's individual, IBD susceptibility gene profile.

In conclusion, it must be considered that if there is an association between IBD and *IL-23R* in Chilean IBD patients, it is very weak. We failed to confirm that the genetic variants of *IL-23R* protect against IBD in the Chilean population. Our results confirm that there seems to exist great diversity between the susceptibility genes in IBD in different ethnic populations. Further research, involving larger and more diverse IBD populations, are necessary for the identification of new candidate genes for these complex diseases, which in turn could lead to more accurate diagnosis and ultimately to the development of better drugs and more effective therapeutic strategies.

**Acknowledgments.** We would like to acknowledge Prof. Fergus Shanahan for his constructive and critical revision of this manuscript.

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