Human Immunology 74 (2013) 658–665

Contents lists available at SciVerse ScienceDirect

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

Cytokine gene polymorphisms in Tunisian endemic pemphigus foliaceus: A possible role of il-4 variants

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ARTICLE INFO

Article history:
Received 28 June 2012
Accepted 14 January 2013
Available online 29 January 2013

ABSTRACT

Polymorphism in the genes of TH2 cytokines and/or theirs receptors can influence serum cytokine levels in and the switch to the pathologic IgG4 auto-antibodies. In order to underline the role of these genes in the aethiopathogenesis of Pemphigus Foliaceus, we conduct a familial and a case control studies including 80 Tunisian patients, 147 related subjects and 160 matched healthy controls. We investigated, by PCR-RFLP technique, seven nucleotide polymorphisms: rs2243250 in promoter region of *IL4* gene, rs47877948, rs3024530 and rs30246223 in the *IL4R* gene, rs1881457and rs205412 SNPs in *IL13* gene and rs535036 in *IL13RA2* gene.

After Bonferroni adjustment, T allele and the TT genotype of IL4–590 were significantly increased in the PF patients group compared to healthy controls. This association was confirmed by the family study. Interestingly, the serum IL-4 levels were significantly increased in patients with the TT genotype compared to CT or CC genotypes.

Interestingly, the IL4/IL13:T–A–C haplotype exhibited a significant effect on PF susceptibility. In addition, a significant gene–gene interaction between the IL4/IL4R (TACA) significantly increases in PF patients as compared to controls.

These findings assess the role of the IL4/IL4R axis in the aethiopathogenesis of Tunisian endemic PF by the induction of a high transcriptional activity which could enhance the T-cell balance and inducing immunoglobulin isotype switching.

1. Introduction

Pemphigus is a tissue-specific autoimmune disease in which antibodies (Abs) against the keratinocyte cell surface induce intra-epidermal skin blisters [1]. Pemphigus foliaceus (PF) is one major clinical variant of pemphigus characterised by the presence of Abs that target desmoglein 1 (Dsg 1), a desmosomal cadherin found predominantly in the superficial layers of stratified squamous epithelia [2,3]. Anti-Dsg1 auto-Abs of IgG4 subclass are pathogenic because, when transferred to normal mice, their in vivo binding to Dsg1 leads to a loss of adhesion between keratinocytes called acantholysis and the formation of intraepidermal blisters [4,5]. The production of anti-Dsg1 Abs is dependent on, not only B lymphocytes (switch of Ig classes and subclasses), but also Dsg1-specific Th lymphocytes, which exhibit a memory T cell phenotype and a Th2-like cytokine profile detected in PF patients [6].

PF can be subclassified into two types: the sporadic form found throughout the world and the endemic one, which is called *fogo selvagem* in Brazil and is also discovered in Columbia and Tunisia [7–9]. The disease develops from interactions between genetic and exogenous factors. The involvement of genetic factors in endemic PF has been suggested by familial clustering [10,11], by the high proportion of individuals living in endemic foci who do not manifest the disease [12,13] and by the strong association of the disease with particular HLA class II molecules: DR4, DR3 [14,15]. The ability of $CD4^+$T lymphocytes to present Dsg-derived peptides to specific B-cell clones has been confirmed and emphasizes the key role of these cells in the production of pathogenic Abs [16,17]. Association studies outside the HLA region have identified individual gene and specific allele that are associated with this endemic form of PF. They are the *Dsg1* [18,19], *CTLA-4*, *CD28*, *CD86* [20], *ICOS* [21], *CD40*, *CD40L*, B-lymphocyte stimulator (*BLYS*) [22] and also *IL4*, *IL6* and *IL10 genes* [23].

The association studies with candidate genes involved in immune responses, such as those encoding elements of T cell

Abbreviations: Abs, antibodies; PF, pemphigus foliaceus; Dsg1, desmoglein 1; SNP, single nucleotide polymorphism; MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium; OR, odds ratios; LD, linkage disequilibrium.

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0198-8859/$36.00 - see front matter © 2013 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humimm.2013.01.009

activation pathway, represents one approach in finding PF disease genes. Several polymorphisms have been identified mainly in the regulatory sequences of cytokine genes, and some of them have been shown to regulate the production level of these immunomodulatory molecules [24,25]. Indeed, cytokines are key components of the immune system and their altered expression levels, either exacerbation or failure in their production, have been implicated in the pathogenesis of many autoimmune conditions [26]. Several studies have reported the association of *IL-4* [27], and *IL-13* with many autoimmunes diseases: allergy, atopic asthma [28], rheumatoid arthritis [29], Crohn's disease [30], type 1 diabetes T1D [31].

Genes for these cytokines are located in a gene cluster at 5q31.1. Their effects are mediated by a heterodimeric receptor composed of the IL-4Rα chain (16p12.1) and either the common γ chain or the IL-13Rα subunit. *IL-13* and *IL-4* are structurally and functionally analogous. Both cytokines similarly have pleiotropic effects in the immune system acting on a number of cellular components [32]. Sharing some of its functions with *IL-13*, *IL-4* induces the activation and differentiation of macrophages, induces B cells to undergo immunoglobulin (Ig) switching to the IgG4 subclass and plays a key role in the polarization of T helper cells toward Th2 differentiation [33]. In the active stages of pemphigus, Abs of the IgG4 subclass are mainly detected and the formation of this subclass of auto-Abs is known to be Th2 cell dependent [34].

Because of the importance of the IL-4/IL-13 axis, we proposed to determine whether polymorphisms in *IL-4*, *IL-4R*, *IL-13* and *IL-13RA2* genes contribute to the development of endemic form of PF in Tunisia. Thus, we examined the potential role of *IL-4*, *IL-4R*, *IL-13* and *IL-13RA2* SNPs in PF susceptibility. We also investigated epistasis between the *IL-4* or *IL-13* and their common receptor *IL-4RA* or *IL-13RA2*.

2. Material and methods

2.1. Study populations

In this case–control study we enrolled 80 patients with PF attending the Dermatology Department of the University Hospital of Sfax. All PF patients are living in the endemic southern area of Tunisia. The period of recruitment started in 2002. All patients with PF were diagnosed on clinical, histological and immunological criteria and were matched by age (±5 years), sex and geographical origin to 160 healthy controls. We also included 147 healthy relatives to PF patients. All patients, relatives and healthy controls gave informed consent to participate in the study.

2.2. SNP selection

Tagging Single Nucleotide Polymorphism (tagSNP) in the *IL-4*, *IL-4R*, *IL-13* and *IL-13R* gene were selected using the genotyping data from the CEU available from the International Hapmap project (Table 2). Selection was undertaken using minor allele frequency (MAF) in Caucasians and sub-Saharan greater than 10%. Tagging of the promoter *IL-4* 590 variant was achieved using rs2243250. In the case for *IL-4R*, we selected three *IL-4R* SNPs that allowed us to define the haplotypes described in the Hapmap project: rs4787948, rs3024530 and rs3024622 localized in intron 1, 2 and 7, respectively. For *IL-13*, two SNP were selected: one SNP capturing five additional SNPs: rs20541 and the promoter polymorphism rs1881457. With respect to the *IL-13RA2* gene, we genotyped the rs535036.

2.3. SNP genotyping

Blood samples were collected in EDTA-anticoagulated tubes and DNA was extracted using standard methods. All SNPs were genotyped by conventional polymerase chain reaction/restriction fragment length polymorphism analysis (Table 2). Digestion products were electrophoresed through 3% agarose and scored following ethidium-bromide staining. The accuracy of the genotyping was confirmed by the direct sequencing of each SNP.

2.4. Measurement of IL-4 levels

IL-4 were detected in the serum of PF patients, relatives and controls by commercially available ELISA Quantikine® kits (R&D, Minneapolis, Minn, USA), according to manufacturer's procedure.

2.5. Statistical analysis

Hardy–Weinberg equilibrium (HWE) of each SNP was assessed in cases and controls separately using a χ^2 test with one degree of freedom. A threshold $P < 0.05$ was regarded to indicate deviation from HWE. Allele and genotype frequencies were calculated and associations with susceptibility to PF were tested by calculating odds ratios (OR) with asymptotic 95% confidence intervals (CIs). Haplotype analysis was carried out using SHEsis program (http://analysis.bio-x.cn) [35,36]. The linkage disequilibrium (LD) coefficients D' = D/Dmax and r^2-values for the pair of the most common alleles at each site were also estimated using the Haploview program version 4.2. The family-based association test (FBAT) was performed with FBAT program v1.5.1. The FBAT program uses generalized score statistics to perform a variety of transmission disequilibrium tests (TDT), including haplotype analyses. Differences were considered to be statistically significant if the *P*-value was $\leqslant 0.05$. Serum IL-4 levels were plotted and analyzed by unpaired *t*-test using SPSS.13 software. The statistical power of detection of the association with the disease at the 0.05 level of significance was determined by using the G* Power software.

3. Results

3.1. Characteristics of PF patients and controls

Clinical and demographic data for our Tunisian study populations are shown in Table 1. The demographic feature of our PF patients was associated with an important sex ratio disequilibrium (female/male, 19/1) and a lower mean age of disease onset (34 years). No statistically significant differences were noted in the demographic analysis of the two groups.

3.2. Information for the IL-4, IL-4R, IL-13 and IL-13RA2 polymorphisms

Genotype frequencies of all SNP tested of control subjects were consistent with those expected from the Hardy–Weinberg equilibrium (HWE) except for rs4787948 polymorphism in the *IL-4R* intron 1 ($P < 0.001$ and $P = 0.002$). MAF of all the seven polymorphisms was consistent with that reported in the HapMap database.

Table 1
Characteristic of study population.

Features	PF	Relatives	Control
Number	80	147	160
Mean age (years)	33.82	40	33
Sex ratio F/M	76/4	86/58	152/8
Origine	Endemic region in the south of Tunisia		
Risk factors	High temperature, desert, poverty		
DIF positif	80	0	0
Anti-Dsg1	80 mainly IgG4	32 IgG2	10 IgG2

DIF: direct Immunofluorescence, anti-Dsg1: anti-desmogleine 1 auto-antibodies detected by ELISA.

Table 2
Primary information of genotyped SNPs.

Gene	SNP[a]	Chr[b]	Base change	Fonction[c]	Primers	ER	MAF		HWE[e]
							HapMap[d]	Control	
IL4	rs2243250	5	C > T	5′ near gene	F: CTAAACTTGGGAGCAACATGGTR: TGGGGAAAGATAGAGTAATA	AvaII	0.485	0.284	0.3638
IL4R	rs4787948	16	A > G	Intron1	F: TGCAGTCAAAGCTATCTTTGATR: CACTCCCAGCTCTCCCTT	EcoRV	0.374	0.456	2.7 10–9
	rs3024530	16	A > G	Intron 2	F: TTCCCTAGGCTGGTCTCAAAR: GTTACAAGTCAGCTTAGTCCGTA	CviQI	0.341	0.412	0.078
	rs3024622	16	C > G	Intron 7	F: TGGGAACTTTCTCACTTGGTACR: AGCAGCCTTTCCTTTCCTTC	KpnI	0.475	0.4	0.3189
IL13	rs1881457	5	A > C	5′ near gene	F: GATAAGGGCGTTGACTCACR: GCTACTTGGCCGTGTGACCGC	Bsh12361	0.203	0.216	0.31
	rs20541	5	C > T	Exon 4	F: TCGCGTTCTACTCATGTGCTR: TTTCGAAGTTTCAGTGGAAC	NlaIV	0.265	0.247	0.227
IL13RA2	rs535036	X	A > C	3′near gene	F: GGTTCACCGACCAAAAGGTAR: GGAAGCTTGGCTCTTGATTG	MnlI	0.215	0.221	0.061

[a] SNP rs No. were taken from NCBI dbSNP (http://www.ncbi.nlm.nih.gov/SNP).
[b] Chr stands for chromosome.
[c] Function entries of the relevant entry for each rs number in the NCBI's Entrez dbSNP database.
[d] MAF from the HapMap databases (http://www.hapmap.org).
[e] HWE *P* value in the control group.

3.3. Genetic association of IL-4, IL-13, IL-4R and IL-13RA2 SNPs with Tunisian endemic PF susceptibility

3.3.1. Case–control study

We successfully genotyped SNPs in *IL-4*, *IL-13*, *IL-4R* and *IL-13RA2* genes (Table 3).

For the *IL-4* C-590T gene polymorphism and after Bonferroni adjustment, a significantly higher frequency of carriers of the variant −590T was observed in patients with PF in comparison to controls (OR = 2.16, $P_c = 0.00011$). The distribution of genotypes in the total sample showed a significant difference. Among the three genotypes, only homozygote TT gave the highest significant OR of PF of about 6.14-fold for cases than for controls under the additive model (OR = 6.14 95% CI: 2.57–14.67, $P_c = 0.00001$). In that, a positive association with TT genotype and T variant as well as negative association with CC genotype and C variant were found with the PF disease. The lower frequency of the C/C genotype in patients suggests a recessive protective effect of the C variant, while the higher frequency of the T variant indicates that it may increase susceptibility in homo- and heterozygosity.

However, in respect to polymorphism rs3024622 of *IL-4R* gene, weak positive and negative associations were found with C/C and C/G genotype, respectively.

In regards to the *IL-13* and *IL-13RA2* genes polymorphisms, our results indicate no significant differences in the distribution of genotypes and alleles between the two groups.

3.3.2. Family study

We also confirmed these results by family study. Indeed, we found a positive significant association in distribution of T allele ($P = 0.013$, $z = 2.461$) and negative significant association in distribution of C allele ($P = 0.013$, $z = -2.461$) with PF disease. Thus, *IL4*–590T allele was associated with a significantly high increased risk of PF.

3.4. Linkage disequilibrium (LD), haplotype and combination analyses

Since *IL-4* and IL-13 genes are located on the same chromosome, it is important to know which alleles are in LD and make haplotypes. The LD analysis measured by r^2 revealed that the three polymorphisms investigated were not in LD and argues for the contribution of the *IL-4* C-590T polymorphism at the occurrence of PF (Fig. 1). On the other hand, the estimated frequencies of the haplotypes (rs2243250–rs1881147–rs20541) on chromosome 5 differed significantly between PF patients and controls (global $\chi^2 = 19.92$, global Pearson's $P = 0.005$) (Table 4a). The results show, after Bonferroni correction, that only haplotype H5; T–A–C is significantly increased ($P = 0.00083$ and $P_c = 0.0066$) in PF patients as compared to controls (Table 4a).

LD values measured by r^2 revealed also no evidence for LD between the three SNP investigated in *IL-4R* gene on chromosome 16 (Fig. 1). On the other hand, PF patients exhibited significantly different frequencies of haplotypes (rs4787948–rs3024530–rs3024622) on chromosome 16 as compared to controls (global $\chi^2 = 16$, global Pearson's $P = 0.025$) (Table 4b). However, after Bonferroni correction, no significant differences were found between haplotypes in PF patients compared to controls (Table 4b).

On the other hand, the estimated frequencies of the *IL4*/*IL4R* combinations (rs2243250, rs4787948, rs3024622 and rs3024530) were depicted in Table 4c. The combinations distribution differed significantly between PF patients and controls (global $\chi^2 = 39.45$, Pearson's $P = 0.0003$). After Bonferroni correction, only the (TACA) combination tested exhibited a significant effect on PF susceptibility (OR = 3.19 and $P_c = 0.0068$) in PF patients as compared to controls.

3.5. Correlation between IL-4 serum level and IL4-C 590T polymorphism

Serum IL-4 levels in 22 PF patients, 17 related and 30 healthy subjects were measured by ELISA. Although mean serum levels of IL-4 were not different between the three groups, we found a significant positive correlation between mean serum levels of IL-4 and genotypes ($r = 0.586$; $P < 10^{-4}$). Interestingly, when serum IL-4 levels were compared with respect to the polymorphism at −590 loci, the IL-4 levels were significantly increased for TT genotype compared to CT or CC genotype ($P_c < 10^{-4}$ in both) (Fig. 2a). Serum IL-4 levels were also increased but not significantly after Bonferroni correction between the three groups according to T/T genotype (Fig. 2b).

4. Discussion

Until now, it is clear that no single genetic risk factor is responsible for the development of PF but the development of this disease in an individual will depend on the interaction of a number of genes and various environmental factors. The purpose of the present study is to investigate if variants of cytokine genes influence susceptibility or resistance to endemic Tunisian PF. Among the candidate genes, the ones coding for *IL-4*, *IL-13* and their antagonist *IL-4RA* and *IL-13RA2* are selected for many reasons: (i) Zeoti et al., suggested that within a Th1/Th2 paradigm, the Th1 profile seems to be inhibited while the Th2 profile predominates in PF [37]. (ii) The investigation of cytokines expressed by $CD4^+$T cells from both peripheral blood and epidermal lesions suggests a predominantly Th2 profile [16,17]. (iii) IL4, IL13 have a crucial and fundamental role for the switch to IgE and IgG4 [38,39]. (iv) IgG4 subclass represents the dominant if not the exclusive auto-Abs in PF, and (v) PF is considered one of the few autoimmune disease in which auto-Abs plays a direct pathogenic role.

In the current data, the C-590T polymorphism in the *IL-4* promoter region was significantly associated with PF. In fact, T allele and the TT genotype were significantly increased in the PF patients group compared to healthy controls. This association was confirmed by case–control and family study. This finding is supported by the report on the endemic Brazilian PF [23] which found weak positive and negative associations with the T/T genotype and the C allele, respectively. IL-4 is a typical Th2 cytokine of decisive significance in regulating Th1/Th2 balance [40]. A C > T exchange has been identified at position −590 in the promoter region of *IL-4* and the variant allele has been suggested to be associated with increased transcriptional activity as well as enhanced IgE secretion or T-cell balance [41–44]. This polymorphism is located in one of the unique binding sites for the nuclear factor of activated T cell (NF–AT) which plays an important role in the transcription of several cytokine genes [42]. Rosenwasser demonstrated an elevated IL-4 activity associated with the rare T-allele of rs2243250 [41]. Such a functional polymorphism in the *IL4* gene may elevate IL-4 levels and thereby influences the IL-4 dependant events which determine disease progression [45]. Associations between this promoter region polymorphism and susceptibility to bronchial asthma, atopic dermatitis, Grave's disease, Crohn's disease and rheumatoid arthritis have been reported, but these associations have not been confirmed in all studies [27,30]. A C > T substitution has been also identified at position −34 in the untranslated region that is in linkage disequilibrium with the polymorphism at position −590 in the promoter region [30,46].

It is pertinent to mention here that, in our study and according to previous hypothesis, besides the strong association with polymorphism −590 of *IL-4* gene, we found a significant correlation

Table 3
Genotypes and allele frequencies of *IL-4*, *IL-4R*, *IL-13* and *IL-13R* polymorphisms and their associations to the risk of Tunisian PF.

Variables	Genotype	PF cases		Controls		OR	95 %CI	P_c
		N = 80	F (%)	*N* = 160	F (%)			
IL-4: rs2243250	CC	27	33.75	79	49.375	1	-	-
	CT	32	40	71	44.375	1.31	0.72–2.41	0.36
	TT	21	26.25	10	6.25	6.14	2.57–14.67	0.00001
	Allele T	86	53.75	229	71.56	2.16	1.46–3.21	0.00011
IL-4R: r s4787948	AA	30	34.66	66	41.25	1	-	-
	AG	26	32.5	42	26.25	1.39	0.72–2.68	0.31
	GG	24	30	52	32.5	1.01	0.53–1.94	0.96
	Allele A	86	53.75	174	54.375	1.02	0.70–1.50	0.89
IL-4R: rs3024622	CC	41	51.25	61	38.125	1	-	-
	CG	24	30	70	43.75	0.51	0.27–0.93	0.029
	GG	15	18.75	29	18.125	0.77	0.36–1.61	0.48
	Allele C	106	66.25	192	60	0.76	0.51–1.13	0.18
IL-4R: rs3024530	AA	34	42.5	62	38.75	1	-	-
	AG	32	40	65	40.625	0.87	0.48–1.57	0.64
	GG	14	17.5	33	20.625	0.76	0.35–1.61	0.47
	Allele A	100	62.5	189	59	0.85	0.57–1.26	0.42
IL-13: rs1881457	AA	51	63.75	101	63.125	1	-	-
	AC	23	28.75	49	30.625	0.93	0.51–1.69	0.81
	CC	6	7.5	10	6.25	1.18	0.40–3.45	0.75
	Allele A	125	78.125	251	78.43	1.01	0.64–1.61	0.93
IL-13: rs20541	CC	55	68.75	92	57.5	1		
	TC	17	21.25	55	34.375	0.54	0.28–1.03	0.06
	TT	8	10	13	8.125	1.05	0.41–2.69	0.91
	Allele C	127	79.375	239	74.7	0.79	0.50–1.25	0.32
IL-13R: rs535036	AA	52	67.53	98	63.63	1	-	-
	AC	22	28.57	44	28.57	0.94	0.51–1.73	0.84
	CC	3	3.9	12	7.8	0.47	0.12–1.74	0.25
	Allele A	126	81.8	240	77.92	0.78	0.48–1.28	0.33

CI, confidence interval; *F*, frequency of alleles or genotypes; *n*, number of alleles or genotypes; OR, odds ratio; P_c: Statistically significant after Bonferroni adjustment (*P* value *x* number of alleles or genotypes) <0.05.

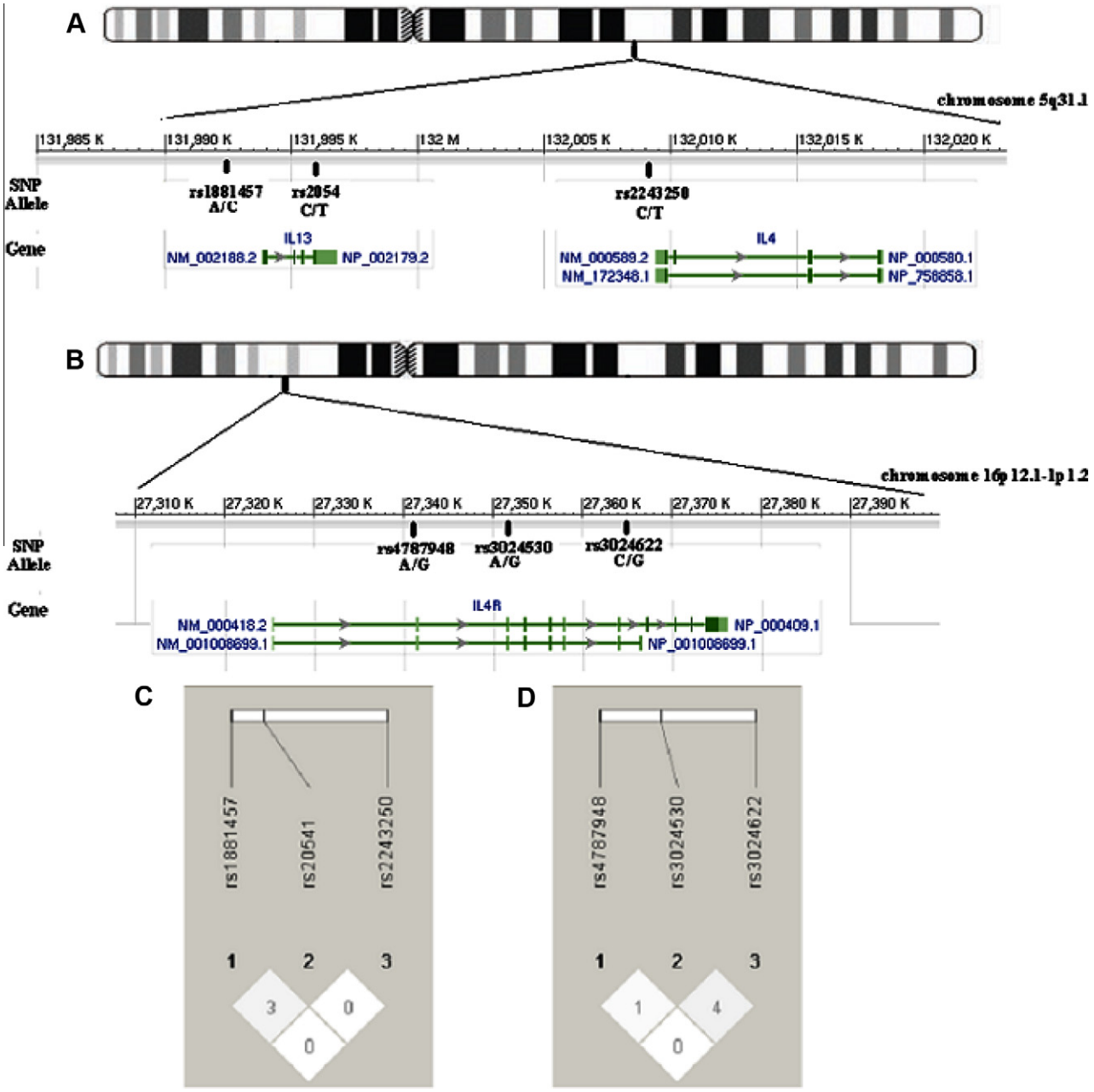

Fig. 1. Overview and linkage disequilibrium (LD). (A) The human susceptibility locus for PF on chromosome 5q31.1 covers a cluster of three SNP in IL-4 and IL-13 genes: rs2243250, rs1881147 and rs20541. (B) The human susceptibility locus for PF on chromosome 16p12.1–11.2 covers a cluster of three SNP in IL-4R gene: rs4787948, rs3024530 and rs20541. LD prime charts generated using Haploview 4.2 software summarise LD ($r2$) patterns between the three SNPs in chromosome 5 (C), and the three SNPs in chromosome 16 (D).

Table 4a
Haplotypes of rs2243250, rs1881147 and rs20541 polymorphisms on chromosome 5 showing significant differences between PF patients and controls.

Haplotypes	PF cases Freq.% $2N = 160$	Controls Freq.% $2N = 320$	Pearson's P	P_c	OR	[95% CI]
H1:C A C	35.6	44.4	0.066	–	0.69	[0.46–1.02]
H2:C A T	7	11.4	0.13	–	0.58	[0.29–1.17]
H3:C C C	7.3	9.2	0.47	–	0.77	[0.38–1.57]
H4:C C T	3.8	6.5	0.21	–	0.56	[0.22–1.42]
H5:T A C	31	17.6	0.0008	0.006	2.1	[1.35–3.27]
H6:T A T	4.5	5	0.78	–	0.88	[0.35–2.17]
H7:T C C	5.5	4.1	0.5	–	1.34	[0.55–3.23]
H8:T C T	5.3	1.7	0.02	0.2	3.24	[1.08–9.64]

H: haplotype, CI: Confidence Interval, Global $\chi^2 = 19.92$, df = 7 (frequency <0.03 in both control and case has been dropped) and Pearson's P value = 0.0057.

Table 4b
Haplotypes of rs4787948, rs3024622 and rs3024530 polymorphisms on chromosome 16 showing significant differences between PF Patients and Controls.

Haplotypes	PF cases Freq.% 2*N* = 160	Controls Freq.% 2*N* = 320	Pearson's *P*	P_c	OR	[95% CI]
H1′:A C A	29.9	23.2	0.11	–	1.41	[0.920–2.161]
H2′:A C G	5.4	9.7	0.10	–	0.52	[0.241–1.148]
H3′:A G A	12.1	9.2	0.31	–	1.36	[0.741–2.499]
H4′:A G G	6.4	12.2	0.046	0.3758	0.49	[0.240–1.003]
H5′:G C A	16.9	16.6	0.93	–	1.02	[0.614–1.697]
H6′:G C G	14.2	10.5	0.24	–	1.40	[0.795–2.487]
H7′:G G A	3.7	9.8	0.01	0.1439	0.35	[0.142–0.865]
H8′:G G G	11.6	8.8	0.32	–	1.36	[0.733–2.534]

H: haplotype, CI: Confidence Interval, Global χ^2 = 16, df = 7 (frequency <0.03 in both control and case has been dropped), and Pearson's *P* value = 0.025.

Table 4c
Combinations of (*IL4*/*IL4R*) rs2243250, rs4787948, rs3024622 and rs3024530 polymorphisms showing significant differences between PF patients and controls.

	PF cases Freq.% 2*N* = 160	Controls Freq.% 2*N* = 320	Pearson's *P*	P_c	OR	[95% CI]
C A C A	16.1	18.6	0.49	–	0.83	[0.50–1.39]
C A C G	2.4	7	0.03	–	0.32	[0.10–0.98]
C A G A	8.8	6.5	0.35	–	1.39	[0.68–2.81]
C A G G	3.2	7.6	0.05	–	0.39	[0.14–1.05]
C G C A	10.2	12.7	0.43	–	0.78	[0.42–1.44]
C G C G	6.8	6.8	0.97	–	1.01	[0.47–2.14]
C G G A	1.3	5.2	0.03	–	0.23	[0.05–1.03]
C G G G	5	7.3	0.34	–	0.67	[0.29–1.54]
T A C A	14	4.9	0.0004	0.0068	3.19	[1.62–6.28]
T A G A	3	2.4	0.67	–	1.28	[0.4–4.06]
T A G G	3.2	4.6	0.48	–	0.69	[0.25–1.93]
T G C A	6.3	3.8	0.20	–	1.72	[0.73–4.07]
T G C G	7.5	3.6	0.06	–	2.17	[0.94–5]
T G G A	2.7	4.7	0.28	–	0.56	[0.18–1.66]
T G G G	6.5	1.7	0.005	0.082	4.04	[1.40–11.64]

CI: Confidence Interval; Global χ^2 is 39.45, df = 14 (frequency <0.03 in both control and case has been dropped) and Pearson's *P* value is 0.00031.

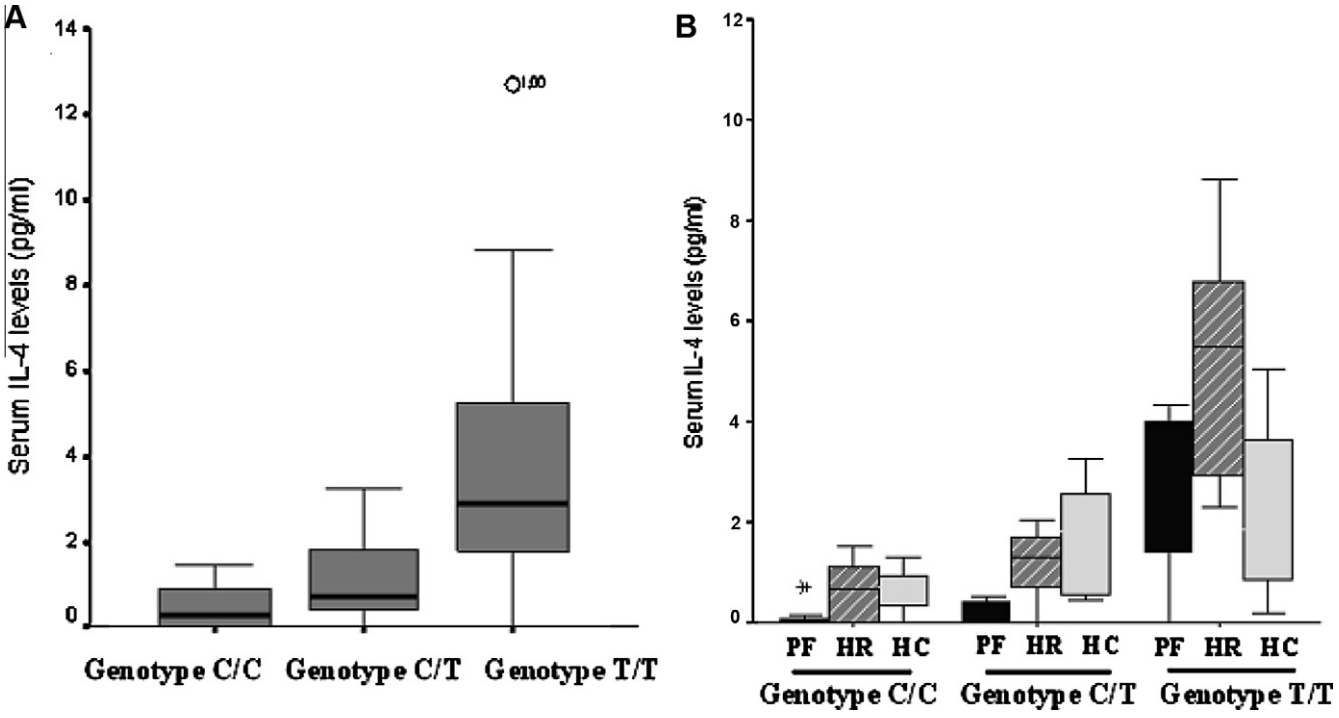

Fig. 2. Correlation of serum IL-4 levels and IL4-C 590T polymorphism. (A) Comparison of serum IL-4 levels (pg/ml) of all Tunisian study population according to C/C, C/T and T/T genotypes (r = 0.587, $P < 10^{-4}$). (B) Comparison of serum IL-4 levels (pg/ml) between PF patients, relatives (HR) and healthy controls (HC) with respect to IL4-C 590T polymorphism.

of serum levels of IL-4 with the presence of T/T genotype instead C/C and C/T genotypes. This functionally relevant T variant could influence the predisposition or the clinical course of PF disease characterised by a predominance of Th2 response inducing autoreactive B cell proliferation and facilitating immunoglobulin class switching to pathogenic IgG4 isotype.

On the other hand, regards to polymorphism of *IL-4R* gene, a positive and negative association was found with C/C and C/G genotype of the rs3024622, respectively. *IL-4R* can be a reliable candidate marker for susceptibility to PF for several reasons: (i) its chromosome position in 16p described in mainly autoimmune disease such as multiple sclerosis, rheumatoid arthritis and type I diabetes [47,48], (ii) the previous identification of SNPs leading to functional alterations of *IL-4R* such as changes in transcription rates, enhanced activity or signaling through the protein, or changes in serum protein levels in diverse cell types [49], and (iii) associations of *IL4R* with other complex diseases characterized by a Th1/Th2 shift. To confirm these hypotheses, we conducted a combination study of SNP polymorphisms on chromosome 16 and then, we investigated epistasis between *IL-4* and *IL-4R* genes. Interestingly, a positive association with the T–A–C–A combination for rs2243250–rs4787948–rs3024622–rs3024530 (*IL-4/IL-4R*) was found associated with PF disease in our population. Thus, although only one polymorphism is not a susceptibility marker to disease, association of several markers could be, in turn, crucial. In our knowledge, there have been no reports on any gene–gene interaction between IL-4 and its receptor, IL-4Ra. Interestingly, our results hypothesis that the IL-4/IL-4R axis could play a central role in the regulation of pathogenic IgG4 Abs production.

In the case of *IL-13*/*IL-13R* polymorphisms, our analysis provide evidence of no association between Tunisian endemic PF development and variation in the *IL-13*/*IL-13R* pathway.

In conclusion, the lack of association in our study suggests that the single nucleotide polymorphisms in *IL-13*/*IL-13R* genes do not have an impact on the risk of developing endemic PF in Tunisian population.

However genetic variability of the *IL4*/*IL4R* could have an effect in the clinical course of the disease instead of increasing or decreasing the susceptibility to endemic PF. Thus, the high association between T-590 allele of IL-4 polymorphism and PF disease and the gene–gene interaction between IL-4 and its receptor, IL-4Ra, might be responsible for elevated IL-4 levels in patients by amplifying the polarisation of autoreactive Th cells towards Th2 pathway, inducing autoreactive B cell proliferation and facilitating immunoglobulin class switching from non pathogenic IgG2 to pathogenic IgG4.

Acknowledgments

This work was supported by a grant from the « Ministère de l'enseignement supérieur et de la recherche scientifique » of Tunisia.

References

[1] Stanley JR, Amagai M. Pemphigus bullous impetigo and the staphylococcal scalded-skin syndrome. N Engl J Med 2006;355:1800–10. Rev.
[2] Koulu L, Kusumi A, Steinberg MS, et al. Human autoantibodies against a desmosomal core protein in pemphigus foliaceus. J Exp Med 1984;160:1509–18.
[3] Eyre RW, Stanley JR. Human autoantibodies against a desmosomal protein complex with a calcium-sensitive epitope are characteristic of pemphigus foliaceus patients. J Exp Med 1987;165:1719–24.
[4] Roscoe JT, Diaz L, Sampaio SA, et al. Brazilian pemphigus foliaceus autoantibodies are pathogenic to BALB/c mice by passive transfer. J Invest Dermatol 1985;85:538–41.
[5] Amagai M, Hashimoto T, Green KJ, et al. Antigen-specific immunoadsorption of pathogenic autoantibodies in pemphigus foliaceus. J Invest Dermatol 1995;104:895–901.
[6] Lin MS, Fu CL, Aoki V, et al. Desmoglein-1-specific T lymphocytes from patients with endemic pemphigus foliaceus (fogo selvagem). J Clin Invest 2000;105:207–13.
[7] Diaz LA, Sampaio SA, Rivitti E, et al. Endemic pemphigus foliaceus (fogo selvagem) I. Clinical features and immunopathology. J Am Acad Dermatol 1989;20:657–69. Rev.
[8] Morini JP, Jomaa B, Gorgi Y, et al. Pemphigus foliaceus in young women. An endemic focus in the Sousse area of Tunisia. Arch Dermatol 1993;129:69–73.
[9] Bastuji-Garin S, Souissi R, Blum L, et al. Comparative epidemiology of pemphigus in Tunisia and France. unusual incidence of pemphigus foliaceus in young Tunisian women. J Invest Dermatol 1995;104:302–5.
[10] Diaz LA, Sampaio SA, Rivitti EA, et al. Endemic pemphigus foliaceus (Fogo Selvagem): II Current and historic epidemiologic studies. J Invest Dermatol 1989;92:4–12. Rev.
[11] Abida O, Masmoudi A, Rebaï A, et al. Familial feature of Tunisian endemic pemphigus foliaceus. Br J Dermatol 2009;161:951–3.
[12] Warren SJ, Lin MS, Giudice GJ, et al. The prevalence of antibodies against desmoglein 1 in endemic pemphigus foliaceus in Brazil. Cooperative group on fogo selvagem research. N Engl J Med 2000;343:23–30.
[13] Abida O, Kallel-Sellami M, Joly P, et al. Anti-desmoglein 1 antibodies in healthy related and unrelated subjects and patients with pemphigus foliaceus in endemic and non-endemic areas from Tunisia. JEADV 2009;23:1073–8.
[14] Pavoni DP, Roxo VM, Marquart Filho A, et al. Dissecting the associations of endemic pemphigus foliaceus (Fogo Selvagem) with HLA-DRB1 alleles and genotypes. Genes Immun 2003;4:110–6.
[15] Abida O, Zitouni M, Kallel-Sellami M, et al. Tunisian endemic pemphigus foliaceus is associated with the HLA-DR3 gene: anti-desmoglein 1 antibody-positive healthy subjects bear protective alleles. Br J Dermatol 2009;161:522–7.
[16] Caproni M, Giomi B, Cardinali C, et al. Further support for a role for Th2-like cytokines in blister formation of pemphigus. Clin Immunol 2001;98:264–71.
[17] Santi CG, Sotto MN. Immunopathologic characterization of the tissue response in endemic pemphigus foliaceus (*fogo selvagem*). J Am Acad Dermatol 2001;44:446–50.
[18] Ayed MB, Martel P, Zitouni M, et al. Tunisian endemic pemphigus foliaceus is associated with desmoglein 1 gene polymorphism. Genes Immun 2002;3:378–9.
[19] Martel P, Gilbert D, Drouot L, et al. A polymorphic variant of the gene coding desmoglein 1, the target autoantigen of pemphigus foliaceus, is associated with the disease. Genes Immun 2001;2:41–3.
[20] Dalla-Costa R, Pincerati MR, Beltrame MH, et al. Polymorphisms in the 2q33 and 3q21 chromosome regions including T-cell coreceptor and ligand genes may influence susceptibility to pemphigus foliaceus. Hum Immunol 2010;71:809–17.
[21] Narbutt J, Lesiak A, Klich I, et al. ICOS gene polymorphism may be associated with pemphigus. J Cutan Med Surg 2010;14:291–7.
[22] Malheiros D, Petzl-Erler ML. Individual and epistatic effects of genetic polymorphisms of B-cell co-stimulatory molecules on suscepti bility to pemphigus foliaceus. Genes Immun 2009;10:547–58.
[23] Pereira NF, Hansen JA, Lin MT, Roxo VM, Braun K, Petzl-Erler ML. Cytokine gene polymorphisms in endemic pemphigus foliaceus: a possible role for IL6 variants. Cytokine 2004;28:233–41.
[24] Bidwell J, Keen L, Gallagher G, et al. Cytokine gene polymorphism in human disease: on-line databases. Genes Immun 2001;2(Suppl. 1):61–70. Rev.
[25] Haukim N, Bidwell JL, Smith AJ, et al. Cytokine gene polymorphism in human disease: on-line databases. Genes Immun 2002;3(suppl. 2):313–30.
[26] Matei I, Matei L. Cytokine patterns and pathogenicity in autoimmune diseases. Rom J Intern Med 2002;40:27–41.
[27] Urcelay E, Santiago JL, Mas A, Martínez A, De Las Heras V, Arroyo R, et al. Role of interleukin 4 in Spanish multiple sclerosis patients. J Neuroimmunol 2005;168(1–2):164–7.
[28] Beghé B, Barton S, Rorke S, Peng Q, Sayers I, Gaunt T, et al. Polymorphisms in the interleukin-4 and interleukin-4 receptor alpha chain genes confer susceptibility to asthma and atopy in a Caucasian population. Clin Exp Allergy 2003;33(8):1111–7.
[29] Cantagrel A, Navaux F, Loubet-Lescoulié P, Nourhashemi F, Enault G, Abbal M, et al. Interleukin-1beta, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms: relationship to occurrence and severity of rheumatoid arthritis. Arthritis Rheum 1999;42(6):1093–100.
[30] Aithal GP, Day CP, Leathart J, Daly AK, Hudson M. Association of single nucleotide polymorphisms in the interleukin-4 gene and interleukin-4 receptor gene with Crohn's disease in a British population. Genes Immun 2001;2(1):44–7.
[31] Bugawan TL, Mirel DB, Valdes AM, Panelo A, Pozzilli P, Erlich HA. Association and interaction of the IL4R, IL4, and IL13 loci with type 1 diabetes among Filipinos. Am J Hum Genet. 2003 Jun; 72(6):1505–14. Epub 2003 May 13.
[32] Wynn TA. IL-13 effector functions. Annu Rev Immunol. 2003;21:425–56. Epub 2001 Dec 19. Review.
[33] Miossec P. The role of the Th1 and Th2 dichotomy in the pathogenesis of juvenile chronic arthritis. Rev Rhum Engl Ed. 1997;64(Suppl. 10):138S–9S.
[34] Mahoney MG, Wang ZH, Stanley JR. Pemphigus vulgaris and pemphigus foliaceus antibodies are pathogenic in plasminogen activator knockout mice. J Invest Dermatol 1999;113(1):22–5.
[35] Shi YY, He L. SHEs is, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005;15(2):97–8.

[36] Li G, Lu X. Comments on: A review on empirical likelihood methods for regression. Test (Madr) 2009;18(3):463–7.
[37] Zeoti DM, Figueiredo JF, Chiossi MP, Roselino AM. Serum cytokines in patients with Brazilian pemphigus foliaceus (fogo selvagem). Braz J Med Biol Res 2000;33(9):1065–8.
[38] Gebhard KL, Veldman CM, Wassmuth R, Schultz E, Schuler G, Hertl M. Ex vivo analysis of desmoglein 1-responsive T-helper (Th) 1 and Th2 cells in patients with pemphigus foliaceus and healthy individuals. Exp Dermatol 2005;14(8): 586–92.
[39] Takahashi H, Amagai M, Nishikawa T, Fujii Y, Kawakami Y, Kuwana M. Novel system evaluating in vivo pathogenicity of desmoglein 3-reactive T cell clones using murine pemphigus vulgaris. J Immunol 2008;181(2):1526–35.
[40] Paul WE, Seder RA. Lymphocyte responses and cytokines. Cell 1994;76(2): 241–51.
[41] Rosenwasser LJ, Klemm DJ, Dresback JK, Inamura H, Mascali JJ, Klinnert M, et al. Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. Clin Exp Allergy 1995;25(Suppl. 2):74–8. Discussion 95–6.
[42] Rosenwasser LJ, Borish L. Genetics of atopy and asthma: the rationale behind promoter-based candidate gene studies (IL-4 and IL-10). Am J Respir Crit Care Med 1997;156(4 Pt 2):S152–5.
[43] Nakashima H, Miyake K, Inoue Y, Shimizu S, Akahoshi M, Tanaka Y, et al. Association between IL-4 genotype and IL-4 production in the Japanese population. Genes Immun 2002;3(2):107–9.
[44] Tangteerawatana P, Pichyangkul S, Hayano M, Kalambaheti T, Looareesuwan S, Troye-Blomberg M, Khusmith S. Relative levels of IL4 and IFN-gamma in complicated malaria: association with IL4 polymorphism and peripheral parasitemia. Acta Trop. 2007 Mar; 101(3):258–65. Epub 2007 Feb 23.
[45] Nguyen DP, Genc M, Vardhana S, Babula O, Onderdonk A, Witkin SS. Ethnic differences of polymorphisms in cytokine and innate immune system genes in pregnant women. Obstet Gynecol 2004;104(2):293–300.
[46] Takabayashi A, Ihara K, Sasaki Y, Kusuhara K, Nishima S, Hara T. Novel polymorphism in the 5'-untranslated region of the interleukin-4 gene. J Hum Genet 1999;44(5):352–3.
[47] Zuvich RL, Bush WS, McCauley JL, Beecham AH, De Jager PL; International Multiple Sclerosis Genetics Consortium, Ivinson AJ, Compston A, Hafler DA, Hauser SL, Sawcer SJ. Interrogating the complex role of chromosome 16p13.13 in multiple sclerosis susceptibility: independent genetic signals in the CIITA-CLEC16A-SOCS1 gene complex. Hum Mol Genet 2011; 20(17): 3517–24.
[48] Martínez A, Perdigones N, Cénit MC, Espino L, Varadé J, Lamas JR, et al. Chromosomal region 16p13: further evidence of increased predisposition to immune diseases. Ann Rheum Dis 2010;69(1):309–11.
[49] Lu MP, Chen RX, Wang ML, Zhu XJ, Zhu LP, Yin M, Zhang ZD, Cheng L. Association study on IL4, IL13 and IL4RA polymorphisms in mite-sensitized persistent allergic rhinitis in a Chinese population. PLoS One. 2011; 6(11):e27363. Epub 2011 Nov 7.