ARTHRITIS & RHEUMATISM
Vol. 54, No. 12, December 2006, pp 4011–4017
DOI 10.1002/art.22218

Interleukin-10 Promoter Polymorphisms in Giant Cell Arteritis

Luigi Boiardi, Bruno Casali, Enrico Farnetti, Nicolò Pipitone, Davide Nicoli, PierLuigi Macchioni, Luca Cimino, GianLuigi Bajocchi, Maria Grazia Catanoso, Laura Pattacini, and Carlo Salvarani

Objective. **To investigate potential associations between interleukin-10 (IL-10) promoter polymorphisms and susceptibility to, and clinical features of, giant cell arteritis (GCA).**

Methods. **A total of 140 patients with biopsy-proven GCA who were residents of Reggio Emilia, Italy, and 200 population-based controls from the same geographic area were genotyped for promoter polymorphisms of the IL-10 gene, by molecular methods. The patients were subgrouped according to the presence or absence of polymyalgia rheumatica (PMR) and ischemic complications (any or all of the following: vision loss, jaw claudication, cerebrovascular accidents, or aortic arch syndrome).**

Results. **The distribution of the C/A 592 genotype differed significantly between the GCA patients and the controls ($P_{corr} = 0.003$). Carriers of the A592 allele (A/A or C/A) were significantly more frequent among the GCA patients than among the controls ($P_{corr} = 0.004$, odds ratio [OR] 2.0 [95% confidence interval (95% CI) 1.3–3.1]). Homozygosity for the A592 allele was significantly more frequent among the GCA patients than among the controls ($P_{corr} = 0.002$, OR 3.4 [95% CI 1.6–7.2]). The distribution of the A/G 1082 genotype was similar in GCA patients and controls. In the haplotype analysis, the frequency of the ATA haplotype was significantly higher in GCA patients than in the controls ($P = 0.0001$), whereas the frequencies of the ACC and GTA haplotypes were significantly lower ($P = 0.0001$ for both comparisons). No significant associations were found for comparisons of GCA patients with and those without PMR or GCA patients with and those without ischemic complications.**

Conclusion. **Our findings show that the −592 C/A promoter polymorphism of the IL-10 gene is associated with susceptibility to GCA.**

Giant cell arteritis (GCA) is considered a T cell–driven disease. In particular, CD4+ T cells are thought to play a central role in inducing and maintaining the vasculitic process. The formation of granulomas is strictly dependent on T cells that stimulate the synthesis of interferon-γ (IFNγ). IFNγ, in turn, appears to exert most of its inflammatory effects by turning macrophages into activated effector cells (1). Since interleukin-10 (IL-10) is a potent suppressor of IFNγ production (2), its role in the immunopathogenesis of GCA may be of relevance.

Recently, 3 biallelic polymorphisms located at positions −1082, −819, and −592 of the promoter region of the IL-10 gene were identified. Two of these single-nucleotide polymorphisms (SNPs), −819 C/T (dbSNP accession no. 3021097) and −592 C/A (dbSNP accession no. 1800872), are in complete linkage disequilibrium in Caucasian populations, forming together with the −1082 G/A SNP (dbSNP accession no. 1800896) only 3 (of the 8 possible) haplotypes (GCC, ACC, and ATA) (3). These polymorphisms have been reported to be functionally important, since they appear to influence the production and plasma concentrations of IL-10 (4–10).

The aim of this study was to examine potential associations of the IL-10 promoter polymorphisms as well as their estimated haplotypes with susceptibility to, and the clinical expression of, GCA, particularly in patients with and those without polymyalgia rheumatica

Luigi Boiardi, MD, PhD, Bruno Casali, MD, Enrico Farnetti, BD, Nicolò Pipitone, MD, PhD, Davide Nicoli, BD, PierLuigi Macchioni, MD, Luca Cimino, MD, GianLuigi Bajocchi, MD, Maria Grazia Catanoso, MD, Laura Pattacini, BD, Carlo Salvarani, MD: Arcispedale Santa Maria Nuova, Reggio Emilia, Italy.

Address correspondence and reprint requests to Carlo Salvarani, MD, Servizio di Reumatologia, Arcispedale Santa Maria Nuova, Viale Risorgimento N80, 42100 Reggio Emilia, Italy. E-mail: salvarani.carlo@asmn.re.it.

Submitted for publication April 13, 2006; accepted in revised form August 16, 2006.

(PMR) and in patients with and those without ischemic complications.

PATIENTS AND METHODS

Study population. We reviewed the computerized registry of the Pathology Laboratory at Arcispedale Santa Maria Nuova, which stores the results of all temporal artery biopsies performed in Reggio Emilia, Italy, between 1986 and 2004. GCA-positive specimens were reviewed by a pathologist. A total of 166 GCA patients residing in the Reggio Emilia area were identified. Their median age was 74 years (range 56–90 years). Of these, 140 patients could be contacted, and all of them were willing to participate in this study.

Patients were diagnosed as having biopsy-proven GCA if histologic examination of the temporal artery biopsy specimen showed disruption of the internal elastic lamina, with infiltration of mononuclear cells into the arterial wall, with or without giant cells. Temporal artery biopsy procedures in Reggio Emilia have been described in detail elsewhere (11,12). Temporal artery biopsy was routinely performed in all patients with clinical manifestations of GCA. Segments longer than 2 cm were generally obtained.

The clinical findings at diagnosis and during followup, the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) values at diagnosis, as well as the initial prednisone dosage, were ascertained through interviews with the patients and by reviewing the patients' medical records. Patients were subgrouped according to the presence or absence of PMR (marked aching and stiffness bilaterally, without other apparent cause, in at least 2 of the 3 following regions: neck, shoulder girdle, or hip girdle) and the presence or absence of ischemic complications (vision loss and/or jaw claudication and/or cerebrovascular accidents and/or aortic arch syndrome).

Controls were randomly recruited from the lists of patients who were under the care of the medical practitioners of the same public health service. Control patients had no evidence of GCA. Stratification by the random-number method according to age and sex was used to approximately match the controls with the patients according to their age and sex distribution. At the end of this selection process, 200 control subjects were identified. The median age of the controls was 69 years (range 50–80 years).

All study subjects were white, of Italian descent, and had been residents of Italy for at least 1 generation. No ethnic differences were found between the patients and the controls. None of the study participants were of Jewish ancestry.

The study was approved by the ethics committee of Reggio Emilia Hospital. Informed consent was obtained from all patients or their relatives.

DNA extraction and genotyping. DNA was extracted from 300 μl of fresh or frozen whole peripheral blood obtained from control subjects and GCA patients. A standard extraction procedure with phenol, chloroform, and isoamyl alcohol was used, and samples were stored at −20°C until used.

Polymerase chain reaction (PCR) amplification of a 480-bp fragment from the IL-10 −592C/A promoter region was performed using the primers 5′-CTCAGTTAGCACTGGTGTAC-3′ and 5′-TGTTCCTAGGTCACAGTGAC-3′. The primers contained 1 mismatched nucleotide that created a restriction site for *Rsa* I (New England Biolabs, Ipswich, MA), as described by Santos et al (13).

A similar strategy was applied to the design of mismatched primers for detecting the IL-10 −1082G/A polymorphism, using the primers 5′-AACACTACTAAGGCTCCTTTGGGA-3′ and 5′-CAAGGAAAAGAAGTCAGGATTCCATGGA-3′. The primers created a restriction site for *Eco* NI (New England Biolabs, Ipswich, MA), consistent with the method described by Tseng et al (14).

PCRs were carried out in a PE 9600 thermal cycler (PerkinElmer Cetus, Emeryville, CA) using a 50-μl reaction volume containing 100 ng of template DNA, 50 m*M* KCl, 10 m*M* Tris HCl, 0.1% Triton X-100, 200 μM each of dATP, dCTP, dGTP, dTTP (Amersham Pharmacia Biotech, Piscataway, NJ), 2.5 m*M* $MgCl_2$, 0.5 μM of each primer, and 1 unit of *Taq* DNA polymerase (PerkinElmer Cetus).

Following an initial denaturation step (2 minutes at 94°C), samples were subjected to 35 cycles at 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 40 seconds, with a final extension time of 5 minutes at 72°C for the IL-10 −592 C/A polymorphism. For the IL-10 −1082 G/A polymorphism, the steps were 35 cycles at 94°C for 20 seconds, 60°C for 20 seconds, and 72°C for 20 seconds, with a final extension time of 3 minutes at 72°C.

The PCR products for the IL-10 −592C/A polymorphism were digested with restriction endonuclease *Rsa* I, which generated fragments of 480 bp and 240 bp. These fragments were visualized by 2% agarose gel electrophoresis and ethidium bromide staining. The PCR products for the IL-10 −1082G/A polymorphism were digested with *Eco* NI, which generated fragments of 82 bp and 20 bp. These fragments were visualized by 3% agarose gel electrophoresis and ethidium bromide staining.

Statistical analysis. Statistical analysis was performed using the SPSS statistical package (SPSS, Chicago, IL). Student's *t*-test and the Mann-Whitney U test were used to compare the means for parametrically and nonparametrically distributed data, respectively. The frequencies of the alleles and genotypes in the patient and the control populations were compared by chi-square test. Odds ratios (ORs) were calculated, together with their 95% confidence intervals (95% CIs). Corrected *P* values (P_{corr}) were calculated by multiplying the *P* value by the number of the alleles compared.

It was previously shown that 2 proximal SNPs in the IL-10 promoter gene (−819 C/T and −592 C/A) are in complete linkage disequilibrium in Caucasian populations, which together with the −1082 G/A SNP represent only 3 of the 8 potentially possible haplotypes (GCC, ACC, and ATA) (3). A fourth haplotype (GTA) has initially been reported in a southern Chinese population (5). Therefore, in this study, only 2 of 3 polymorphic sites, −1082 and −592, were analyzed in order to determine IL-10 promoter haplotypes.

Haplotypes from unrelated individuals were constructed using the World Wide Web source (available at http://research.calit2.net/hap/WebServer.htm), described by Halperin and Eskin (15).

We also performed power calculations for an unmatched case–control study and estimated the relative risk using Power and Sample Size Calculation software version

Table 1. Demographic data and clinical features of the 140 patients with biopsy-proven giant cell arteritis*

Male/female	21.4/78.6
Age at disease onset, mean ± SD years	74 ± 7
Headache	82.9
Abnormalities of the temporal arteries†	66.9
Scalp tenderness	42.3
Jaw claudication	47.9
Visual manifestations	30.7
Vision loss	20.0
Ischemic complications‡	57.9
Systemic symptoms and/or signs§	77.9
Polymyalgia rheumatica	45.0
Duration of therapy, mean ± SD months	20 ± 15
Duration of followup, mean ± SD months	25 ± 20
ESR at diagnosis, mean ± SD mm/hour	91 ± 30
CRP at diagnosis, mean ± SD mg/dl	10 ± 7

* Except where indicated otherwise, values are the percentage of patients. ESR = erythrocyte sedimentation rate; CRP = C-reactive protein (normal <0.5 mg/dl).
† Artery tenderness and/or decreased or absent temporal artery pulsation.
‡ Any or all of the following features: vision loss, jaw claudication, cerebrovascular accident, or aortic arch syndrome.
§ Fever, anorexia, and weight loss.

2.1.31 (available at http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize).

RESULTS

Table 1 shows the clinical and demographic characteristics of the 140 patients with GCA. Sixty-three of the patients had PMR, and 76 had ischemic complications. Vision loss was diagnosed in 28 patients, jaw claudication in 67, cerebrovascular accidents in 4, and aortic arch syndrome in 4 patients.

The allele and genotype frequencies of the IL-10 −592 C/A promoter polymorphism in GCA patients and in the control group are shown in Table 2. The distribution of the C/A 592 genotype differed significantly between the GCA patients and the controls (P_{corr} = 0.003). The distribution of the genotype in the C/A 592 polymorphism indicated that the differences in allele distribution were related to a higher frequency of A/A homozygosity in GCA patients as compared with the controls, whereas C/C homozygosity was less frequent in GCA patients.

Allele A592 was significantly more frequent in GCA patients than in the controls (P_{corr} = 0.0002, OR 2.0 [95% CI 1.4–2.8]). Carriers of the A592 allele (A/A plus A/C) were significantly more frequent in the GCA patient group than in the control group (P_{corr} = 0.006, OR 2.0 [95% CI 1.3–3.1]). Homozygosity for the A592 allele was significantly more frequent in GCA patients than in controls (P_{corr} = 0.002, OR 3.4 [95% CI 1.6–7.2]). Given the sample sizes (140 patients with GCA and 200 controls) and the allele frequencies of the polymorphism examined, we can exclude with 80% certainty that there is a genetic relative risk of 1.68 for GCA in carriers of the −592 C/A IL-10 promoter polymorphism.

The distribution of allele and genotype frequencies of the −1082 G/A polymorphism did not differ significantly between GCA patients and controls (Table 3). Given the sample sizes (140 patients with GCA and 200 controls) and the allele frequencies of the polymorphism examined, we can exclude with 80% certainty that there is a genetic relative risk of 1.44 for GCA in carriers of the −1082G/A IL-10 promoter polymorphism.

In the haplotype analysis, the frequency of the ATA haplotype was significantly higher in GCA patients

Table 2. Frequencies of alleles, genotypes, and carriage rates of the interleukin-10 promoter polymorphism at position −592 in patients with GCA and in controls*

Variable	Controls (n = 200)	GCA patients (n = 140)	*P* (P_{corr})	OR (95% CI)
Allele				
A	86/400 (21.5)	98/280 (35.0)	0.0001 (0.0002)	2.0 (1.4–2.8)
C	314/400 (78.5)	182/280 (65.0)		
Genotype				
A/A	11/200 (5.5)	23/140 (16.4)	0.001 (0.003)	–
A/C	64/200 (32.0)	52/140 (37.1)		
C/C	125/200 (62.5)	65/140 (46.4)		
Carriage rate				
A/A + A/C	74/200 (37.0)	75/140 (53.6)	0.003 (0.006)	2.0 (1.3–3.1)
C/C	126/200 (63.0)	65/140 (46.4)		
C/C + A/C	189/200 (94.5)	117/140 (83.6)	0.001 (0.002)	3.4 (1.6–7.2)
A/A	11/200 (5.5)	23/140 (16.4)		

* Values are the number/total number examined (%). GCA = giant cell arteritis; P_{corr} = corrected *P* (corrected for the number of alleles compared); OR = odds ratio; 95% CI = 95% confidence interval.

Table 3. Frequencies of alleles, genotypes, and carriage rates of the interleukin-10 promoter polymorphism at position −1082 in patients with GCA and in controls*

Variable	Controls (n = 200)	GCA patients (n = 140)	*P*	OR (95% CI)
Allele				
G	144/400 (36.0)	95/280 (33.9)	NS	0.9 (0.7–1.3)
A	256/400 (64.0)	185/280 (66.1)		
Genotype				
G/G	27/200 (13.5)	17/140 (12.1)	NS	–
G/A	90/200 (45.5)	61/140 (43.6)		
A/A	83/200 (41.5)	62/140 (44.3)		
Carriage rate				
G/G + A/G	117/200 (58.5)	78/140 (55.7)	NS	0.9 (0.6–1.4)
A/A	83/200 (41.5)	62/140 (44.3)		
A/A + A/G	173/200 (85.6)	123/140 (87.9)	NS	0.9 (0.7–1.3)
G/G	27/200 (13.5)	17/140 (12.1)		

* Values are the number/total number examined (%). GCA = giant cell arteritis; OR = odds ratio; 95% CI = 95% confidence interval; NS = not significant.

than in the controls ($P = 0.0001$). The frequencies of the ACC and GTA haplotypes were significantly lower ($P = 0.0001$ for both comparisons) (Table 4).

The associations between the −592 C/A and the −1082 G/A polymorphisms of IL-10 and the clinical features of GCA were evaluated by comparing the 63 GCA patients who had PMR with the 77 patients who did not have PMR as well as by comparing the 81 GCA patients who had ischemic complications with the 59 patients who did not have ischemic complications. No significant associations were found (data not shown). In the haplotype analysis, the frequency of ATA, ACC, GCC, and GTA did not differ significantly for comparisons of patients with and those without PMR and for patients with and those without ischemic complications (data not shown).

DISCUSSION

IL-10 is a pleiotropic cytokine produced by numerous cell types, including Th2 cells, B cells, monocytes, macrophages, and dendritic cells (16). IL-10 regulates Th1 and Th2 immunity in vitro and in vivo by skewing immune responses toward a predominantly Th2 phenotype (16) via the inhibition of IL-12 production and other mechanisms (17). Although the pathways implicated in immune regulation are extremely complex, since they involve not only the synthesis of soluble factors but also fine-tuning by distinct subgroups of lymphocytes (CD4+, CD8+, and natural killer T cells) (18), there is convincing evidence that the net effect of IL-10 is to suppress inflammation in a variety of diseases that are characterized by a prevalent Th1 response (19,20).

GCA is a primary systemic vasculitis considered to be largely driven by CD4+ T cells located at the junction of the media and the adventitia that display a strong bias toward a Th1 phenotype (21). In particular, local synthesis by these cells of the key Th1 cytokine IFNγ is germane to the differentiation and activation of tissue-invading macrophages and to the formation of granulomata. Conversely, deficiency of IFNγ has been shown to prevent the formation of granulomata (22). Similarly, in vivo treatment with IL-10 of T cell cultures

Table 4. Haplotype distribution of the interleukin-10 promoter in GCA patients and in controls*

Haplotype	Controls (n = 200)	GCA patients (n = 140)	*P*	OR (95% CI)
ATA	36/400 (9.0)	80/280 (28.6)	0.0001	4.0 (2.6–6.2)
ACC	220/400 (55.0)	93/280 (33.2)	0.0001	0.4 (0.3–0.6)
GCC	95/400 (23.7)	72/280 (25.7)	NS	1.1 (0.8–1.6)
GTA	49/400 (12.3)	11/280 (3.9)	0.0001	0.3 (0.2–0.6)

* The −819 single-nucleotide polymorphism is assumed on the basis of the known complete linkage disequilibrium between the −819 (T or C) and the −592 (A or C) polymorphisms, resulting in 1 of 2 haplotypes (T-A or C-C). Values are the number/total number examined (%). GCA = giant cell arteritis; OR = odds ratio; 95% CI = 95% confidence interval; NS = not significant.

derived from patients with Wegener's granulomatosis (WG) has been shown to prevent the development of a Th1 response by inhibiting IFNγ in a dose-dependent manner (23). The relevance of IFNγ to the pathogenesis of GCA is further borne out by the demonstration of elevated concentrations of IFNγ messenger RNA in temporal artery biopsy samples from GCA patients with ischemic symptoms, such as jaw claudication and visual symptoms (24), which suggests a role of this cytokine in the process of lumen obstruction (25). Consistent with these findings, González-Gay et al (25) demonstrated an association between the 126-bp allele 3, which causes the production of high levels of IFNγ, and visual ischemic manifestations in Spanish patients with GCA. In contrast, other cytokine polymorphisms, such as those of the IL-1 locus and the TNFα gene, have not been implicated in the risk of developing GCA or, in particular, visual ischemic complications in Spanish patients (26).

Since IL-10 can inhibit Th1 responses and can suppress IFNγ synthesis by both direct (27) and indirect (16,17) mechanisms, we examined in this study potential associations between IL-10 promoter polymorphisms and susceptibility to, and clinical features of, GCA. The IL-10 promoter is highly polymorphic. Among 3 SNPs at promoter regions -1082 G/A, -592 C/A, and -819 C/T, SNPs -592 C/A and -819 C/T are in complete linkage disequilibrium, with 3 haplotypes reported in Caucasian populations (GCC, ACC, ATA) (3). A GTA haplotype has also been reported in Caucasians, but only infrequently (5,28). IL-10 polymorphisms -592 C/A and -1082 G/A and estimated haplotypes have been associated with a variety of diseases, such as Alzheimer's disease (29–31), cardiovascular diseases (32–34), cancer (35), sepsis (36,37), and rheumatic disorders, including rheumatoid arthritis (38), Sjögren's syndrome (39–41), systemic sclerosis (42), systemic lupus erythematosus (43,44), WG, and microscopic polyangiitis (45,46).

We studied these polymorphisms in Italian patients with GCA and found an association between -592 C/A, but not -1082 G/A, and susceptibility to the development of GCA. The significance of this association is further strengthened by the evidence of a consistent susceptibility among individuals homozygous for the A allele. We found a higher frequency of the ATA haplotype in GCA patients than in controls, whereas frequencies of the ACC and GTA haplotypes were decreased. Therefore, the increased frequency of the ATA haplotype found in GCA seems mainly to be due to the higher frequency of the -582 A allele. However, replication studies in other populations are required to arrive at more robust conclusions.

In vitro studies of human peripheral blood cells primed with bacterial lipopolysaccharide (LPS) have revealed a genetic component in the large interindividual differences (estimated at $>70\%$) in the production of IL-10 (47). Variable associations between IL-10 production and IL-10 gene polymorphisms and estimated haplotypes have been reported (3–10). More specifically, the biallelic polymorphism at position -592 has been found to be associated with altered levels of IL-10 synthesis. In healthy individuals, the A allele was correlated with low levels of LPS-stimulated IL-10 production, whereas the homozygous C/C genotype was associated with high levels of IL-10 synthesis upon stimulation (36). The A allele of the -1082 G/A polymorphism has been found to be associated with reduced synthesis of IL-10 upon stimulation, whereas the opposite holds true for the G/G genotype (3,6,8). On the same line, the ATA haplotype has been suggested to be associated with decreased production of IL-10 (3,6–8), while the relationship between the GCC or ACC haplotype and IL-10 production is more controversial. Recent findings, however, suggest a link between the ACC haplotype and high levels of IL-10 production (5,6,9).

Taken together, these data suggest that a genetically determined down-regulation of IL-10 synthesis may be implicated in the pathogenesis of GCA. In particular, in GCA patients, the high frequency of the ATA haplotype, which is associated with low production of IL-10, and the low frequency of the ACC haplotype, which is associated with high production of IL-10, consistently point to a down-regulation of IL-10 synthesis. A relative impairment of IL-10 synthesis, in turn, could favor the development of a full-blown Th1 response, leading to the classic histologic and clinical features of GCA.

Interestingly, a significant shift toward the A/A low-producer genotype of the IL-10 G/A -1082 polymorphism has been observed in WG (45,46). Similar to GCA, the granulomatous inflammation in WG is characterized by T cells that exhibit a Th1 cytokine pattern. In cultures of T cells from WG patients, it has been shown that IL-10 can prevent the development of a Th1 response by inhibiting IFNγ in a dose-dependent manner (23). Therefore, as in GCA, a genetically determined relative lack of IL-10 may predispose to the development of WG.

A second aim of this study was to determine whether these IL-10 polymorphisms might be associated with the presence of ischemic complications (vision loss and/or jaw claudication and/or aortic arch syndrome) or with PMR. However, when patients with and those

without these manifestations were compared, no associations were found. These findings are consistent with the results of a previous study, which demonstrated that IL-10 expression in temporal artery tissues of patients with GCA could not be mapped to any particular clinical phenotype (24).

Taken together, our findings may thus suggest that while impaired IL-10 production may predispose to the development of GCA, once the disease is established, its effect on the local inflammatory response is negligible. However, further studies are required both to replicate our findings in other populations and to investigate other cytokines and their respective polymorphisms in the pathogenesis of GCA.

REFERENCES

1. Weyand CM, Goronzy JJ. Medium- and large-vessel vasculitis. N Engl J Med 2003;349:160–9.
2. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;19:683–765.
3. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997;24:1–8.
4. Eskdale J , Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. Proc Natl Acad Sci U S A 1998;95:9465–70.
5. Eskdale J, Keijsers V, Huizinga T, Gallagher G. Microsatellite alleles and single nucleotide polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus. Genes Immun 1999;1:151–5.
6. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5′ flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis Rheum 1999;42:1101–8.
7. Edwards-Smith CJ, Jonsson JR, Purdie DM, Bansal A, Shorthouse C, Powell EE. Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis C to interferon alfa. Hepatology 1999;30:526–30.
8. Koss K, Satsangi J, Fanning GC, Welsh KI, Jewell DP. Cytokine (TNFα, LTα and IL-10) polymorphisms in inflammatory bowel diseases and normal controls: differential effects on production and allele frequencies. Genes Immun 2000;1:185–90.
9. Kurreeman FA, Schonkeren JJ, Heijmans BT, Toes RE, Huizinga TW. Transcription of the IL10 gene reveals allele-specific regulation at the mRNA level. Hum Mol Genet 2004;13:1755–6.
10. Keijsers V, Verweij CL, Westendorp RG, Breedveld FC, Huizinga TW. IL-10 polymorphisms in relation to production and rheumatoid arthritis (RA) [abstract]. Arthritis Rheum 1997;40 Suppl 9:S179.
11. Gonzalez-Gay MA, Boiardi L, Garcia-Porrua C, Macchioni P, Amor-Dorado JC, Salvarani C. Geographical and genetic factors do not account for significant differences in the clinical spectrum of giant cell arteritis in southern Europe. J Rheumatol 2004;31:520–3.
12. Salvarani C, Macchioni PL, Tartoni PL, Rossi F, Baricchi R, Castri C, et al. Polymyalgia rheumatica and giant cell arteritis: a 5-year epidemiologic and clinical study in Reggio Emilia, Italy. Clin Exp Rheumatol 1987;5:205–15.
13. Santos AR, Suffys PN, Vanderborght PR, Moraes MO, Vieira LM, Cabello PH, et al. Role of tumor necrosis factor-α and interleukin-10 promoter gene polymorphisms in leprosy. J Infect Dis 2002;186:1687–91.
14. Tseng LH, Chen PJ, Lin MT, Singleton K, Martin EG, Yen AH, et al. Simultaneous genotyping of single nucleotide polymorphisms in the IL-6, IL-10, TNFα and TNFβ genes. Tissue Antigens 2002;59:280–6.
15. Halperin E, Eskin E. Haplotype reconstruction from genotype data using Imperfect Phylogeny. Bioinformatics 2004;20:1842–9.
16. Skapenko A, Niedobitek GU, Kalden JR, Lipsky PE, Schulze-Koops H. Generation and regulation of human Th1-biased immune responses in vivo: a critical role for IL-4 and IL-10. J Immunol 2004;172:6427–34.
17. Trinchieri G. Regulatory role of T cells producing both interferon γ and interleukin 10 in persistent infection. J Exp Med 2001;194:F53–7.
18. Jiang H, Chess L. Regulation of immune responses by T cells. N Engl J Med 2006;354:1166–76.
19. Van Roon JA, Lafeber FP, Bijlsma JW. Synergistic activity of interleukin-4 and interleukin-10 in suppression of inflammation and joint destruction in rheumatoid arthritis [review]. Arthritis Rheum 2001;44:3–12.
20. McInnes IB, Illei GG, Danning CL, Yarboro CH, Crane M, Kuroiwa T, et al. IL-10 improves skin disease and modulates endothelial activation and leukocyte effector function in patients with psoriatic arthritis. J Immunol 2001;167:4075–82.
21. Weyand CM, Goronzy JJ. The pathogenic role of T lymphocytes in vasculitis. Sarcoidosis Vasc Diffuse Lung Dis 1996;13:217–20.
22. Dorman SE, Holland SM. Mutation in the signal-transducing chain of the interferon-γ receptor and susceptibility to mycobacterial infection. J Clin Invest 1998;101:2364–9.
23. Ludviksson BR, Sneller MC, Chua KS, Talar-Williams C, Langford CA, Ehrhardt RO, et al. Active Wegener's granulomatosis is associated with HLA-DR+ CD4+ T cells exhibiting an unbalanced Th1-type T cell cytokine pattern: reversal with IL-10. J Immunol 1998;160:3602–9.
24. Weyand CM, Tetzlaff N, Bjornsson J, Brack A, Younge B, Goronzy JJ. Disease patterns and tissue cytokine profiles in giant cell arteritis. Arthritis Rheum 1997;40:19–26.
25. Gonzalez-Gay MA, Hajeer AH, Dababneh A, Garcia-Porrua C, Amoli MM, Llorca J, et al. Interferon-γ gene microsatellite polymorphisms in patients with biopsy-proven giant cell arteritis and isolated polymyalgia rheumatica. Clin Exp Rheumatol 2004;22 Suppl 36:S18–20.
26. Gonzalez-Gay MA, Di Giovine FS, Silvestri T, Amoli MM, Garcia-Porrua C, Thomson W, et al. Lack of association between IL-1 cluster and TNF-α gene polymorphisms and giant cell arteritis. Clin Exp Rheumatol 2002;20:431.
27. Ito S, Ansari P, Sakatsume M, Dickensheets H, Vazquez N, Donnelly RP, et al. Interleukin-10 inhibits expression of both interferon α- and interferon γ-induced genes by suppressing tyrosine phosphorylation of STAT1. Blood 1999;93:1456–63.
28. Kingo K, Koks S, Silm H, Vasar E. IL-10 promoter polymorphisms influence disease severity and course in psoriasis. Genes Immun 2003;4:455–7.
29. Lio D, Licastro F, Scola L, Chiappelli M, Grimaldi LM, Crivello A, et al. Interleukin-10 promoter polymorphism in sporadic Alzheimer's disease. Genes Immun 2003;4:234–8.
30. Arosio B, Trabattoni D, Galimberti L, Bucciarelli P, Fasano F, Calabresi C, et al. Interleukin-10 and interleukin-6 gene polymorphisms as risk factors for Alzheimer's disease. Neurobiol Aging 2004;25:1009–15.
31. Ma SL, Tang NL, Lam LC, Chiu HF. The association between promoter polymorphism of the interleukin-10 gene and Alzheimer's disease. Neurobiol Aging 2005;26:1005–10.
32. George S, Ruan XZ, Navarrete C, Turner D, Reynard M, Sweny P, et al. Renovascular disease is associated with low producer

genotypes of the anti-inflammatory cytokine interleukin-10. Tissue Antigens 2004;63:470–5.
33. Bown MJ, Burton PR, Horsburgh T, Nicholson ML, Bell PR, Sayers RD. The role of cytokine gene polymorphisms in the pathogenesis of abdominal aortic aneurysms: a case-control study. J Vasc Surg 2003;37:999–1005.
34. Lio D, Candore G, Crivello A, Scola L, Colonna-Romano G, Cavallone L, et al. Opposite effects of interleukin 10 common gene polymorphisms in cardiovascular diseases and in successful ageing: genetic background of male centenarians is protective against coronary heart disease. J Med Genet 2004;41:790–4.
35. Shih CM, Lee YL, Chiou HL, Hsu WF, Chen WE, Chou MC, et al. The involvement of genetic polymorphism of IL-10 promoter in non-small cell lung cancer. Lung Cancer 2005;50:291–7.
36. Lowe PR, Galley HF, Abdel-Fattah A, Webster NR. Influence of interleukin-10 polymorphisms on interleukin-10 expression and survival in critically ill patients. Crit Care Med 2003;31:34–8.
37. Stanilova SA, Miteva LD, Karakolev ZT, Stefanov CS. Interleukin-10 –1082 promoter polymorphism in association with cytokine production and sepsis susceptibility. Intensive Care Med 2006;32: 260–6.
38. Pawlik A, Kurzawski M, Szklarz BG, Herczynska M, Drozdzik M. Interleukin-10 promoter polymorphism in patients with rheumatoid arthritis. Clin Rheumatol 2005;24:480–4.
39. Font J, Garcia-Carrasco M, Ramos-Casals M, Aldea AI, Cervera R, Ingelmo M, et al. The role of interleukin-10 promoter polymorphisms in the clinical expression of primary Sjögren's syndrome. Rheumatology 2002;41:1025–30.
40. Hulkkonen J, Pertovaara M, Antonen J, Lahdenpohja N, Pasternack A, Hurme M. Genetic association between interleukin-10 promoter region polymorphisms and primary Sjögren's syndrome. Arthritis Rheum 2001;44:176–9.
41. Origuchi T, Kawasaki E, Ide A, Kamachi M, Tanaka F, Ida H, et al. Correlation between interleukin 10 gene promoter region polymorphisms and clinical manifestations in Japanese patients with Sjögren's syndrome. Ann Rheum Dis 2003;62:1117–8.
42. Crilly A, Hamilton J, Clark CJ, Jardine A, Madhok R. Analysis of the 5′ flanking region of the interleukin 10 gene in patients with systemic sclerosis. Rheumatology (Oxford) 2003;42:1295–8.
43. Khoa PD, Sugiyama T, Yokochi T. Polymorphism of interleukin-10 promoter and tumor necrosis factor receptor II in Vietnamese patients with systemic lupus erythematosus. Clin Rheumatol 2005;24:11–3.
44. Rood MJ, Keijsers V, van der Linden MW, Tong TQ, Borggreve SE, Verweij CL, et al. Neuropsychiatric systemic lupus erythematosus is associated with imbalance in interleukin 10 promoter haplotypes. Ann Rheum Dis 1999;58:85–9.
45. Murakozy G, Gaede KI, Ruprecht B, Gutzeit O, Schurmann M, Schnabel A, et al. Gene polymorphisms of immunoregulatory cytokines and angiotensin-converting enzyme in Wegener's granulomatosis. J Mol Med 2001;79:665–70.
46. Bartfai Z, Gaede KI, Russell KA, Murakozy G, Muller-Quernheim J, Specks U. Different gender-associated genotype risks of Wegener's granulomatosis and microscopic polyangiitis. Clin Immunol 2003;109:330–7.
47. Reuss E, Fimmers R, Kruger A, Becker C, Rittner C, Hohler T. Differential regulation of interleukin-10 production by genetic and environmental factors: a twin study. Genes Immun 2002;3:407–13.