IL-6 promoter polymorphism at position -174 modulates the phenotypic expression of polymyalgia rheumatica in biopsy-proven giant cell arteritis

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Abstract

Objectives

Giant cell arteritis (GCA) and polymyalgia rheumatica (PMR) are related diseases in which diverse genetic and environmental factors are implicated. Both GCA and PMR are characterized by an intense acute phase reaction. In these syndromes the increased production of IL-6 has been observed. To investigate further the genetic influence of IL-6 in GCA and PMR we have examined the IL-6 promoter polymorphism (G to C) at position -174 in the 5' region in a series of patients from Northwest Spain diagnosed with GCA and/or PMR.

Methods

Sixty-two biopsy-proven GCA patients (30 of them with associated PMR) and 84 patients with isolated PMR were studied. Patients and ethnically matched controls (n = 124) were from the Lugo region (Galicia, Northwest Spain). Patients and controls were genotyped for HLA-DRB1 and IL-6 polymorphism at position -174 by molecular methods.

Results

*IL-6-174 allele C was marginally increased in frequency in GCA patients with PMR manifestations compared with isolated GCA (p_{corr}: 0.06; OR = 2.3). The increase in the frequency of the CC genotype in GCA patients with PMR versus those with isolated GCA was statistically significant (p_{corr}: 0.02). The increased frequency of allele C in GCA patients with PMR was more commonly observed in HLA-DRB1*04 negative patients. However, this polymorphism was not associated with a higher risk of ischemic events in GCA or with relapses in PMR.*

Conclusion

*Allele C at position -174 in the 5' promoter region of the IL-6 gene may be associated with PMR in biopsy-proven GCA patients not carrying HLA-DRB1*04 alleles.*

Key words

Giant cell arteritis, polymyalgia rheumatica, HLA-DRB1*04, IL-6 polymorphism.

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Introduction

Giant cell arteritis (GCA) (temporal arteritis) constitutes a common vasculitic syndrome that involves the large and middle-sized blood vessels with a predisposition to the cranial arteries in persons generally over 50 years of age (1-3). Polymyalgia rheumatica (PMR) is a common syndrome consisting of pain and stiffness in the neck, shoulders and pelvic girdle (4,5). As with GCA, PMR also occurs in subjects older than 50 years (6). PMR and GCA are related diseases as PMR may be the presenting manifestation of GCA, and it may be observed in up to 50% of patients with GCA (1, 2). However, PMR is sometimes an isolated condition unrelated to GCA.

An important concern for clinicians who often see elderly people is to differentiate isolated PMR from that associated with GCA. This point is of special interest as GCA is associated with permanent visual loss in more than 7% of cases (1-3). Another point of potential interest is to search for differences in genetic associations that may shed light on whether GCA and PMR have an identical or different genetic bases.

GCA and PMR are complex diseases in which diverse genetic and environmental factors are implicated in the development of an inflammatory response. Both GCA and PMR are characterized by an intense acute phase reaction. In these syndromes the increased production of IL-6 has been observed (7-9). Expression of IL-6 was found in more than 60% of circulating monocytes in patients with untreated GCA. Twenty percent of macrophages in temporal artery tissue biopsies were found to synthesise IL-6-specific mRNA and to produce IL-6 (10). In GCA/PMR patients Dasgupta *et al.* observed that although there was a significant decline in IL-6 in serum after adequate corticosteroid therapy, almost 50% of their patients maintained elevated IL-6 serum levels for up to 6 months after the onset of treatment (7). This occurred despite normalization of the acute phase reactants. Also, Roche *et al.* showed that in PMR short-term withdrawal of corticosteroids was followed by a rapid elevation in plasma IL-6 concentrations. (9). In addition, Weyand *et al.* observed that plasma IL-6 was a more sensitive parameter than an elevated erythrocyte sedimentation rate (ESR) for assessing disease activity in untreated and treated GCA patients (8).

An association between GCA and PMR and genes that lie within the HLA-class II region has been reported (11, 12). In Northwest Spain GCA and isolated PMR have different patterns of HLA-DRB1 association (13). GCA patients exhibited a significant increase in HLA-DRB1*04. This pattern of association was seen in GCA with or without PMR. In contrast, in PMR patients without GCA (i.e., isolated PMR) a significant increase in HLA-DRB1*13/14 alleles was observed. In these patients HLA-DRB1*04 was only marginally increased compared with ethnically matched controls (13), although it was observed to be a marker for disease severity in both GCA and PMR. HLA-DRB1*04 was associated with an increased risk of ischemic visual manifestations in GCA and with relapses of PMR (14, 15).

Other genetic polymorphisms may be implicated in the susceptibility and severity to GCA and PMR. Salvarani *et al.* have described a higher frequency of the allele R at codon 241 of the intercellular adhesion molecule-1 in HLA-DRB1*04 negative patients with GCA and PMR (16). In the human IL-6 gene, a single base change variation at the promoter region (transition G to C position -174 in the 5' region) has been observed (17). To investigate further the genetic influence of IL-6 in GCA and PMR, we have examined the -174 IL-6 promoter polymorphism in an unselected population of patients diagnosed with PMR and GCA from Northwest Spain.

Materials and methods

Patients and controls

Patients (n = 146) and controls (n = 124) were recruited from the area of Lugo in Northwest Spain. All were of caucasoid origin. Sixty-two biopsy-proven GCA patients (30 of them with associated PMR features) and 84 patients diagnosed with isolated 'pure' PMR were available for study.

Patients with GCA were only included in this study if they had a positive temporal artery biopsy showing infiltration of mononuclear cells into the arterial wall with or without giant cells.

All patients with PMR met the following criteria: 1) severe bilateral pain associated with morning stiffness for more than 1 month in at least 2 of 3 areas: neck, shoulder, and/or pelvic girdles; 2) an ESR at the time of diagnosis of at least 40 mm/1st hour; 3) resolution of the syndrome in less than 7 days following treatment with 10-20 mg/day of prednisone; and 4) exclusion of other diseases except for GCA that may present with polymyalgic manifestations or that may mimic PMR (4, 15, 18). The possibility of GCA in patients with isolated PMR was excluded by either a negative temporal artery biopsy or by a resolution of the syndrome following low dose prednisone therapy, and the absence of GCA manifestations after a follow-up of at least 36 months. In our study population the proportion of patients diagnosed with isolated PMR, without any symptom of GCA at the time of diagnosis, who developed GCA after 2 years of follow-up was lower than 1.5%.

A temporal artery biopsy was performed on all patients with clinical manifestations of GCA. In patients with PMR without any cranial or ischemic manifestations of GCA, a temporal artery biopsy was usually considered if there was a constitutional syndrome (asthenia, anorexia and weight loss of at least 4 kg) and/or an ESR > 80 mm/hour (15). Patients who at any time during the course of the disease fulfilled the 1987 ACR criteria for RA (19) were also excluded. Finally, those patients fulfilling the above criteria but with a positive rheumatoid factor (by nephelometry) were also excluded.

Visual ischemic complications were considered to be present if GCA patients had at least one of the following: 1) permanent visual loss (partial or complete permanent visual loss linked to GCA despite any possible improvement following corticosteroid therapy); 2) amaurosis fugax (transient visual loss that was followed by complete recovery or normal vision), or 3) diplopia (related to palsy of the extrinsic ocular muscles) (14).

At the time of diagnosis all patients with isolated PMR received an initial dose of prednisone ranging between 10 and 20 mg/day (median initial dose at the time of diagnosis in this group: 15 mg/day). Reductions were individualized. In general, a rate of 2.5 mg every 3 months was attempted. Relapses occurred when the dose of prednisone was low (median 2.5 mg/day) or had been discontinued.

A relapse of PMR was considered to be present if after definite objective improvement with corticosteroid therapy there was a flare of PMR features (frequently associated with an increase in the ESR) that was again suppressed by a resumption or increase in the corticosteroid dose (15). Relapses that occurred after at least 1 year after steroid therapy had been discontinued were defined as recurrences of PMR (15). In patients with relapses prednisone was increased by 2.5 or 5 mg above the former dose. In those patients with recurrences a dose of 5 mg/prednisone/day was prescribed.

Molecular analysis of IL-6 promoter polymorphism

DNA was extracted from anticoagulated blood collected in EDTA using a phenol-chloroform extraction method. Biallelic G/C polymorphism in the IL-6 promoter at position -174 was examined using the following PCR primers (17)

Forward 5'TTG TCA AGA CAT GCC AAG TGC T 3'

Reverse 5' GCC TCA GAG ACA TCT CCA GTC C 3'

A total of 100 ng genomic DNA (5 l) was amplified in a 25 l PCR reaction containing 2.5 l buffer-NH4 (Bioline), 1 l $MgCl_2$, 2.5 l dNTP's (Bioline), 0.25 l of each primer, 0.2 l Taq polymerase (Bioline) and 13.3 l distilled water. Thermal cycling was performed using a Hybaid OmniGene PCR machine. Cycles consisted of 10 minutes denaturation 95°C followed by 35 rounds of 95°C for 1 minute, 61°C for 1 minute, 72° for 1 minute, and a final extension at 72°C for 10 minutes. The presence of product was verified on a 1% agarose gel stained with ethidium bromide. The IL-6 promoter polymorphism (G to C) at position -174 in the 5' region creates a restriction site for *NIa*III. Due to this, PCR products were digested with *NIa*III in a 10 l final volume (17). The digest was incubated overnight at 37°C and the products of the digest were then visualized on a 4% agarose gel stained with ethidium bromide.

HLA typing

DNA was extracted from anti-coagulated blood collected in EDTA using a phenol-chloroform extraction method. HLA-DRB1 phenotype data for some of the patients included in this study have been described previously (14, 15, 20). They were determined using a semi-automated reverse dot blot method, INNO-LiPA (Abott Laboratories, UK), following the manufacturers' instructions. Reaction patterns were interpreted using INNO-LiPA software.

Statistical analysis

The strength of the association between GCA or PMR and alleles or genotypes of the -174 IL-6 was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined using contingency tables by either Chi-square or Fisher exact analysis. The same methods were used to examine the strength of the association between GCA and PMR subgroups (HLA-DRB1*04 positive and negative) and the -174 IL-6 polymorphism. Statistical significance was defined as $p \leq 0.05$. P values with Bonferroni's correction (p_{corr}) are shown. These were calculated by multiplying the p value by the number of alleles or genotypes compared. Calculations were performed with the statistical package Stata V6.

Results

Allele and genotype frequencies of the -174 IL-6 promoter polymorphism in GCA patients

The frequencies of alleles and genotypes for IL-6 promoter polymorphism at position -174 in the whole group of GCA patients were compared with

controls. No significant differences in frequency were found between the patients and controls (Table I). However, when GCA patients were stratified according the presence of PMR, allele C was marginally increased in frequency in patients with GCA with PMR (GCA+PMR+) compared with isolated GCA patients (GCA+PMR-) ($p = 0.03$, p_{corr} for the number of alleles tested = 0.06; OR = 2.3). The frequency of CC homozygotes in GCA+PMR+ patients was slightly higher than in controls (corrected p value = NS). The increase in the frequency of the CC genotype in GCA+PMR+ patients was statistically significant when these patients were compared with isolated GCA patients ($p = 0.008$; $p_{corr} = 0.02$) (Table I).

Allele and genotype frequencies of the -174 IL-6 promoter polymorphism in PMR patients

The frequencies of alleles and genotypes for IL-6 promoter polymorphism at position -174 in PMR patients were compared with those of controls (Table II). No significant differences in the frequency between the different disease groups and controls were found.

*Allele frequencies of the -174 IL-6 promoter polymorphism in HLA-DRB1*04 positive and negative patients with GCA and PMR*

The distribution of alleles for the -174 IL-6 biallelic polymorphism was examined in GCA and PMR patients stratified by HLA-DRB1*04 status (Table II A). In isolated GCA (GCA+PMR-) no significant differences in the -174 IL-6 allele C frequency were found. Also, no differences between HLA-DRB1*04 positive and negative patients with isolated PMR were found. However, the frequency of allele C in the group of HLA-DRB1*04 negative patients with GCA plus PMR (GCA+PMR+) was significantly increased compared with HLA-DRB1*04 positive ones (p_{corr} for the number of alleles tested = 0.04). In addition, when p values were corrected for the number of alleles tested to take into account the number of possible comparisons a significant increase of allele C in HLA-DRB1*04 negative patients with GCA+PMR+ compared with HLA-DRB1*04 negative isolated GCA patients was still observed ($p_{corr} = 0.01$). Thus, the increased frequency of allele C in the whole group of GCA+PMR+ patients compared with patients with isolated GCA was mainly due to the subgroup of HLA-DRB1*04 negative patients. Moreover, GG genotype was decreased and CC increased in HLA-DRB1*04 negative patients with GCA+PMR+ compared with HLA-DRB1*04 negative isolated GCA. However, these associations were slightly out of the range of significance if a correc-

Table I. The allele and genotype frequencies (%) of IL-6 promoter polymorphism at position -174 in patients with GCA and controls.

	Control (2N = 248)	A11GCA (2N = 124)	GCA+PMR- (2N = 64)	GCA+PMR+ (2N = 60)
Alleles				
G	67.3	67.7	76.6	58.3
C	32.7	32.3	23.4*	41.7*
Genotypes	(N = 124)	(N = 62)	(N = 32)	(N=30)
GG	46.0	45.2	53.1	36.7
CC	11.3	9.7	0.0**	20.0**
GC	42.7	45.2	46.9	43.3

*p = 0.03, $p_{corr} = 0.06$; OR = 2.3 (95% CI: 1.1 - 5.1) (GCA+PMR+ compared with GCA+PMR-).
**p = 0.008; $p_{corr} = 0.02$; OR = (GCA+PMR+ compared with GCA+PMR-).

Table II.

A. Allele frequencies (%) of IL-6 polymorphism at position -174 in HLA-DRB1*04 positive and negative patients with GCA and PMR.

IL-6 allele	GCA+PMR (-) DRB1*04		GCA+PMR+ DRB1*04		Isolated PMR DRB1*04	
	(+)	(-)	(+)	(-)	(+)	(-)
2N=	30	34	22	38	50	118
G	73.3	79.4	77.3	47.4	70.0	62.7
C	26.7	20.6[2]	22.7[1]	52.6[1,2]	30.0	37.3

[1]p = 0.02; OR = 3.8 (95%CI: 1.2 -11.9) (GCA+PMR+ HLA-DRB1*04 negative compared with GCA+PMR+ HLA-DRB1*04 positive).
p_{corr}: 0.04
[2]p = 0.005; OR = 4.3 (95%CI: 1.5 - 12.2) (GCA+PMR+ HLA-DRB1*04 negative compared with GCA+PMR negative HLA-DRB1*04 negative).
p_{corr}: 0.01

B. Genotype frequencies (%) of IL-6 polymorphism at position -174 in HLA-DRB1*04 positive and negative patients with GCA and PMR.

IL-6 genotype	GCA+PMR (-) DRB1*04		GCA+PMR+ DRB1*04		Isolated PMR DRB1*04	
	(+)	(-)	(+)	(-)	(+)	(-)
N=	15	17	11	19	25	59
Genotypes						
GG	46.7	58.8[1]	63.6	21.1[1]	52.0	39.0
CC	0	0[2]	9.1	26.3[2]	12.0	13.6
GC	53.3	41.2	27.3	52.6	36.0	47.4

[1] p = 0.02; OR = 0.2 (95%CI: 0.04 - 0.8) (GCA+PMR+ HLA-DRB1*04 negative compared with GCA+PMR negative HLA-DRB1*04 negative).
p_{corr}: 0.06
[2] p = 0.02; (GCA+PMR+ HLA-DRB1*04 negative compared with GCA+PMR negative HLA-DRB1*04 negative).
p_{corr}: 0.06

Table III. Allele frequencies of IL-6 polymorphism at position ~174 in GCA with and without visual complications and isolated PMR patients with or without relapses*.

IL-6 allele	GCA Visual ischemic complications		Isolated PMR Relapses or recurrences	
	YES	No	YES	No
2N=	28	96	36	132
G	75.0	65.6	63.9	65.2
C	25.0	34.4	36.1	34.8

* No statistically significant differences were found.

tion for the number of genotypes tested was used ($p_{corr} = 0.06$) (Table II B).

Influence of IL-6 polymorphism at position -174 on ischemic visual complications in GCA

Fourteen of the 62 biopsy-proven GCA patients had ischemic visual complications. As previously reported (14), visual complications in GCA were primarily associated with carriage of an HLA-DRB1*04 allele. However, the polymorphism of IL-6 at position -174 was not associated with a higher risk of ischemic events (Table III).

Influence of HLA-DRB1 and ICAM-I polymorphisms on relapses in isolated PMR

After a follow-up of at least 3 years none of the 84 patients with isolated PMR developed clinical manifestations of GCA. However, 16 of them had relapses and in another 2 there was a recurrence of the disease at least 1 year after steroid therapy had been discontinued. We have previously reported that in Northwest Spain relapses in PMR were primarily associated with carriage of an HLA-DRB1*0401 allele (15). The polymorphism of IL-6 at position -174 in patients with isolated PMR, however, was not associated with an increased risk for relapses or recurrences of the disease (Table III).

Discussion

Increased levels of IL-6 have been observed not only in GCA and PMR but also in other rheumatic diseases such as rheumatoid arthritis (21). In this study we have observed that the presence of allele C at position -174 in the promoter region of the IL-6 gene may be a marker for PMR in biopsy-proven GCA patients. This was particularly apparent in the subgroup of HLA-DRB1*04 negative patients. Although serum levels of IL-6 in this cohort were not available in the active phase of the disease, no apparent association of this biallelic polymorphism and disease severity was found. This absence of association between this IL-6 biallelic polymorphism is similar to the observations by Boiardi *et al.* who described a significant association between PMR and IL-1N gene biallelic polymorphism but an absence of functional significance of this polymorphism in terms of disease severity (22).

Associations of the -174 IL-6 polymorphism with the development of PMR features in GCA but not with isolated PMR on first examination suggests a paradox, especially when elevated IL-6 levels appear to be a generic feature of PMR. This can perhaps in part be explained by isolated PMR and PMR in the context of GCA having different genetic bases to their etiologies. Initial studies certainly suggest different underlying HLA-DRB1 associations (13). Furthermore, although no clinical distinction regarding musculoskeletal manifestations is made between isolated PMR and PMR associated with GCA, subtle differences in cytokine expression may occur (23). Similar variations may be present in GCA patients with and without PMR.

In keeping with other investigators (24), we have observed that an increased inflammatory response, manifested by the presence of clinical features such as asthenia, anorexia and weight loss, and abnormal laboratory tests were more common in the group of GCA patients without severe ischemic complications (14). By multivariate logistic regression analysis we observed that the absence of anemia and HLA-DRB1*04 phenotype constituted the best predictive model for ischemic visual events in 161 biopsy-proven patients (14). Paradoxically, the presence of anemia, a classic marker of the inflammatory response, was associated with a low risk of visual complications in GCA (14).

Why IL-6 encoded risk should be particularly confined to HLA-DRB1*04 negative patients with PMR is difficult to explain and warrants further investigation. One possibility may be that in oligogenic conditions such as GCA, complex gene-gene interactions between HLA-DRB1 and IL-6 regulatory region polymorphism may contribute to disease expression. A further paradox within these data is that earlier studies have suggested that the IL-6 -174 C allele is associated with lower levels of IL-6 expression, whereas our data demonstrates increased risk of PMR with the presence of the C allele. However, studies are now beginning to demonstrate that multiple regulatory elements exist in the promoter region of cytokine genes and that the relationship between genotypes and levels of expression is both complicated and dependent on the producing cell type.

IL-6 transcription is regulated by the coordination of several factors binding at distinct sites in the promoter. Polymorphisms in the IL-6 promoter region may result in inter-individual variations in the levels of transcription for the IL-6 gene. This may result in the different clinical expression of a particular disease. For example, in systemic onset juvenile chronic rheumatoid arthritis the significantly lower frequency of the IL6 -174 C allele was associated with significantly lower levels of IL-6 (25). Thus, the CC genotype was considered to be potentially protective against the development of the disease. However, the increased frequency of the IL6 -174 allele C among HLA-DRB1*04 negative GCA+PMR+ patients may suggest an opposite effect.

These results may suggest new avenues for investigating the association between GCA and PMR. Further studies on the implication of the -174 IL-6 pro-

moter biallelic polymorphism in other populations are required.

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