Epistatic Interactions Between HLA-DRB1 and Interleukin 4, But Not Interferon-γ, Increase Susceptibility to Giant Cell Arteritis

MAHSA M. AMOLI, MIGUEL A. GONZALEZ-GAY, ELEFTHERIA ZEGGINI, FIONA SALWAY, CARLOS GARCIA-PORRUA, and WILLIAM E.R. OLLIER

ABSTRACT. ***Objective.*** To assess the roles of the interleukin 4 (IL-4) and interferon-γ (IFN-γ) gene polymorphisms in a series of patients with biopsy-proven giant cell arteritis (GCA).

Methods. Eighty-two patients with biopsy-proven GCA and 102 ethnically matched controls from the Lugo region (Northwest Spain) were studied. The following single nucleotide polymorphisms (SNP) were assessed: IL-4 (SNP1: rs2070874, SNP2: rs2227284, SNP3: rs2227282, SNP4: rs2243266, and SNP5: rs2243267) and IFN-γ (SNP1: rs1861494, SNP2: rs1861493, and SNP3: rs2069718).

Results. Significant differences in allele and genotype frequencies were observed for the IL-4 SNP between HLA-DRB1*04 negative patients and controls. Epistatic interaction between SNP2 (rs2227284) with HLA-DRB1 showed a significant interaction ($p = 0.001$) and carriage of the SNP2*T allele in the absence of HLA-DRB1*04 resulted in a 4-fold risk of developing GCA (OR 4.2, 95% CI 1.1–15.6). Also, a significant increase in the frequency of the T-T-C-A-C IL-4 haplotype was observed in HLA-DRB1*04 negative GCA patients compared to the controls ($p = 0.02$; OR 2.0, 95% CI 1.0–3.9). Similar distributions of allele and genotype frequencies were observed for the IFN-γ polymorphisms in both GCA patients and controls.

Conclusion. Our results suggest an association with IL-4 gene polymorphism that is dependent on HLA-DRB1 genotype in GCA susceptible individuals. These data indicate an interaction between HLA-DRB1 and IL-4 that contributes to pronounced disease susceptibility. (J Rheumatol 2004;31:2413–7)

Key Indexing Terms:
GIANT CELL (TEMPORAL) ARTERITIS SINGLE NUCLEOTIDE POLYMORPHISM
INTERLEUKIN 4 INTERFERON-γ HLA-DRB1*04 DISEASE SUSCEPTIBILITY

Giant cell arteritis (GCA), also referred to as temporal arteritis, is a systemic vasculitis that involves large and medium-size vessels in the elderly[1,2]. The medium-size and large extracranial arteries are most frequently affected clinically, but in some cases, the aorta and its primary branches to the upper extremities and neck are also involved[3]. The inflammatory lesions are usually scattered irregularly along the course of involved vessels but longer segments may be affected in a continuous manner. Histologically, a granulomatous inflammatory process is seen, usually focused along the internal elastic lamina, that can lead to occlusion of blood vessels or weakening of the vessel wall and subsequent rupture[4].

The unbalanced production of Th1/Th2 cytokines is thought to have a role in the initiation phase and in the evolution of autoimmune vasculitis[5]. Two major Th1 and Th2 cytokines, interferon-γ (IFN-γ) and interleukin 4 (IL-4), have been identified as being important factors playing major roles in many conditions[6]. Functional polymorphisms resulting in high levels of IFN-γ production could change the Th1/Th2 balance, increasing the likelihood of developing Th1-type disorders and decreasing the probability of Th2-driven processes. The reciprocal of this could happen with regard to IL-4 functional polymorphisms, which correlate with increased production[6], but it may be possible to disturb the Th1/Th2 balance by the presence of functional polymorphisms that encode particularly low levels of IFN-γ or IL-4.

Cytokine production profiles in GCA show a pattern compatible with the Th1 model. Arterial lesions in GCA are composed of T cells and macrophages that form granulomas. Stimulated T cells release cytokines, particularly IFN-

From the Centre for Integrated Genomic Medical Research, School of Epidemiology and Health Sciences, University of Manchester, Manchester, United Kingdom; and the Division of Rheumatology, Hospital Xeral-Calde, Lugo, Spain.

M.M. Amoli, MD, PhD; E. Zeggini, PhD; F. Salway, MPhil; W.E.R. Ollier, PhD, FRCPath, Centre for Integrated Genomic Medical Research, School of Epidemiology and Health Sciences, University of Manchester; M.A. Gonzalez-Gay, MD, PhD; C. Garcia-Porrua, MD, PhD, Rheumatology Division, Hospital Xeral-Calde.

Address reprint requests to Dr. M.A. Gonzalez-Gay, Rheumatology Division, Hospital Xeral-Calde, c/ Dr. Ochoa s/n, 27004 Lugo, Spain. E-mail: miguelaggay@hotmail.com

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γ, that recruit macrophages to the vessel wall, leading to a granulomatous reaction[7].

The clonal expansion of selected T cell populations obtained from the site of inflammation suggests a possible role of T cells in recognizing a disease-relevant antigen in GCA[8,9]. The dramatic effects of steroid treatment and the clear association of HLA encoded polymorphisms further support a central role for T cells in GCA susceptibility.

In studies of HLA class II polymorphisms in GCA, an association with HLA-DRB1*04 alleles has been seen[10]. HLA-DRB1*04 alleles have also been found to be associated with disease severity[11,12].

Several polymorphisms have been characterized in both IL-4 and IFN-γ genes and these have been associated with disease susceptibility in several immune and inflammatory conditions[6]. We examined the roles of IL-4 and IFN-γ genes in GCA. Five polymorphisms in IL-4 and 3 polymorphisms in the IFN-γ gene were genotyped in patients with GCA and controls from Northwest Spain.

MATERIALS AND METHODS

Patients. The study group comprised patients diagnosed with biopsy-proven GCA (n = 82) treated in the Division of Rheumatology of the Hospital Xeral-Calde (Lugo, Spain). Ethnically matched controls (n = 102) from the Lugo region in Galicia, Northwest Spain, were available for comparison. The main characteristics of this population have been reported[10,12-14].

Patients were included if they had a positive temporal artery biopsy showing infiltration of mononuclear cells into the arterial wall with or without giant cells. GCA patients and controls had previously been HLA-DRB1 typed[15] and the frequencies of HLA-DRB1*04 were 40% and 26%, respectively.

Single nucleotide polymorphism (SNP) selection. SNP were selected from the National Center for Biotechnology Information SNP database (www.ncbi.nlm.nih.gov/SNP/) as follows: IL-4 (exon 1: SNP1: rs2070874; intron 2: SNP2: rs2227284, SNP3: rs2227282, SNP4: rs2243266, and SNP5: rs2243267) and IFN-γ (intron 3: SNP1: rs1861494, SNP2: rs1861493, and SNP3: rs2069718).

The criteria used for selecting the SNP assessed within IFN-γ and IL-4 were the following: (1) they gave reasonable coverage of the candidate genes tested, (2) the control frequencies of the 2 alleles for each SNP were frequent enough to give reasonable statistical power and chance of finding something in the cohort size tested, and (3) we could devise a Taqman assay for the SNP.

Genotyping. Genotyping was performed using the Taqman system (Applied Biosystems, Foster City, CA, USA). All reactions were performed using 2.5 µl Taqman universal polymerase chain reaction (PCR) master mix and 20 ng genomic DNA.

Two of the SNP within IL-4 and 2 of the SNP within IFN-γ were genotyped using ABI Assays-by-Design with 0.125 µl of 40× mix of unlabelled PCR primers and Taqman MGB probes (FAM and VIC dye-labeled) for each reaction. Primers and probes used for each SNP are as follows: (1) IL-4 (rs2227282): forward primer CGGCAGGGATGGAGTGT; reverse primer CAGCTTTAGTGCAAGGCCTTAAC; probes VIC CAGAGCAACTAAAC and FAM CAGAGCAAGTAAAAC; (2) IL-4 (rs2243266): forward primer GTGCCTCAGTCTGGGTCTAG; reverse primer AATCTTTGTGTTCATCTCTTATTTATGTAGGCT; probes VIC CAGTTCATATTTTAACGTTTTT (reverse probe) and FAM CAGTTCATATTTTAATGTTTTT (reverse probe); (3) IFN-γ (rs1861494): forward primer AGTGAAGAGGAAGTAGGTGAGGAA; reverse primer CAAGGGACAATGAGAGAACTGCTT; probes VIC TCAGTACTCCCCGCTTC (reverse probe) and FAM TCAGTACTCCCTGCTTC (reverse probe); (4) IFN-γ (rs1861493): forward primer CCACCAAGGAACTGTGATGAATCA; reverse primer GTTTTGGAGCAAAGAAGGTCATCAA; probes VIC CTTATACAGTGAGCCTAAC (reverse probe) and FAM CTTATACAGTGAACCTAAC (reverse probe).

IL-4 (rs2070874, rs2227284, rs2243267) and IFN-γ (rs2069718) genotyping was performed by ABI Assays-on-Demand using 0.25 µl of a 20× mix of primers and probes for each reaction.

Thermal cycling conditions were 95°C for 10 minutes then 50 cycles of 95°C for 15 seconds and 60°C for 1 minute. Genotypes were obtained by reading fluorescent signals of the reaction end product. Taqman PCR products were read directly in an ABI 7700 analyzer (Applied Biosystems). DNA were grouped according to genotype. The PCR reaction was initially validated by real-time PCR. Subsequently, genotypes were obtained by reading the fluorescent signal from the PCR reaction end product.

Statistical analysis. All SNP were checked for deviation from Hardy-Weinberg equilibrium. Strength of association between alleles or genotypes 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's exact tests. Statistical significance was defined as $p < 0.05$. Linkage disequilibrium (LD) patterns were determined through the HelixTree™ and EHplus packages. Inferred haplotypes were estimated using the expectation-maximization algorithm through the EHplus, snphap, and HelixTree™ packages. Epistatic interactions were investigated through unconditional logistic regression using the Stata package. We have only presented uncorrected p values and they should be interpreted accordingly.

RESULTS

Genotype frequencies for all polymorphisms were in Hardy-Weinberg equilibrium in both case and control populations.

Single-point association analysis of the IL-4 SNP. Allele and genotype frequencies for the IL-4 SNP were compared between GCA patients and controls (Table 1). A significant increase in the carriage of the IL-4 SNP3 (rs2227282) C allele was observed in GCA patients compared to controls ($p = 0.03$, OR 1.8, 95% CI 0.9–3.5). A marginally significant increase in carriage of the IL-4 SNP2 (rs2227284) T allele was also observed in GCA patients compared to controls ($p = 0.04$, OR 1.8, 95% CI 0.9–3.4; Table 1). No significant differences for allele and genotype frequencies were observed between GCA patients and controls for other IL-4 SNP tested.

Single-point association analysis of the IFN-γ SNP. Allele and genotype frequencies for all IFN-γ SNP were compared in GCA patients and controls. Similar distributions of allele and genotype frequencies were observed for IFN-γ polymorphisms in both GCA cases and controls (Table 2).

LD patterns of the IL-4 and IFN-γ SNP. Hplus revealed strong LD among the IL-4 SNP in both the GCA and control populations ($p < 0.0001$). The IFN-γ SNP were also found to be in strong LD with each other ($p < 0.0001$). Additional pairwise analysis through HelixTree confirmed these results.

*Single-point association analysis of the IL-4 and IFN-γ SNP stratified by HLA DRB1*04 status.* GCA patients genotyped for HLA-DRB1 were stratified based on the presence or absence of HLA-DRB1*04. Allele and genotype frequen-

Table 1. Genotype frequencies of IL-4 polymorphisms in patients with GCA and controls.

IL-4	Controls, (%)	GCA, (%)	DR4+, (%)	DR4–, (%)
SNP1 (rs2070874)	n = 99	n = 77	n = 31	n = 45
Genotype				
CC	73 (74)	54 (70)	26 (84)	27 (60)
CT	25 (25)	20 (26)	5 (16)	15 (33)
TT	1 (1)	3 (4)	0 (0)	3 (7)[3]
SNP2 (rs2227284)	n = 102	n = 82	n = 33	n = 48
Genotype				
GG	61 (60)[1]	37 (45)[1]	19 (57)	18 (37)
GT	31 (30)	35 (43)	10 (31)	24 (50)
TT	10 (10)[4]	10 (12)	4 (12)	6 (13)
SNP3 (rs2227282)	n = 101	n = 82	n = 33	n = 49
Genotype				
CC	10 (10)[5]	10 (12)	3 (9)	7 (14)[5]
CG	31 (30)	36 (44)	12 (36)	24 (48)
GG	60 (60)[2]	36 (44)[2]	18 (55)	18 (37)
SNP4 (rs2243266)	n = 102	n = 81	n = 32	n = 48
Genotype				
GG	74 (73)	55 (68)	27 (84)	27 (56)
GA	26 (26)	23 (28)	5 (16)	18 (38)
AA	2 (2)	3 (4)	0 (0)	3 (6)[6]
SNP5 (rs2243267)	n = 101	n = 77	n = 29	n = 47
Genotype				
CC	1 (1)[7]	3 (4)	0 (0)	3 (6)[7]
CG	24 (24)	23 (30)	5 (17)	18 (38)
GG	76 (75)	51 (66)	24 (83)	26 (56)

[1] TT + GT vs GG: p = 0.04, OR 1.8, 95% CI 0.9–3.4. [2] CG + CC vs GG: p = 0.03, OR 1.8, 95% CI 0.9–3.5. [3] TT vs CC + CT: p = 0.05, OR 7.0, 95% CI 0.5–371.0. [4] TT + GT vs GG: p = 0.01, OR 2.4, 95% CI 1.1–5.3. [5] CG + CC vs GG: p = 0.01, OR 2.4, 95% CI 1.1–5.4. [6] GA + AA vs GG: p = 0.04, OR 2.0, 95% CI 0.9–4.4. [7] CC + CG vs GG: p = 0.01, OR 2.4, 95% CI 1.1–5.4.

Table 2. Genotype frequencies of IFN-γ polymorphisms in patients with GCA and controls. No statistically significant differences were observed.

IFN-γ	Controls, (%)	GCA, (%)	DR4+, (%)	DR4–, (%)
SNP1 (rs1861494)	n = 101	n = 82	n = 33	n = 48
Genotype				
GG	9 (9)	6 (7)	3 (9)	3 (6)
GA	31 (31)	24 (29)	9 (27)	15 (31)
AA	61 (60)	52 (63)	21 (64)	30 (62)
SNP2 (rs1861493)	n = 99	n = 77	n = 32	n = 45
Genotype				
CC	10 (10)	6 (8)	3 (9)	3 (6)
TC	28 (28)	25 (32)	9 (28)	15 (33)
TT	61 (61)	46 (60)	20 (62)	27 (60)
SNP3 (rs2069718)	n = 101	n = 80	n = 32	n = 47
Genotype				
TT	18 (18)	13 (16)	4 (13)	9 (19)
CT	45 (44)	32 (40)	15 (47)	17 (36)
CC	38 (38)	35 (44)	13 (41)	21 (45)

cies for all IL-4 and IFN-γ polymorphisms were determined in both groups (Tables 1 and 2). When stratified single-point analyses were carried out, no significant differences between the case groups and the controls were observed for any of the IFN-γ SNP. However, significantly different allele and genotype frequencies were observed for the IL-4 SNP tested between DRB1*04 negative patients and controls.

Investigation of epistatic interactions. When interactions

between the IL-4 SNP most significantly associated (SNP2 and SNP5) with HLA-DRB1*04 were investigated, evidence for a significant interaction between SNP2 and HLA-DRB1 was obtained (p = 0.001). Specifically, carriage of the SNP2*T allele in the absence of HLA-DRB1*04 resulted in a 4-fold risk of developing GCA (OR 4.2, 95% CI 1.1–15.6).

Haplotype analysis of IL-4. Estimated haplotype frequencies for all SNP were determined in GCA patients and controls and also in GCA patients stratified according to HLA-DRB1*04 status. No significant differences in haplotype frequencies were observed between the total group of GCA patients and controls. A significant increase in the frequency of the T-T-C-A-C IL-4 haplotype was observed in HLA-DRB1*04 negative GCA patients compared to the controls (p = 0.02, OR 2.0, 95% CI 1.0–3.9; Table 3).

DISCUSSION

The antagonistic effect of Th1 and Th2 responses is useful in the design of cytokine therapies for the treatment of disease[16]. IFN-γ is a prototypic Th1 cytokine involved in host defense against intracellular pathogens with a role in organ-specific autoimmune response. IL-4, in contrast, is a prototypic Th2 cytokine involved in antibody responses in host defense and mediation of allergic reaction[17].

Several reports have described SNP within the IL-4 gene forming 2 major haplotypes, recognized as high and low IL-4 producer haplotypes, confirmed by several functional assays[6]. Associations have been observed between the high producer IL-4 haplotype and several different conditions such as pulmonary dysfunction and severe asthma[18,19], which were in keeping with the disease pattern showing its role as an enhancer in Th2-type disease. In Th1-type disorders such as rheumatoid arthritis[20] and multiple sclerosis[21], high producer haplotypes have been found to be associated with either a less severe or nondestructive clinical course. Similarly, polymorphisms within the IFN-γ gene seem to form the same pattern of high producer and low producer haplotypes, showing associations with inflammatory conditions with the same disease-modifying pattern as the IL-4 haplotypes for both Th1 and Th2-type disorders[6].

HLA-DRB1 genes represent significant risk factors in GCA. An association between GCA and genes within the HLA class II region has been reported[22] and most studies have shown an association between HLA-DRB1*04 and GCA[10,11,23,24]. Patients with biopsy-proven GCA in our study were also associated with HLA-DRB1*04[15]. We observed marginally significant associations between GCA and the IL-4 (rs2227282) and IL-4 (rs2227284) SNP regardless of HLA-DRB1*04 status. However, the results were more significant when the HLA-DRB1*04 status was considered. Frequencies for all 5 SNP tested within the IL-4 gene were significantly different in GCA HLA-DRB1*04 negative patients compared to controls. This suggests that association with IL-4 gene polymorphism is dependent on HLA-DRB1 genotype in GCA susceptible individuals. These data indicate an interaction between HLA-DRB1 and IL-4 that contributes to pronounced disease susceptibility. Significant evidence for an epistatic interaction between these 2 genes has been obtained through logistic regression analysis. Strong LD among the SNP tested has been observed, and increased frequency for the T-T-C-A-C IL-4 haplotype found in HLA-DRB1*04 negative GCA patients compared to controls indicates the presence of a functional haplotype in IL-4 that is implicated in GCA susceptibility in association with HLA-DRB1.

It has been suggested that the tissue cytokine profile within the inflammatory lesion of GCA may be orchestrating an inflammatory response in this condition compatible with the Th1 pattern[7]. The observation that a particular IL-4 haplotype but not IFN-γ polymorphism is associated with GCA susceptibility in HLA-DRB1*04 negative individuals appears to be at odds with the reported immunohistological evidence of increased IFN-γ expression in temporal artery biopsies[25]. However, one explanation could be that genetically encoded low production of IL-4 shifts the Th1/Th2 balance predominantly towards a Th1 profile and gives a tendency to produce more IFN-γ, especially within sites of cell mediated damage. Such a predisposition could constitute an increased risk for GCA, and this could be more likely to be observed in HLA-DRB1*04 negative individuals, where other GCA risk factors would be required. IL-4, as a prototypic Th2 cytokine against the Th1 immune response, can be regarded as a key molecule in modulating inflammatory response in GCA. However, confirmation of this hypothesis would require further investigation. Investigating the association between GCA and other polymorphisms within the promoter region of IL-4, and also exam-

Table 3. Estimated IL-4 SNP haplotype frequencies in patients with GCA and controls.

SNP1 rs2070874	SNP2 rs2227284	SNP3 rs2227282	SNP4 rs2243266	SNP5 rs2243267	Controls, 2N = 198	GCA, 2N = 154	DR4+, 2N = 58	DR4–, 2N = 90
C	G	G	G	G	0.730392	0.662503	0.712121	0.614484
T	T	C	A	C	0.127451*	0.162501	0.090909	0.229064*
C	T	C	G	G	0.117647	0.155730	0.181818	0.135522
C	G	G	A	G	0.014706	0.000000	0.000000	0.000000

* p = 0.02, OR 2.0, 95% CI 1.0–3.9.

ining the involvement of other Th2 cytokine gene polymorphisms localized to the chromosome 5 cluster close to the IL-4 gene in GCA, would be useful.

Although strong LD was found among 3 polymorphisms across the IFN-γ gene, no associations were found between these polymorphisms and GCA when testing the polymorphisms either individually or as haplotypes.

Our study suggests new avenues for investigating the association between Th2 cytokines and GCA in the context of HLA-DR and their interactions. However, it is important for others to replicate our results in an independent cohort to ultimately confirm our findings.

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