Lack of Association of a Functional –94ins/delATTG *NFKB1* Promoter Polymorphism with Susceptibility and Clinical Expression of Biopsy-Proven Giant Cell Arteritis in Northwest Spain

JAVIER MARTIN, CRISTINA PEREZ-ARMENGOL, JOSE A. MIRANDA-FILLOY, JOSE R. VILCHEZ, MIGUEL A. LOPEZ-NEVOT, CARLOS GARCIA-PORRUA, and MIGUEL A. GONZALEZ-GAY

ABSTRACT. ***Objective.*** Giant cell arteritis (GCA) is a vasculitis preferentially involving large and middle-sized arteries in the elderly. The nuclear factor of k-light polypeptide gene enhancer in B cells (NF-kB) is a family of 5 proteins expressed in most cells that function to regulate gene transcription. *NFKB1* gene plays a critical role in the coordination of the immune system by regulating the transcription of a broad variety of genes implicated in the immune response. A *NFKB1* promoter polymorphism consisting of a common insertion/deletion (–94ins/delATTG) located between 2 putative key promoter regulatory elements and showing functional effects on the transcription of the *NFKB1* gene has been described. Since GCA is a polygenic disease, we sought to assess the potential role of the –94ins/delATTG *NFKB1* promoter polymorphism in susceptibility to GCA and to determine if this polymorphism is implicated in the clinical expression of this vasculitis.

Methods. Ninety-six patients with biopsy-proven GCA and 204 ethnically matched Caucasian controls from the Lugo region (Northwest Spain) were studied. Genotyping of the –94ins/delATTG *NFKB1* promoter polymorphism was performed by fluorescent polymerase chain reaction (PCR).

Results. No significant differences in allele or genotype frequencies for this *NFKB1* promoter polymorphism were observed between patients with GCA and controls even when patients were stratified according to gender, presence of polymyalgia rheumatica (n = 38), severe ischemic manifestations (n = 49), or other clinical manifestations of GCA.

Conclusion. Our results do not support a role for –94ins/delATTG *NFKB1* promoter polymorphism in susceptibility and clinical expression of GCA in a Northwestern Spanish population. (J Rheumatol 2006;33:285–8)

Key Indexing Terms:
GIANT CELL (TEMPORAL) ARTERITIS SUSCEPTIBILITY POLYMORPHISM
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Giant cell arteritis (GCA), a disease that preferentially affects medium and large-sized arteries, is the most common systemic vasculitis in the elderly in Western countries, in particular in individuals with Northern European ancestry[1,2]. GCA has proved to be a polygenic disease, and different genes may influence the phenotype and the outcome of this condition[3].

The nuclear factor of k-light polypeptide gene enhancer in B cells (NF-kB) is a family of 5 proteins expressed in most cells that function to regulate gene transcription[4]. Active NF-kB is released from the cytoplasm of most cells[5]. The protein NF-kB is restrained in cytoplasm bound to an inhibitor of NF-kB (IkB)[6]. NF-kB can be freed from IkB by the phosphorylation and ubiquitination of the IkB by an IkB kinase. This ultimately targets the IkB for proteosomal degradation and allows NF-kB to dimerize and translocate into the nucleus to activate gene transcription[4].

The *NFKB1* gene plays a critical role in coordinating the immune system through its ability to regulate the transcription of a broad variety of genes implicated in the immune response, including those of proinflammatory cytokines, such as interleukin (IL)-1ß, IL-6, and tumor necrosis factor (TNF)-α, adhesion and intercellular adhesion molecules, and inducible effector enzymes, such as inducible nitric oxide synthase (NOS2) and cyclooxygenase 2[7]. Due to this, polymorphisms near the *NFKB1* coding region could affect its expression and the immune equilibrium.

Corticosteroids are the cornerstone of treatment of GCA[2]. These drugs can suppress NF-kB[7]. Inflammatory

From the Instituto de Parasitologia y Biomedicina Lopez-Neyra. CSIC; the Division of Immunology, Hospital Virgen de las Nieves, Granada; and the Division of Rheumatology, Hospital Xeral-Calde, Lugo, Spain.

J. Martin, MD, PhD; C. Perez-Armengol, PhD, Instituto de Parasitologia y Biomedicina Lopez-Neyra, CSIC; J.R. Vilchez, PhD; M.A. Lopez-Nevot, MD, PhD, Servicio de Inmunología, Hospital Virgen de las Nieves; M.A Gonzalez-Gay, MD, PhD; J.A. Miranda-Filloy, MD; C. Garcia-Porrua, MD, PhD, Division of Rheumatology, Hospital Xeral-Calde.

Dr. Gonzalez-Gay and Dr. Martin share senior authorship of this report.

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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cytokines are expressed in temporal artery tissues from patients with biopsy-proven GCA[8]. Weyand, *et al* found that temporal artery specimens from patients with GCA with ocular ischemia expressed high amounts of interferon (IFN)-γ mRNA[9]. By regulating giant cell formation IFN-γ could indirectly control intimal hyperplasia and lead to the development of lumenal obstruction[10]. Interestingly, administration of dexamethasone to temporal artery-severe combined immune deficiency (SCID) chimeras caused a partial suppression of T cell and macrophage function as indicated by the reduced tissue concentrations of IL-2, IL-1ß, and IL-6 mRNA, and by the diminished expression of NOS2. In contrast, synthesis of IFN-γ mRNA was only slightly decreased, and expression of transforming growth factor (TGF)-ß1 was unaffected. These findings correlated with activation of the IkB gene and blockade of the nuclear translocation of NF-kB in xenotransplanted tissue[11]. Also, *in vitro* studies using T cell clones and monocytes derived from patients with GCA showed that dexamethasone preferentially targeted NF-kB-regulated monokines[12].

In light of these findings, it is possible that NF-kB may play an important role either in the development of GCA or in the phenotypic expression of the disease. Interestingly, a *NFKB1* gene promoter polymorphism consisting of a common insertion/deletion (–94ins/delATTG) located between 2 putative key promoter regulatory elements, showing functional effects on the transcription of the *NFKB1* gene, has been described[13]. The presence of this 4-base pair (bp) deletion resulted in loss of binding to nuclear proteins and reduced promoter activity of *NFKB1* promoter. This deletion was found to increase the risk of ulcerative colitis in a North American population[13].

We sought to assess whether this –94ins/delATTG *NFKB1* polymorphism is implicated in susceptibility and clinical expression of GCA.

MATERIALS AND METHODS

Study population. The study group comprised 96 patients diagnosed with biopsy-proven GCA at the Division of Rheumatology of the Hospital Xeral-Calde (Lugo, Northwest Spain). All patients fulfilled the 1990 American College of Rheumatology classification criteria for GCA[14]. Two-hundred and four ethnically matched controls from the same area of Lugo were studied. All individuals assessed in this study were of Spanish Caucasian origin.

As with former genetic studies on GCA from Lugo[3], only patients with a positive temporal artery biopsy showing disruption of the internal elastic laminae with infiltration of mononuclear cells into the arterial wall with or without giant cells were included.

As reported[15], patients with GCA were considered to have associated polymyalgia rheumatica (PMR) if they had severe bilateral pain and aching involving at least 2 of the 3 following: the neck, the shoulder, and the pelvic regions, associated with morning stiffness. Patients were considered to have severe ischemic manifestations if they suffered at least one of the following complications: visual manifestations (transient visual loss including amaurosis fugax, permanent visual loss, or diplopia), cerebrovascular accidents (stroke and/or transient ischemic attacks), jaw claudication, or large-artery stenosis of the extremities that caused signs of occlusive manifestations (limb claudication) of recent onset[15].

Patients and controls gave informed consent, which was approved by the local institutional committee, prior to participation in the genetic studies.

NFKB1 genotyping. DNA was isolated from anticoagulant-treated peripheral blood mononuclear cells using standard methods. We determined the –94ins/delATTG *NFKB1* genotypes by a polymerase chain reaction (PCR)-based method[13]. Briefly, a 289 bp PCR fragment was amplified from genomic DNA using forward primer 5´-TTT AAT CTG TGA AGA GAT GTG AAT G-3´ and reverse primer 5´-CTC TGG CTT CCT AGC AGG G-3´. The forward primer was 5´labeled with the fluorescent dye 6-FAM. Presence or absence of the 4 bp deletion was determined by the size of the labeled PCR product on an ABI 3100 sequencer, using Genescan 672 software (Applied Biosystems, Foster City, CA, USA). Selected samples were sequenced on the ABI 3100 sequencer. Sequence results accurately confirmed the molecular weight determined by fluorescence labeling.

Statistical analysis. Allelic and genotypic frequencies of the –94ins/delATTG *NFKB1* polymorphism were obtained by direct counting. Strength of association between patient and control groups and alleles or genotypes of this polymorphism 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 analysis. Statistical significance was defined as $p \leq 0.05$. Calculations were performed using the statistical package Stata V6.

RESULTS

Clinical features of patients with GCA. The main clinical characteristics of this series of 96 patients with biopsy-proven GCA are summarized in Table 1. This study comprised 53 women and 43 men (median age at diagnosis 75 yrs; range: 60-92 yrs). Between the onset of GCA symptoms until 1 month after the onset of steroid therapy 38 had PMR and 49 developed severe ischemic manifestations. Among them, 37 had jaw claudication, 23 visual ischemic manifestations (10 with irreversible visual loss despite corticos-

Table 1. Clinical features of 96 patients with biopsy-proven GCA from Lugo (Northwest Spain). Values in parentheses indicate the total proportion of patients with a particular variable. Values are no. (%) unless otherwise indicated.

Variable	%
Age, yrs	
Range	60–92
Median	75
Women:men	53:43
Proportion of women	55.2
Headache	82 (85.4)
Abnormal temporal artery on physical examination	73 (76.0)
Polymyalgia rheumatica	38 (39.6)
Jaw claudication	37 (38.5)
Visual manifestations*	23 (24.0)
Permanent visual loss	10 (10.4)
Stroke	1 (1.0)
Arm claudication due to ischemia of the humeral artery	1 (1.0)
Severe ischemic manifestations**	49 (51.0)
ESR > 40 mm/h	96 (100.0)

* Transient visual loss including amaurosis fugax, permanent visual loss, or diplopia. ** Visual manifestations, cerebrovascular accidents (stroke and/or transient ischemic attacks), jaw claudication, or limb claudication of recent onset.

teroid therapy), 1 stroke, and 1 arm claudication due to ischemia caused by vasculitic occlusion of the humeral artery. In all cases the erythrocyte sedimentation rate (Westergren ESR) at the time of disease diagnosis was greater than 40 mm/hour.

–94ins/delATTG NFKB1 promoter polymorphism genotypic and allelic frequencies. No evidence of departure from Hardy-Weinberg equilibrium was observed in controls. Table 2 shows the allele and genotype frequencies of the *NFKB1* promoter polymorphism in patients with biopsy-proven GCA and controls. No statistically significant differences between patients and controls were found. This polymorphism also failed to discriminate GCA patients according to specific disease characteristics. No significant differences were observed when patients were stratified according to gender, PMR, or severe ischemic manifestations (Table 3), or when compared according to presence of headache, abnormal temporal artery on physical examination, jaw claudication, or visual ischemic manifestations (data not shown).

DISCUSSION

Inappropriate activation of NF-kB has been implicated in inflammation associated with a variety of human diseases and pathologic conditions such as asthma, septic shock, lung fibrosis, diabetes, cancer, AIDS, atherosclerosis, stroke, and inflammatory bowel disease[16]. NF-kB is also activated in the inflamed synovium of patients with rheumatoid arthritis (RA)[17].

Since the –94ins/delATTG *NFKB1* promoter polymorphism has shown functional effects on the transcription of the *NFKB1* gene[13], analysis of the potential implication of this gene polymorphism might improve our understanding of mechanisms associated with development of inflammatory and autoimmune diseases. Interestingly, Karban, *et al* showed for the first time the genetic association of the –94ins/delATTG *NFKB1* promoter polymorphism with a common human disease, ulcerative colitis[13].

Because GCA is associated with a high inflammatory response, we examined the potential implication of the functional –94ins/delATTG *NFKB1* promoter polymorphism in patients with histologically confirmed vasculitis. However, no differences between patients with GCA and controls were observed. This was also the case when patients were stratified according to specific features of the disease. Accordingly and considering the frequency of this vasculitis and the number of patients we assessed, we may exclude a potential role for this polymorphism in susceptibility to GCA in Northwest Spain. It is unlikely that our negative results could have arisen due to a Type II error (false-negative results) since our sample size had 98% power to detect the effect of a polymorphism conferring an OR of 1.8 at the 5% significance level. Interestingly, our data are in accordance with former observations from our group in Spanish patients with RA and systemic lupus erythematosus (SLE). Orozco, *et al* found no evidence of association between the above mentioned *NFKB1* polymorphism and any of the demographic and clinical variables tested in Spanish patients with either RA or SLE[18].

With respect to the functional relevance of the *NFKB1* gene variation, the –94ins/delATTG *NFKB1* alleles appear to affect promoter activity of the *NFKB1* gene and differential nuclear protein binding[13]. Nevertheless, caution should

Table 2. Frequencies of –94ins/delATTG *NFKB1* promoter polymorphism genotypes and alleles in patients with biopsy-proven GCA and controls. No statistically significant differences between GCA patients and controls were observed.

–94ins/delATTG	GCA Patients n = 96 (%)	Controls n = 204 (%)
Genotypes		
del/del	19 (19.8)	30 (14.7)
del/ins	49 (51.0)	96 (47.1)
ins/ins	28 (29.2)	78 (38.2)
Alleles		
del	87 (45.3)	156 (38.2)
ins	105 (54.7)	252 (61.8)

Table 3. Frequencies of –94ins/delATTG *NFKB1* promoter polymorphism genotypes and alleles in patients with biopsy-proven GCA according to the main clinical manifestations. No statistically significant differences according to the different subgroups of GCA were observed.

NFKB1 Polymorphism	GCA and Severe Ischemic Manifestations		GCA Gender		GCA and PMR	
	Yes n = 49 (%)	No n = 47 (%)	Female n = 53 (%)	Male n = 43 (%)	Yes n = 38 (%)	No n = 58 (%)
–94 ins/del ATTG						
Genotype						
del/del	11 (22.4)	8 (17.0)	10 (18.9)	9 (20.9)	11 (29.0)	8 (13.8)
del/ins	27 (55.1)	22 (46.8)	26 (49.1)	23 (53.5)	17 (44.7)	32 (55.2)
ins/ins	11 (22.4)	17 (36.2)	17 (32.1)	11 (25.6)	10 (26.3)	18 (31.0)
Allele						
del	49 (50.0)	38 (40.4)	46 (43.4)	41 (47.7)	39 (51.3)	48 (41.4)
ins	49 (50.0)	56 (59.6)	60 (56.6)	45 (52.3)	37 (48.7)	68 (58.6)

be exercised in extrapolating results of *in vitro* experiments to the individual patient, since other factors within the disease environment may affect NF-kB production and biologic activity. In addition, further detailed molecular promoter studies using cell lines of different origins are needed to define the overall functional importance of –94ins/delATTG *NFKB1* polymorphism, bearing in mind that other polymorphisms in linkage disequilibrium might also be influencing promoter activity.

It is clear that NF-kB plays an important role in autoimmunity and inflammation[19-21], but pathologic processes involved are complex and further genetic studies are required to assess the relative importance of *NFKB* gene polymorphism in relation to genetic predisposition to autoimmunity. It would be of interest to examine polymorphism within other NF-kB molecules such as NFkB2 and NFkB3, or genes encoding for other components of the NF-kB cascade such as inhibitors of NF-kB in relation to GCA.

The search for other genes implicated in susceptibility to GCA should continue to further improve our understanding of the pathogenesis of this vasculitis. Since strong evidence points to an association of GCA with HLA-class II and class III genes, it is possible that other genes in the nearby region might be critical in the loss of immune-homeostasis that leads to development of GCA in the elderly.

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