Interferon-gamma gene microsatellite polymorphisms in patients with biopsy-proven giant cell arteritis and isolated polymyalgia rheumatica

M.A. Gonzalez-Gay[1], A.H. Hajeer[2], A. Dababneh[2], C. Garcia-Porrua[1], M.M. Amoli[2], J. Llorca[3], W.E.R. Ollier[2]

[1]*Division of Rheumatology, Hospital Xeral-Calde, Lugo, Spain;*
[2]*Centre for Integrated Genomic Medical Research, School of Epidemiology, and Health Sciences, University of Manchester, Manchester, United Kingdom;*
[3]*Department of Preventive Medicine and Public Health, University of Cantabria School of Medicine, Santander, Spain.*

Miguel A. Gonzalez-Gay MD, PhD; Carlos Garcia-Porrua, MD, PhD; Ali H. Hajeer, PhD, MRCPath; Adele Dababneh, PhD; Mahsa M. Amoli, MD, PhD; William E.R. Ollier, PhD, FRCPath; Javier Llorca, MD, PhD.

Please address correspondence and reprint requests to: Miguel A. González-Gay, MD, PhD, Rheumatology Division, Hospital Xeral-Calde, c) Dr. Ochoa s/n, 27004, Lugo, Spain.
E-mail: miguelaggay@hotmail.com

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ABSTRACT

Objectives. *Inflammatory cytokines are implicated in the pathogenesis of giant cell arteritis (GCA) and polymyalgia rheumatica (PMR). In this study we have examined the potential association of a CA repeat polymorphism in the first intron of the interferon gamma (IFN-γ) gene with disease susceptibility and clinical expression of these conditions.*

Methods. *Seventy-nine patients with isolated PMR, 59 biopsy-proven GCA patients and 129 ethnically matched controls from Lugo (NW Spain) were studied. Patients and controls were genotyped by molecular methods for the microsatellite dinucleotide (CA) repeat within the first intron of IFN-γ gene.*

Results. *No significant differences in the distribution of alleles for the IFN-γ gene polymorphism between GCA and isolated PMR patients and controls were found. However, the frequency of IFN-γ allele *4 (128 bp) was reduced in GCA patients (33.1%) compared with isolated PMR patients (46.2%). Also, GCA patients with visual ischemic manifestations exhibited a significantly reduced frequency of IFN-γ allele *4 compared with those without visual manifestations (17.9% versus 42.5%; p = 0.05 [OR: 0.36, 95% CI: 0.13 – 1.00]). Moreover, allele *3 (126 bp) was over-represented in the GCA patients with visual ischemic manifestations (71.4% versus 44.4% in the remaining GCA patients; p = 0.01 [OR = 3.13, 95% CI: 1.27–7.68]).*

Conclusions. *In GCA, INF-γ functional polymorphisms are associated with clinical manifestations of severity rather than susceptibility to this vasculitis.*

Introduction

Polymyalgia rheumatica (PMR) and giant cell arteritis (GCA) are common related diseases in the elderly (1, 2). PMR may occur alone as an isolated condition or in the setting of GCA (3). Inflammatory cytokines are implicated in the pathogenesis of both diseases. Interferon-gamma (IFN-γ) plays a role in the pathogenesis of GCA (4). In this regard, while IFN-γ is expressed in almost 70% of the temporal artery biopsy (TAB) samples from GCA patients it was not detected in TAB samples from patients with isolated PMR (4). High transcription of IFN-γ mRNA was associated with the formation of giant cells and with the evidence of cranial ischemic symptoms in GCA patients (5). The absence of IFN-γ expression in TAB samples from patients with isolated PMR suggests that its production may be crucial to the development of GCA(4).

To further investigate the role of IFN-γ in GCA and PMR, we have examined the potential association of a CArepeat polymorphism in the first intron of the IFN-γ gene with disease susceptibility and clinical expression in a series of unselected patients diagnosed with either biopsy-proven GCA or isolated PMR.

Patients and methods

Patients and controls

Seventy-nine patients with isolated PMR, 59 biopsy-proven GCA patients and 129 ethnically matched controls from Lugo (NW Spain) were studied.

Isolated PMR patients were included in this study if they fulfilled the following criteria: 1) severe and bilateral pain associated with morning stiffness (>30 minutes) for more than 1 month in at least 2 of the 3 areas: neck, shoulder,

and/or pelvic girdles; 2) erythrocyte sedimentation rate at the time of diagnosis of at least 40 mm/hour; 3) resolution of the syndrome in < 7 days following treatment with 10-20 mg/day of prednisone and 4) exclusion of other diseases that may present with polymyalgia manifestations (6). Patients with positive rheumatoid factor, clinical signs of GCA at the time of diagnosis or during the follow-up (at least 2 years) or a positive TAB were excluded from this category. The TAB procedure was performed as previously described (3). GCApatients were included in this study if they had a TAB showing infiltration of mononuclear cells into the arterial wall with or without giant cells. Visual ischemic complications were considered to be present if patients had at least one of the following: 1) Permanent visual loss (partial or complete permanent visual loss involvement related to GCA despite any possible improvement related to corticosteroid therapy), 2) Amaurosis fugax (transient visual loss that was followed by complete recovery or normal vision), or 3) Diplopia (related to palsy of extrinsic ocular muscles) (7). Biopsy-proven GCA patients were considered to have an associated diagnosis of PMR if they also had marked aching and stiffness bilaterally without other apparent cause in at least 2 of 3 regions: neck, shoulder girdle, and pelvic girdle (3, 6).

Molecular analysis of IFN-γ microsatellite gene polymorphism

DNA was obtained from EDTA anticoagulated blood samples using a phenol-chloroform extraction procedure.

A microsatellite dinucleotide (CA) repeat within the first intron of IFN-γ gene was analyzed. Ten ng DNA samples were amplified in 10 µl reaction, using the following primers: HEX-labelled Forward 5' AAA AGA TAG TTC CAA AC 3' and reverse 5' TTA TAATTATAG CTG TC 3'.

PCR reactions were carried out at 55ºC with 5 min denaturation followed by 35 cycles of 1 min at 95ºC, 1 min annealing, and 1 min extension at 72ºC and a final step of 5 min at 72ºC. PCR products were mixed with loading buffer and internal standard (Genescan 350) (Applied Biosystems, Foster City, CA), denatured at 95ºC for 2 min. Microsatellite alleles were visualized on 6% polyacrylamide gel using a 373 DNA sequencer (Applied Biosystems, Foster City, CA), Genescan and Genotyper software (Applied Biosystems, Foster City, CA). Microsatellite alleles were scored with reference to the internal standard and reference samples.

Statistical analysis

Strength of association between isolated PMR and GCA and alleles of the IFN-γ microsatellite polymorphism and between visual ischemic complications of GCA and IFN-γ polymorphism were estimated using odds ratios (OR) and 95% confidence intervals (CI). Chi-square or Fisher exact analyses were used. Statistical significance was defined as $p \leq 0.05$.

Results

No significant differences in the distribution of alleles for the IFN-γ polymorphism between GCA and isolated PMR patients and controls were found (Table I).

However, the frequency of IFN-γ allele *4 (128 base pairs [bp]) was reduced in GCA patients (33.1%) compared with that observed in isolated PMR patients (46.2%) (Table I).

When GCA patients were stratified by the presence (n= 30) or absence (n = 29) of PMR features no differences were found (data not shown). However, GCA patients with visual ischemic manifestations (n = 14) exhibited a significantly reduced frequency of IFN-γ allele *4 (128 bp) compared with the remaining patients without visual ischemic complications (17.9% versus 42.5%; p = 0.05 [OR: 0.36 (95% CI: 0.13–1.0)]) (Table II). Moreover, allele *3 (126 bp) was over-represented in GCA patients with visual ischemic manifestations (71.4% versus 44.4% in the remaining GCA patients; p = 0.01 [OR = 3.13 (95% CI: 1.27–7.68)]) (Table II).

Discussion

The DNAsequence of the human IFN-γ gene contains a variable-length CA repeat in the first intron of the gene. In a study conducted in the UK, Pravica *et al.* (8) assessed the allelic frequency of this microsatellite marker and its association with IFN-γ production in 164 unrelated healthy individuals. These authors specifically demonstrated a correlation between *in vitro* production of IFN-γ and this allele polymorphism (8). Their finding reinforces the potential functional role of this polymorphism.

IFN-γ gene microsatellite polymorphism has been implicated in the pathogenesis of autoimmune diseases such as Graves' disease and systemic lupus erythematosus (SLE) (9-11), in the acute and chronic kidney transplant outcome (12), and in the development of fibrosis in lung allograft and tuberculoid leprosy (13, 14).

Lee *et al*. genotyped 136 patients with SLE and 99 controls for the CA repeat in the first intron of the IFN-γ gene (10). In keeping with the observations from our series of GCA patients and

Table I. IFN-γ microsatellite allele distribution in biopsy-proven GCA, isolated PMR and controls[a].

Allele	Size in base pairs	Controls 2N = 288	GCA 2N = 118	Isolated PMR 2N = 158
*1	122	0 (0%)	0 (0%)	0 (0%)
*2	124	1 (0.3%)	1 (0.8%)	0 (0%)
*3	126	133 (46.2%)	60 (50.9%)	70 (44.3%)
*4	128	116 (40.3%)	39 (33.1%)	73 (46.2%)
*5	130	22 (7.6%)	9 (7.6%)	11 (7.0%)
*6	132	14 (4.9%)	8 (6.8%)	4 (2.5%)
*7	134	2 (0.7%)	1 (0.8%)	0 (0%)

[a]No significant differences in the distribution of alleles for the IFN-γ gene polymorphism between GCAand isolated PMR patients and controls were found.

Table II. IFN-γ microsatellite allele distribution in biopsy-proven GCA with or without visual ischemic complications.

Allele	Size in base pairs	GCAwith visual ischemic manifestations 2N = 28	GCAwithout visual ischemic manifestations 2N = 90
*1	122	0 (0%)	0 (0%)
*2	124	0 (0%)	1 (0.8%)
*3	126	20 (71.4%)[a]	40 (44.4%)[a]
*4	128	5 (17.9%)[b]	34 (42.5%)[b]
*5	130	2 (7.1%)	7 (7.8%)
*6	132	1 (3.6%)	7 (7.8%)
*7	134	0 (0%)	1 (1.1%)

[a]Allele *3 in GCAwith visual ischemic complications compared with GCA without visual ischemic complications: p = 0.01 (OR = 3.13 [95% CI: 1.27 – 7.68]).
[b]Allele *4 in GCA with visual ischemic complications compared with GCAwithout visual ischemic complications: p = 0.05 (OR: 0.36 [95% CI: 0.13 – 1.00]).

matched controls, these authors did not find statistically significant differences in the allele frequencies between SLE patients and controls, suggesting that these polymorphic variants do not influence disease susceptibility. However, in SLE patients different IFN-γ alleles were associated with specific features of the disease such as gastrointestinal or articular manifestations (10). More recently, Miyake *et al*. have confirmed that the IFN-γ gene is associated with the histological phenotype in SLE nephritis (11). This genetic polymorphism seems to be functional, as genotypic variations influence the level of IFN-γ and the percentage of IFN-γ–producing CD4 (+) T cells, which are closely related to the development of nephritis (11).

Weyand *et al*. found that TAB specimens from GCA patients with ocular ischemia expressed high amounts of IFN-γ mRNA, whereas those from GCApatients with fever had less IFN-γ mRNA (5). Thus, clinical correlates suggest a role of IFN-γ in the process of lumenal obstruction. By regulating giant cell formation IFN-γ could indirectly control intimal hyperplasia (15). According to these authors, IFN-γ may dictate the functional properties of other cell populations in vascular infiltrates and, by means of this mechanism, guide the response-to-injury reaction of the artery (15).

Our study indicates that the CA repeat polymorphism in the first intron of the IFN-γ gene may be associated with some differences between biopsy-proven GCA and isolated PMR. This is especially true for specific clinical manifestations of GCA such as visual ischemic complications. In this regard, the main finding of this work is the association between the 126 bp allele-allele 3 (high INF-γ producer) in GCA patients with visual ischemic manifestations, and the inverse correlation with the 128 bp allele-allele 4 (low INF-γ producer).

It makes sense biologically that IFN-γ functional polymorphisms in GCA would be associated with clinical manifestations of severity rather than with susceptibility to this vasculitis. Polymorphism in the IFN-γ gene could be involved in GCA by directly affecting IFN-γ production. Alternatively, it is possible that these alleles may be in linkage disequilibrium with alleles at other loci directly implicated in the regulation of IFN-γ production.

Further studies in different populations are required to confirm the implication of the CA repeat polymorphism in the first intron of the IFN-γ gene in the pathogenesis of GCA.

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