Corticotropin releasing hormone promoter polymorphisms in giant cell arteritis and polymyalgia rheumatica

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Abstract

Objective

Giant cell (temporal) arteritis (GCA) and polymyalgia rheumatica (PMR) are different but overlapping diseases of unknown etiology affecting the elderly. Corticotropin-releasing hormone (CRH) helps to regulate the immune response and maintain homeostasis during inflammatory stress. CRH promoter region polymorphisms in the 5' regulatory region of the CRH gene have been described. To investigate the possible implications of the CRH promoter polymorphisms in PMR and GCA susceptibility we have examined a series of patients with these conditions.

Methods

Sixty-two patients with biopsy-proven GCA, 86 patients with isolated PMR and 147 ethnically matched controls from the Lugo region of Northwest Spain were included in this study. Patients and controls were genotyped for CRH polymorphism in the 5' regulatory region of the gene at position 1273 (alleles A1 and A2) and at position 225 (alleles B1 and B2) by PCR-restriction fragment length polymorphism. Allele frequencies and genotype distribution were evaluated by the chi-square test.

Results

When GCA and PMR patients were examined for alleles and genotypes for each CRH polymorphism no significant differences in frequency were found compared with controls. A higher CRH-A2 allele frequency was observed in GCA patients with visual complications (21.4%) compared with controls (9.2%) and GCA cases without eye involvement [6.3%; p= 0.017, p_{corr} = 0.034, O.R: 4.1 (95% CI 1.2- 13.9)], although this was based on a small sample of patients with ischemic visual complications (n = 14) and should be interpreted with caution. No differences in CRH allele or genotype frequencies were observed in isolated PMR patients stratified by relapses and recurrence of disease symptoms.

Conclusion

Polymorphisms in the CRH gene regulatory region do not appear to be associated with increased susceptibility to PMR or GCA. The CRH-A2 allele may encode risk for the development of visual complications in GCA, although further studies to confirm this will be required.

Key words

Giant cell (temporal) arteritis, polymyalgia rheumatica, CRH, disease susceptibility, visual complications.

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Introduction

Giant cell (temporal) arteritis (GCA) and polymyalgia rheumatica (PMR) are different but overlapping diseases (1). Giant cell arteritis is a common vasculitic syndrome in Western countries that involves the large and middle-sized blood vessels with predisposition to the cranial arteries in the elderly (2). Polymyalgia rheumatica (PMR) is also a common syndrome in people over the age of 50. Symptoms consist of pain, aching and morning stiffness involving the neck, shoulder girdle, and hip girdle, which are generally associated with an elevated erythrocyte sedimentation rate (ESR) (3). In addition, PMR is observed in up to 50% of patients with GCA (2, 3). In this regard, PMR may represent the first manifestation of GCA. The development of GCA in patients adequately treated for a long period of time due to PMR symptoms has also been reported. Polymyalgia rheumatica, however, is sometimes an isolated condition unrelated to GCA (4).

The etiology of these overlapping conditions remains unknown. The incidence of both PMR and GCA is correlated with age. Thus, the incidence of GCA, with or without PMR, and isolated PMR is much higher in the age group ≥ 70 than in younger age groups (4, 5). This may be due, at least in part, to the natural decline in the levels of several hormones during aging (6). Piredda *et al.* have reported a reduced production of adrenal steroids in patients with PMR (7). Of note, these authors observed a negative correlation between the number of relapses and the duration of steroid therapy (7). The latter conclusion supports former clinical observations that described a direct correlation between the rate of steroid tapering in patients with isolated PMR and the development of relapses (4).

Corticotropin-releasing hormone (CRH) is produced by the hypothalamus and this process is stimulated by the action of inflammatory cytokines such as IL-1, IL-6 or TNF . CRH is the best activator of the hypothalamic-pituitary-adrenal (HPA) axis through stimulation of ACTH release. CRH regulates the immune response and helps to maintain homeostasis during inflammatory stress.

The chromosomal locus of the human CRH gene has been assigned to chromosome 8q13 by somatic cell hybrids and *in situ* hybridization studies (8). CRH promoter region polymorphisms in the 5' regulatory region of the CRH gene have been reported by restriction length fragment polymorphism (RLFP) in healthy controls and in rheumatoid arthritis (RA) patients (9). Polymorphisms in the CRH promoter might be implicated in the functioning of the HPA axis in PMR and GCA and thus they might play a role in the pathogenesis of these conditions. To investigate the possible implications of the CRH promoter polymorphisms in PMR and GCA, we have examined a series of patients with these conditions.

Patients and methods

Patients and controls

The study group comprised patients diagnosed with biopsy-proven GCA (n = 62; 30 men and 32 women; mean age 73.7 ± 6.5 years at the onset of the disease) or isolated PMR (n = 86; 35 men and 51 women; mean age 70.1 ± 7.3 years at the onset of the disease) in the Department of Medicine of the Hospital Xeral-Calde (Lugo, Spain). Thirty of the 62 biopsy-proven GCA patients also had PMR manifestations. Ethnically matched controls (n = 147) were from the same area. The Hospital Xeral-Calde provides medical care to a very specific area of the interior of Galicia in Northwest Spain (2, 10). This area has been isolated from the rest of Galicia and the rest of Spain for many centuries for geographic reasons. Due to this, special interest was paid in the study to these polymorphisms in the group of patients and controls whose families have lived in this area for several generations (11).

Inclusion criteria

Patients with GCA were 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. 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 due to GCA, despite any possible improvement after corticosteroid therapy); (2) amaurosis fugax (transient visual loss followed by complete recovery of normal vision); or 3) diplopia (related to palsy of extrinsic ocular muscles) (2).

Patients with PMR were included in this study if they met the following criteria: (a) aged 50 years or older; (b) severe, bilateral pain associated with morning stiffness (> 30 minutes) for more than 1 month in at least 2 out of 3 areas: neck, shoulder and/or pelvic girdles; (c) a rapid resolution of the syndrome in less than 7 days with low dose prednisone (10-20 mg/daily); (d) ESR at the time of diagnosis of 40 mm/1st hour or higher; and (e) the exclusion of other diseases apart from GCA that may present with polymyalgia manifestations or mimic PMR (4, 10,12). Furthermore, the possibility of GCA in patients with isolated PMR was excluded either by a negative temporal artery biopsy, which was generally performed in those patients with PMR who presented at the hospital with asthenia anorexia and a weight loss of at least 4 Kg and in those cases with ESR on admission of higher than 80 mm/1st hour (10), or by a resolution of the syndrome after low dose prednisone therapy and the absence of manifestations of GCA after a follow-up of at least 36 months. In addition, patients who at any time during the course of the disease were rheumatoid factor positive or fulfilled the 1987 ACR criteria for RA (13) were also excluded. Although classically more than 4 weeks are required for the diagnosis of PMR, we arbitrarily estimated two weeks for PMR in biopsy-proven GCA patients (4, 10).

Rheumatologists from Lugo diagnosed all the patients with isolated PMR reported in this study. For this reason they were uniformly treated and followed. At the time of diagnosis most patients with isolated PMR were initially treated with prednisone 15 mg/day. Reductions were individualized. A rate of 2.5 mg every 2 months or, more commonly, every 3 months was attempted. Relapses occurred when the dose of prednisone was low (generally lower than 5 mg/day) or when it had been discontinued (median 2.5 mg/day).

A relapse of PMR was considered to be present if, after attaining definite objective improvement with prednisone 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 (4, 10, 12). An isolated increase in the ESR was not considered sufficient to justify an increase in the corticosteroid dose. Relapses that occurred at least 1 year after steroid therapy had been discontinued were defined as recurrences of PMR (4). 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 CRH promoter polymorphisms

Bi-allelic polymorphism in the 5' regulatory region of the CRH gene characterized as a T C base substitution located at position 1273 (alleles A1 and A2) was examined. This substitution leads to the destruction of the recognition site for *Afl*III (position 1273) restriction enzyme (9). In addition, bi-allelic polymorphism in the 5¨flanking region of the CRH gene characterized as a T G base substitution located at position 225 (alleles B1 and B2) was also examined. This substitution leads to destruction of the recognition site for the *Xmn* I restriction enzyme (14). Polymorphisms in the CRH promoter region were examined using the following PCR primers:

CRH-A: Forward 5' GCT GTT CTT GTG ATA GTA AAT A 3'
Reverse 5'CCC CAG AGG AAG AGA AGC 3'
CRH-B: Forward 5' TGA AGG TAC AAG GTG ATA CAA G TG ACA A 3'
Reverse 5' ACA CAA ACT GAG GTG AAA AGA TGA A 3'

A total of 100 ng genomic DNA (5 l) was amplified in a 25 l PCR reaction containing 2.5 l KCl buffer containing 1.5 mM $MgCl_2$ for CRH-A, and 3.5 mM $MgCl_2$ for CRH-B, 2.5 l dNTP's (Bioline), 0.5 l of each primer for CRH-A and 1.0 l of each primer for CRH-B, 0.1 l Taq polymerase (Bioline), Betaine (4M) 6.0 l for CRH-A, and distilled water (7.9 l for CRH-A and 10.9 l for CRH-B). Thermal cycling was performed using a Hybaid OmniGene PCR machine. Cycles consisted of 10 minutes denaturation at 95° C followed by 35 rounds at 95°C for 1 minute each, annealing temperature of 52°C for 1 minute for CRH-A and 66° C for 1 minute for CRH-B, 72°C for 1 minute and a final extension at 72°C for 10 minutes. The presence of the product was verified on a 1% agarose

Table I. CRH-A and CRH-B marker allele and genotype frequencies in GCA and PMR*.

CRH	Controls	GCA	isolated PMR	PMR with or without GCA
Allele	2N=294	2N=124	2N=172	2N=232
A1	90.8	90.3	90.7	90.1
A2	9.2	9.7	9.3	9.9
Genotype	N=147	N=62	N=86	N=116
A1/A1	82.3	82.3	81.4	81.0
A1/A2	16.3	16.3	18.6	18.1
A2/A2	1.4	1.6	0	0.9
Allele	2N=284	2N=124	2N=172	2N=232
B1	94.7	95.2	95.3	95.3
B2	5.3	4.8	4.7	4.7
Genotype	N=142	N=62	N=86	N=116
B1/B1	89.4	90.3	90.7	90.5
B1/B2	10.6	9.7	9.3	9.5
B2/B2	0	0	0	0

* No statistically significant differences among the different groups were found.

gel stained with ethidium bromide. PCR products were digested with *Afl* III for CRH-A and *Xmn* I for CRH-B in a 10 l final volume. PCR products were incubated overnight at 37°C and the products of the digest were then visualized on a 4% agarose gel stained with ethidium bromide.

Statistical analysis

The strength of the association between GCA or PMR and alleles or genotypes of CRH-A and CRH-B were 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 association between GCA and PMR subgroups and CRH polymorphisms. Corrected p values (p_{corr}) by Bonferroni correction were performed. They were calculated by multiplying the p value by the number of alleles or genotypes compared. Statistical significance was defined as $p \leq 0.05$. Calculations were performed with the statistical package Stata V6.

Results

Allele and genotype frequencies of CRH-A and CRH-B polymorphisms in PMR and GCA

When GCA and PMR patients were examined for alleles and genotype distribution for each CRH polymorphism no significant differences in frequency were found compared with controls (Table I).

Allele and genotype frequencies of CRH polymorphisms in biopsy-proven GCA patients with or without ischemic visual complications

Allele A2 was increased in GCA patients with visual manifestations (6 of 28; 21.4%) compared to GCA patients without visual manifestations (6 of 96; 6.3%). The difference was statistically significant [p = 0.017; O.R: 4.1 (95% CI 1.2- 13.9)] (Table II). This difference was still statistically significant when p values were corrected by the number of CRH-A alleles compared (p_{corr} = 0.034) (Table II).

Four of the 14 (28.6%) GCA patients who had visual complications exhibited the A1/A2 genotype compared to only 6 of 48 (12.5%) without these complications. Of note, when GCA patients with visual manifestations who carried the A1/A2 or A2/A2 genotype were considered together (5 of 14; 35.7%) and compared with those GCA patients without visual manifestations carrying the same genotypes (6 of 48; 12.5%) a statistically significant difference was found (p = 0.045; O.R. = 3.8 (95% CI: 1.0-15.6(). However, this difference was not significant if Bonferroni's correction was applied.

No differences in the frequencies of alleles and genotypes for CRH-B polymorphism between GCA patients with or without visual manifestations were observed.

Influence of CRH polymorphisms on relapses or recurrences in isolated PMR

After a follow-up of at least 4 years none of the 86 patients with isolated PMR developed clinical manifestations of GCA. However, 18 of them had relapses and in another 3 there was a recurrence of the disease after at least 1 year since steroid therapy had been discontinued. No statistically significant differences in CRH-A or B alleles and genotypes were observed between these groups (Table III).

Discussion

In PMR a correlation between inflammatory cytokines and adrenal hormones has been observed (15). Straub *et al.* observed that in these patients the increase in IL-6 serum levels was associated with elevated serum levels of androstenedione (ASD) or dehydroepiandrosterone sulphate (DHEAS) and cortisol. However, the cortisol serum levels in PMR patients with and without corticosteroids were lower than expected under an inflammatory status related to the increased IL-6 (15). These findings indicated a change in the hypothalamic-pituitary-adrenal (HPA) axis responsiveness to inflammatory stimuli such as IL-6 at disease onset, before steroid therapy and during the course of the disease (15, 16). Also, these authors found a shift in bio-

Table II. CRH-A and CRH-B biallelic marker allele and genotype frequencies in biopsy-proven GCA patients with and without visual ischemic complications.

CHR	With visual complications	Without visual complications
Allele	2N=28	2N=96
A1	78.6	93.7
A2	21.4*	6.3*
Genotype	N=14	N=48
A1/A1	64.3	87.5
A1/A2	28.6	12.5
A2/A2	7.1	0
Allele	2N=28	2N=96
B1	96.4	94.8
B2	3.6	5.2
Genotype	N=14	N=48
B1/B1	92.9	89.6
B2/B2	0	0
B1/B2	7.1	10.4

*GCA patients with visual complications compared to those without: p= 0.017; p_{corr} = 0.034; OR = 4.1 (95% CI: 1.2 - 13.9).

synthesis to cortisol in relation to DHEAS or ASD in chronic disease (15, 16).

Genetic polymorphisms have been considered to be important candidate susceptibility factors for GCA and PMR. Among them, ICAM-1 (17), RANTES (18), and IL-1 receptor antagonist (19) gene polymorphisms seem to play a role in the pathogenesis of GCA and

Table III. CRH-A and CRH-B biallelic marker allele and genotype frequencies in isolated PMR patients with and without relapses or recurrences of the disease*.

CRH	With relapses/recurrences	Without relapses/recurrences
Allele	2N=42	2N=130
A1	85.7	92.3
A2	14.3	7.7
Genotype	N=21	N=65
A1/A1	71.4	84.6
A2/A2	0	0
A1/A2	28.6	15.4
Allele	2N=42	2N=130
B1	90.4	94.8
B2	9.5	5.2
Genotype	N=21	N=65
B1/B1	80.9	93.8
B2/B2	0	0
B1/B2	19.1	6.2

* No statistically significant differences between both groups were found

PMR in some populations.

CRH polymorphisms have been investigated in RA, a disease where diverse genetic and environmental factors are implicated in the development of an inflammatory response. The CRH-A2B1 haplotype was protective against the development of RA in UK Caucasoids (20). The CRH-A2B1 haplotype was also correlated with a later onset of disease in UK patients (20). In contrast, in South African blacks the CRH-A1B1 haplotype was associated with RA (20).

Giant cell arteritis is a good example of an association between vasculitis and genes that lie within the HLA-class II region and most studies have shown an association with HLA-DRB1*04 alleles (21). However, unlike PMR associated with GCA, which is mostly associated with HLA-DRB1*04, the HLA-class II genetic susceptibility to isolated PMR varies from one population to another (11). Of note, in the Caucasoid population of the Lugo region in Northwest Spain HLA-DRB1*04 is associated with disease incidence and severity in RA and GCA (2, 11, 22). In the present study no differences between GCA patients and controls were observed for CRH polymorphism. However, an association with the CRH allele A2 appeared to exist in those patients who developed ischemic visual complications. This was not the case for isolated PMR as in these patients only a non-significantly increased frequency of alleles A2 and B2 was observed in patients with relapses or recurrences of the disease.

To further investigate the possible role of the CRH-A bi-allelic polymorphism in the risk of visual manifestations, biopsy-proven GCA were stratified by HLA-DRB1*04 status. However, although the frequency of the CRH allele A2 was increased in HLA-DRB1*04 positive patients (7 of 26 patients carried the A2 allele [26.9%] versus 4 of 36 [12.5%] HLA-DRB1*04 negative patients) the difference was not statistically significant. Thus, HLA-DRB1*04 and CRH-A2 alleles might be independent risk factors for ischemic visual complications in GCA.

This possible contribution of CRH polymorphism to the susceptibility for visual ischemic complications in this population of biopsy-proven GCA patients from Northwest Spain is not yet understood. It is possible that CRH peptides encoded by specific CRH alleles may modulate either protection or susceptibility to ischemic phenomena in these patients. In this regard, ovine CRF has proved to be a selective mesenteric vasodilator in dogs (23). In rats CRH increases neuroendocrine changes induced by subarachnoid hemorrhage, which is believed to be related to activation of immune cells involved in the patomechanism of chronic vasoespasm (24). Also, simulated ischemia is lethal to primary cultures of neonatal rat myocytes (25). In these cases, the protective effect of exogenous urocortin in protecting cardiac myocytes from necrotic and apoptotic death induced by ischemia through the cardiac receptor CRH-R2 is abrogated by antagonists to the CRH family of peptides (25). Moreover, sublethal hypoxia in fetal hippocampal neurons *in vitro* yields the induction and expression of CRF-receptor 1. These investigations support a role of CRH peptides in the ischemic phenomena of various conditions (26). Thus, some CRH alleles may be implicated in the synthesis of specific CRH peptides that might be unable to protect GCA patients against the ischemia related to the inflammatory process associated with vasculitis. Further investigations on the role of CRH allelic polymorphisms in the susceptibility to ischemic manifestations GCA in other populations are necessary.

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