Association of CD24 Gene Polymorphisms with Susceptibility to Biopsy-Proven Giant Cell Arteritis

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ABSTRACT. ***Objective.*** To investigate the possible implication of *CD24* gene in the genetic predisposition to giant cell arteritis (GCA).

Methods. A total of 120 patients diagnosed with biopsy-proven GCA and 195 ethnically matched controls from the same region were studied. Two putative functional polymorphisms, a C to T coding polymorphism (rs8734) and a TG deletion in the 3' untranslated region (rs3838646) were used as *CD24* genetic markers and genotyped using a Taqman 5' allelic discrimination assay.

Results. The 2 genetic variants showed statistically significant differences between patients with GCA and controls. The strongest association was observed for the rs3838646 TG/del polymorphism, conferring on the "del" allele an increased risk of GCA genetic susceptibility (odds ratio 1.94, 95% confidence interval 1.15–3.27, p = 0.01). In addition, genotypes carrying the rs3838646 "del" allele showed an increased frequency among GCA patients compared to controls (OR 2.31, 95% CI 1.30–4.1, p = 0.003). For the rs8743, an increased frequency of Val/Val homozygous individuals in patients with GCA compared to controls (OR 6.08, 95% CI 1.50–24.63, p = 0.001) was observed. A high degree of linkage disequilibrium was estimated between the 2 polymorphisms (D' = 0.7) and the C/del haplotype was associated with an increased risk of GCA susceptibility (OR 2.10, 95% CI 1.23–3.60, p = 0.005), whereas the C/TG haplotype showed a protective effect (OR 0.63, 95% CI 0.45–0.87, p = 0.005).

Conclusion. Our results suggest a potential role for the *CD24* gene in the susceptibility to GCA in our population. (First Release Mar 15 2008; J Rheumatol 2008;35:850–4)

Key Indexing Terms:
GIANT CELL ARTERITIS DISEASE SUSCEPTIBILITY CD24 GENE POLYMORPHISMS

Giant cell temporal arteritis (GCA) is the most common vasculitis in Western countries in Caucasians over age 50 years[1,2]. It is characterized by the granulomatous involvement of large and medium-size blood vessels of the aorta, with predilection for the extracranial branches of the carotid artery[1,2].

Clinical manifestations of GCA are the result of inflammation of the arterial wall, leading to fragmentation of internal elastic laminae, intimal hyperplasia, and luminal occlusion[3]. Although polymyalgia rheumatica (PMR) is observed in up to 40%–50% of the patients with GCA[4], severe ischemic complications, especially blindness in the setting of ischemic optic neuropathy, are the most feared complications of this vasculitis[5].

GCA is a polygenic disease[6,7]. In keeping with data from other populations, GCA was associated with HLA-DRB1*04 alleles in the Lugo region of Northwestern Spain[8]. Moreover, a number of genes have been implicated in both susceptibility to[6,7,9] and severity of this vasculitis in Northwestern Spain[10,11].

CD24 is a cell-surface protein anchored to the plasma membrane by a glycosylphosphatidylinositol (GPI) linkage[12]. CD24 is expressed on a wide subset of immune cells including activated T cells, B cells, granulocytes, macrophages, and dendritic cells[13-16]. Although the exact biological function of CD24 is still unclear, this molecule has been involved in relevant physiological processes such as the B and T cell differentiation process and the activation of CD4 and CD8 T cells providing a CD28-independent costimulatory signal[15,17]. In addition, CD24 is implicated in the pathogenesis of cancer and inflammatory diseases due to its ability to bind for P-selectin, allowing tumor cell dissemination and lymphocyte recruitment to inflamed tissues[18,19]. To some extent, all these mechanisms may contribute to the pathogenesis of GCA, since GCA is an antigen-driven disease with local T cell and macrophage activation in the vessel wall, with an important role of proinflammatory cytokines[20,21].

Interestingly, 2 genetic variants of *CD24* gene, a C to T coding polymorphism leading to an alanine amino acid (Ala) to valine (Val) change at nucleotide 57 (rs8734), and a TG deletion in the 3'-untranslated region (UTR) (rs3838646), have been shown to be associated with different autoimmune conditions such as multiple sclerosis (MS) and systemic lupus erythematosus (SLE)[22-25]. In addition, both rs8734 and rs3838646 polymorphisms seem to have functional relevance,

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altering *CD24* expression levels and CD24 mRNA stability, respectively[22,24].

Taking into consideration the role of *CD24* polymorphisms in autoimmunity, we investigated the possible implication of *CD24* genetic variants in the genetic predisposition to GCA.

MATERIALS AND METHODS

Patients. A total of 120 patients diagnosed with biopsy-proven GCA in the Division of Rheumatology of the Hospital Xeral-Calde and 195 healthy controls were included in the study. The control population was matched by ethnicity, age, and sex with GCA patients. Patients and controls were of Spanish Caucasian origin from the Lugo region of Northwest Spain. All patients had 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. In addition, all of them met the 1990 American College of Rheumatology criteria for the classification of GCA[26].

Patients with GCA were considered to have an associated PMR if they had severe bilateral ache and pain involving the neck, the shoulder, or the pelvic girdles, associated with morning stiffness[27,28]. As described[29,30], patients were considered to have severe ischemic manifestations if they experienced at least 1 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. The main clinical features of this series of patients with biopsy-proven GCA are summarized in Table 1. Patients and controls in our study all gave written informed consent. We obtained approval for the study from the local ethics committee.

CD24 polymorphism genotyping. DNA was obtained from peripheral blood, using standard methods. Samples were genotyped for the *CD24* rs8734 and rs3838646 genetic variants using a Taqman 5' allelic discrimination assay[31]. For the rs8734 polymorphism the primer sequences were 5'-CCC AAA TCC AAC TAA TGC C-3', and 5'-TAA GAG TAG AGA TGC AGA AGA G-3', and the TaqMan minor groove binder (MGB) probe sequences were 5'-ACC AAG GCG GCT GGT GGT G-3', and 5'-ACC AAG GTG GCT GGT GGT G-3'; the probes were labelled with the fluorescent dyes FAM and JOE, respectively (Sigma-Aldrich, St. Louis, MO, USA). The polymerase chain reaction (PCR) was carried out in a total reaction volume of 10 µl as reported[25]. For the rs383646, primers and probes were provided by Custom-Taqman-SNP-Genotyping-Assay service (Applied Biosystems, Foster City, CA, USA). The primer sequences were 5'-GCT ATT CTG ATC CAT AGT TGT TTT TTA AAG AAA AAA AAG TA-3' and 5'-GAA GGC AAA AAT GTA AAG GAG TCA AAA CTA TAA-3', and the Taqman MGB probes were 5'-ATT AGC TTC CAG TCT TC-3' and 5'-CAA ATT AGC TTC GTC TTC-3'. The PCR was performed in a total volume of 10 µl with the following amplification protocol: denaturation at 92°C for 10 min, followed by 50 cycles of denaturation at 92°C for 15 s and annealing and extension at 58°C for 90 s. Post-PCR, the genotype of each sample was automatically attributed by measuring the allele-specific fluorescence in the ABI Prism 7500 Sequence Detection System, using SDS 1.2.3 software for allele discrimination (Applied Biosystems). To confirm the genotype obtained by TaqMan allelic discrimination assay, direct sequencing of selected samples of each genotype was performed using ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

To verify the genotyping consistency, the control population was genotyped twice for both rs8734 and rs3838646 genetic variant, showing 98% identical genotypes.

Since several CD24 homologs have been reported on chromosomes 6, 15, 20, and Y, the genotype of rs8734 and rs3838646 heterozygous individuals was verified using a specific PCR for the *CD24* 6q21 location before Taqman allelic discrimination assay, as described[22].

Statistical analysis. We tested Hardy-Weinberg equilibrium (HWE) for each genetic variant in our population by using the program Fineti (Wienker TF and Strom TM, unpublished data; available from: http://ihg.gsf.de/cgi-bin/hw/hwa2.pl). In the case of no departure from HWE, significance was calculated by 2×2 contingency tables and Fisher's exact test, to obtain p values, odds ratios (OR) and 95% confidence intervals (95% CI) by using Statcalc software (Epi Info 2002; Centers for Disease Control and Prevention, Atlanta, GA, USA). When the distribution of genotypes deviated from Hardy-Weinberg proportions we performed an Armitage trend test that considers the individual genotypes rather than alleles, implemented in the Fineti software (available from: http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Although it has been suggested that deviation from HWE increases the chance of a false-positive result, the Armitage trend test based on genotypes remains valid even in the case of departure from HWE[32,33].

Statistical significance was defined as p less than 0.05. Pair-wise linkage disequilibrium measures were investigated and haplotypes constructed using the expectation-maximization algorithm implemented in Haploview 3.32 software[34].

Table 1. Clinical features of 120 patients with biopsy-proven giant cell arteritis from the Lugo region of northwest Spain.

Characteristic	Variable
Age at the time of diagnosis, yrs, mean ± SD, median (range)	75 ± 6, 75, (60–92)
Women/men	69/51
Women, %	57.5
Headache	104 (87)
Abnormal temporal artery on examination	90 (75)
Polymyalgia rheumatica	46 (38)
Jaw claudication	46 (38)
Visual manifestations*	26 (22)
Permanent visual loss	12 (10)
Stroke	3 (2)
Arm claudication due to ischemia of the humeral artery	1 (1)
Severe ischemic manifestations**	60 (50)
ESR > 40 mm/h	120 (100)

Values are n (%) unless otherwise indicated. * Transient visual loss including amaurosis fugax, permanent visual loss, or diplopia. ** At least one of the following features: visual manifestations, cerebrovascular accidents (stroke and/or transient ischemic attacks), jaw claudication, or limb claudication of recent onset.

RESULTS

The distribution of genotypic and allelic frequencies of *CD24* rs8734 and rs3838646 polymorphisms in patients with GCA and healthy controls is shown in Table 2. Interestingly, the 2 genetic variants showed statistically significant differences between patients with GCA and controls, considering either allelic or genotypic frequencies.

The strongest association was observed for the rs3838646 TG/del polymorphism, conferring the "del" allele with an increased risk to GCA genetic susceptibility (OR 1.94, 95% CI 1.15–3.27, p = 0.01), whereas the TG allele was associated with a significant protective effect against GCA susceptibility (OR 0.51, 95% CI 0.30–0.86, p = 0.01; Table 2). In addition, genotypes carrying the rs3838646 "del" allele showed an increased frequency among patients with GCA compared to controls (OR 2.31, 95% CI 1.30–4.1, p = 0.003; Table 2).

Regarding the *CD24* rs8734 genetic variant, we observed that the control population was not in agreement with HWE.

Table 2. Distribution of *CD24* rs8734 (A57V) and rs3838646 (P1527 TG/del) polymorphisms in patients with giant cell arteritis (GCA) and controls.

CD24 Polymorphisms	Patients with GCA n = 120 (%)	Controls, n = 185 (%)	p	OR (95% CI)
rs8734				
(A57V)				
Genotype				
Ala/Ala	53 (44.2)	93 (50.3)	0.29	1.28 (0.78–2.08)
Ala/Val	57 (47.5)	90 (48.6)	0.84	0.96 (0.60–1.51)
Val/Val	10 (8.3)	2 (1.1)	0.001	6.08 (1.50–24.63)
Ala/Val + Val/Val	67 (55.8)	92 (49.7)	0.29	0.78 (0.48–1.27)
Allele*				
Ala	163 (67.9)	276 (74.6)	0.07	0.72 (0.50–1.03)
Val	77 (32.1)	94 (25.4)	0.07	1.39 (0.97–1.98)
rs3838646				
(P1527 TG/del)	n = 119 (%)	n = 185 (%)		
Genotype				
TG/TG	86 (72.3)	159 (86.0)	0.003	0.43 (0.31–0.87)
TG/del	32 (26.9)	23 (12.4)	0.001	2.55 (1.41–4.60)
del/del	1 (0.8)	3 (1.6)	0.56	0.77 (0.11–5.28)
del/del + TG/del**	33 (27.7)	26 (13.5)	0.003	2.31 (1.30–4.10)
Allele				
TG	206 (86.6)	341 (92.2)	0.01	0.51 (0.30–0.86)
del	34 (13.4)	29 (7.8)	0.01	1.94 (1.15–3.27)

* Armitage's trend test p = 0.05. ** del/del + TG/del versus TG/TG. OR: odds ratio; CI: confidence interval.

Therefore, as described above, we applied an appropriate statistical test for this case, and no statistically significant deviation of rs8734 allelic frequencies between patients with GCA and controls was found (Armitage's trend test p = 0.05; Table 2). However, we observed a statistically significant increased frequency of *CD24* rs8734 Val/Val homozygous individuals in the GCA group (OR 6.08, 95% CI 1.50–24.63, p = 0.001; Table 2).

In addition to the single-marker analysis we performed a *CD24* haplotype analysis (Table 3). A high degree of linkage disequilibrium was observed between rs8734 and rs3838646 polymorphisms both in patients with GCA and in the controls (D' = 0.7). The C/del haplotype was associated with an increased risk of GCA susceptibility (OR 2.10, 95% CI 1.23–3.60, p = 0.005) and the C/TG haplotype with a protective effect (OR 0.63, 95% CI 0.45–0.87, p = 0.005), in accord with that found in the single-marker analysis (Table 3).

The possible implication of *CD24* gene in the clinical spectrum of the disease was investigated further. However, no statistically significant differences in the distribution of *CD24* rs8734 and rs3838646 alleles, genotypes, or haplotypes were observed after stratification of patients with GCA by the presence of PMR, severe ischemic complications, or visual ischemic manifestations (data not shown).

Table 3. Distribution of *CD24* haplotypes in patients with giant cell arteritis and controls.

Haplotype rs8734/rs3838646	GCA, 2n = 268, %	Controls, 2n = 432, %	p	OR (95% CI)
Ala/TG	0.56	0.64	0.005	0.63 (0.45–0.87)
Val/TG	0.30	0.25	0.23	1.24 (0.87–1.76)
Ala/del	0.13	0.09	0.005	2.10 (1.23–3.60)

DISCUSSION

Our study constitutes the first attempt to establish the potential role of CD24 gene polymorphisms in GCA.

Based on a large series of biopsy-proven cases, our analyses showed that a dinucleotide deletion at position rs3838646 in the *CD24* 3'-UTR is associated with a significantly increased risk for susceptibility to GCA. According to our results, a destabilizing dinucleotide deletion in the 3'-UTR of the *CD24* gene confers a significant increased risk for GCA. In this regard, Spanish individuals with GCA were twice as likely to have this dinucleotide deletion as controls (OR 2.31). However, this finding is in contrast to a recent report showing that the TG deletion in the 3'-UTR of the *CD24* gene confers a significant protective effect against the risk of and progression of MS and SLE[22]. A possible explanation for this lack of concordance might be due to differences in the genetic background between the 2 populations. In this regard, we observed a high degree of linkage disequilibrium between rs8734 and rs3838646 polymorphisms in our population, whereas these 2 polymorphisms seem to be independent in the North American population. Thus, it is possible that differences in haplotype structures of the *CD24* gene across populations might exist. Due to these differences, alleles and single-nucleotide polymorphisms associated with inflammatory diseases might be in different haplotypes depending on the spe-

cific population. In addition, it might be possible that the implication of *CD24* gene could not be the same in all autoimmune diseases. The exact role of CD24 in immune system regulation is unclear. CD24 functions as an important regulator of B and T cell development and selection[17,35]. In addition, CD24 exerts an important role in T cell activation, providing a costimulatory signal[14,15]. Therefore, it might be speculated that the rs3838646 deletion allele, associated with lower CD24 mRNA levels and stability, could exert protective or deleterious effects in different situations. Thus, in the case of GCA the lower *CD24* mRNA levels associated with the deletion allele might alter the apoptosis in the B and T cell selection process, allowing release of potential autoreactive cells; whereas in other disease states, lower CD24 levels could act in a protective way, inhibiting T cell activation. It is known that GCA is a cell-mediated process. Unlike SLE, in GCA the inflammatory infiltrate in affected vessels is predominantly composed of lymphocytes and macrophages, and also frequently contains multinucleated giant cells. Also, most involved lymphocytes are CD4-positive T cells[3].

We also observed that the frequency of the rs8734 Val/Val genotype in Spanish patients with GCA was significantly increased compared to the controls. A potential dose-dependent effect of the rs8734 polymorphism in predisposition to GCA was found, since homozygosity for the Val allele confers a 7-fold increased risk of GCA (OR 6.08). Similarly, the rs8734 Val allele is associated in Spanish individuals with susceptibility to SLE and MS[23,25]. However, the association of rs8734 polymorphism with GCA should be considered with caution, since a departure from HWE for this genetic variant was observed in the control population.

Several factors can cause departure from HWE such as genotyping errors, chance, and failure of assumptions underlying HWE expectations, or the underlying genetic disease model[36]. We discarded the possibility of genotyping errors since all controls were genotyped twice for this polymorphism and 98% genotyping accuracy was observed. In our control population there was a markedly decreased frequency of Val/Val (1.1%), causing deviation of HWE. In agreement with this observation, an independent control population from another region of Northern Spain showed a similar distribution of genotypes (Val/Val 1.4%)[23]. Therefore, it is plausible that factors such as inbreeding, nonrandom mating, or migration might affect the segregation of rs8734 polymorphisms in populations from Northern Spain. In addition, the Spanish population shows a decreased frequency of the Val/Val genotype compared with control North European populations (4.3% vs 5.7% in German, 8.9% in Swedish, and 12% in UK individuals)[25,37]. It seems that a north-south gradient of different geographic origins may affect the distribution of the *CD24* rs8734 genotypes. This is a common effect that has been reported for several other genetic variants[38].

Our results suggest a potential role for the *CD24* gene in the susceptibility to GCA. The different results in terms of *CD24* gene polymorphism association between different autoimmune diseases support the notion that different pathogenic mechanisms are involved in the development of polygenic diseases like GCA.

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