**RANTES gene polymorphism in polymyalgia rheumatica, giant cell arteritis and rheumatoid arthritis**

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**ABSTRACT**

**Objective**

To investigate whether a biallelic polymorphism (A or G) occurring within the promoter region of the RANTES gene (position-403) is associated with polymyalgia rheumatica (PMR), giant cell arteritis (GCA) and rheumatoid arthritis (RA).

**Methods**

A PCR-RFLP method was used to genotype cases and controls for this polymorphism. 3 groups of patients were examined; these comprised GCA patients who did not exhibit features of PMR (n = 30), PMR patients who did not exhibit features of GCA (n = 53) and RA patients (n = 99). All patients and controls (n = 65) originated from the area surrounding Lugo, Galicia, NW Spain.

**Results**

A significant increase in the frequency of allele A was found in PMR patients compared with normal controls. A marginal increase of this allele frequency was observed in RA but not in GCA patients.

**Conclusion**

This is the first report of an association of a RANTES gene polymorphisms with PMR and RA. Our data suggest a possible role for RANTES in the development of both PMR and RA.

**Introduction**

Polymyalgia rheumatica (PMR), is a rheumatic disorder which predominantly affects the elderly. PMR is associated with moderate to severe muscle pain and stiffness in the neck, shoulder and hip area (1). The cause of PMR is not known, however, possibilities include immune system abnormalities and genetic factors. Giant cell arthritis (GCA), also known as temporal arthritis, is a disorder that results in the swelling and inflammation of arteries in the head, neck and arms. PMR and GCA often coexist in patients and it is reported that 50% of GCA patients develop PMR and 5-10% of PMR patients develop GCA (1-3). Several studies suggest that the immune system is involved in the pathology of these conditions. Elevated levels of soluble interleukin-2 receptor (IL-2R) (4), interleukin-6 (IL-6) (5), circulating intercellular adhesion molecule-1 (ICAM) (6), and soluble CD8 (6), and a decreased percentage of circulating CD8+ lymphocytes (7, 8) have all been found in PMR patients during the active stage of the disease. RANTES (regulated upon activation normal T-cell expressed and secreted) is a potent chemotactic factor for monocytes, CD45RO+ memory T-cells, basophils, eosinophils and mast cells (12, 13). A recent study demonstrated elevated serum concentrations of the chemokine RANTES in patients with PMR (9). In addition, there is increasing evidence to suggest the a role for RANTES in the pathogenesis of RA (10, 11).

Recently, we have identified a novel polymorphism in the human RANTES gene promoter; a single base change was identified at position - 403 (G → A) (14). This a frequent polymorphism with a heterozygosity of 48% in UK Caucasoid controls, thus making it a suitable marker for association studies of RANTES with disease.

We have examined the polymorphism at position - 403 in the RANTES gene promoter in clinically well characterised PMR, GCA and RA patients and compared these with an ethnically matched control group.

**Methods**

**Study subjects**

All patients and controls originated from the area surrounding Lugo, Galicia, NW Spain. Controls (n = 65) were ethnically matched to the cases and judged to be healthy at the time of venepuncture. All patients were referred to the Xeral-Calde Hospital of Lugo by general practitioners or were self referred to the emergency unit. The main characteristics of the patients are described in Table I, other clinical details have been described in detail elsewhere (15). Three groups of patients were examined; these comprised GCA patients who did not exhibit features of

<table>
<thead>
<tr>
<th>Patient group</th>
<th>N</th>
<th>F:M</th>
<th>Mean age at onset ± SD</th>
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<tbody>
<tr>
<td>RA</td>
<td>99</td>
<td>67:32</td>
<td>48.9 ± 13.4</td>
</tr>
<tr>
<td>GCA+/PMR-</td>
<td>30</td>
<td>15:15</td>
<td>73.4 ± 6.8</td>
</tr>
<tr>
<td>PMR+/GCA-</td>
<td>53</td>
<td>28:25</td>
<td>69.9 ± 7.0</td>
</tr>
</tbody>
</table>

Table I. Clinical details of patient groups.
PMR (n = 30), PMR patients who did not exhibit features of GCA (n = 53) and RA patients (n = 99). For isolated GCA patients, the average age at onset was 73.4 years with an equal female to male ratio. All patients underwent biopsy examination. A biopsy was considered positive when there was interruption of the internal elastic laminae with infiltration of mononuclear cells into the arterial wall with or without the presence of giant cells. No GCA patient exhibited clinical features of PMR. For isolated PMR patients, the average age at onset was 69.9 years, with a slight excess of female patients. All patients fulfilled the following criteria: (i) more than 50 years age at disease onset; (ii) severe pain for more than one month in at least 2 of 3 areas (neck, shoulder, pelvic girdle); (iii) resolution of the syndrome in less than 7 days following low dose treatment with prednisone (10-20 mg/daily); and (iv) an ESR of ≥40 mm/first hour at the time of diagnosis. The possibility of GCA in the isolated PMR patients was excluded by either a negative temporal artery biopsy or by resolution of the syndrome following low dose steroid treatment and absence of GCA manifestations after a follow up of 18 months.

For RA patients, the mean age at disease onset was 48.9 years. The female: male ratio was approximately 2:1. All RA patients satisfied the 1987 ACR diagnostic criteria (16).

**RANTES genotyping**

The details of the PCR RFLP have been described elsewhere (14). Briefly, the following primers were used to amplify the promoter region of RANTES gene: forward 5’ GCC TCA ATT TAC AGT GTG 3’ and reverse 5’ TGC TTA TTC ATT ACA GAT GTT3’. PCR reactions were carried out in a 25 µl final volume, containing 1x NH4 buffer (Bioline), 1.5 mM Mgs2+, 0.2 mM dNTPs (Bioline), 6.3 pmol of each primer, 1 unit of Taq polymerase (Bioline) and 1 mM Betaine (Sigma). PCR cycles were as follows: 95°C for 2 min followed by 35 cycles each of 95°C for 40 sec, 50°C for 40 sec and 72°C for 40 sec. A final extension step was carried out at 72°C for 5 min. PCR products were digested with Mae III enzyme in a 15 µl final volume using 4 units of Mae III enzyme and 5 µl of PCR product. The reactions were incubated at 55°C overnight. The wild type allele (G) yields two bands of 112 and 23 bp while the mutant allele (A) yields one uncut band (135 bp).

**Results**

Genotype and allele frequencies in patients and controls are summarised in Table II. The frequency of allele A was significantly increased in PMR patients versus controls (OR = 2.2 95%, CI 1.0-4.8, p = 0.03). The A allele frequency was also marginally increased in the RA patient group (OR-1.8, p = 0.07) compared with controls. No significant differences in allele frequencies were observed in GCA patients compared with controls. Individuals homozygous for the A allele were detected among the RA and PMR patients, but none were observed among the GCA patients or controls. A difference in the genotype distribution was observed between PMR patients and controls, but this did not reach statistical significance (Fisher’s exact p = 0.09). Similar findings were observed for RA patients, but again this did not reach statistical significance.

**Discussion**

In the present study, we have demonstrated a weak association between RANTES gene polymorphism and PMR. This may also extend to RA, but this was not apparent with GCA. In both PMR and RA patients the A allele frequency was increased, this being a reflection of the increased AA and GA genotype frequencies.

High levels of RANTES chemokine have been demonstrated in the blood of both PMR and RA patients (9, 17). In PMR patients, the levels of RANTES returned to normal following treatment with corticosteroids (9), thus suggesting a role for RANTES in the pathogenesis of the disease. Increased levels of RANTES in GCA patients have not as yet been reported.

RANTES is expressed immediately after stimulation of different cells including fibroblasts and macrophages, but its release is delayed in activated T cells (3-5 days post-activation) (18). Corticosteroids are known potent T cell inhibitors and were shown recently to inhibit the release of RANTES by human bronchial epithelial cells (19). Treatment of PMR patients with corticosteroids might inhibit the release of RANTES by T cells and any other cell type. The direct effect of RANTES in the pathology of PMR is not fully understood, but it could possibly play a role for RANTES in the recruitment of T cells and other inflammatory cells to the site of inflammation. A similar role may apply in RA where RANTES is abundant in the synovial tissues and is believed to play a major role in the inflammatory process within the synovial tissues (10, 11).

In our PMR patients, synovitis was quite rare (20) and it was difficult to conclude any associations with the RANTES gene polymorphisms. In addition, analysis of our data in PMR and GCA according to HLA-DRB1 types and RANTES gene polymorphisms did not show any statistically significant associations (data not shown).

Our results suggest a possible role for RANTES gene regulation in the development of PMR and possession of the A allele at position -403 may be a risk factor for the development of the disease. This is the first report on the association

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**Table II.** Genotype and allele frequencies of the RANTES gene polymorphism in PMR, GCA and RA.

<table>
<thead>
<tr>
<th></th>
<th>Genotype frequency</th>
<th>Allele frequency</th>
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<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GA</td>
</tr>
<tr>
<td>Control (n = 65)</td>
<td>0.77</td>
<td>0.23</td>
</tr>
<tr>
<td>RA (n = 99)</td>
<td>0.69</td>
<td>0.26</td>
</tr>
<tr>
<td>PMR (n = 53)</td>
<td>0.64</td>
<td>0.30</td>
</tr>
<tr>
<td>GCA (n = 30)</td>
<td>0.77</td>
<td>0.23</td>
</tr>
</tbody>
</table>

* OR = 2.2; 95% CI (1.0 - 4.8), p = 0.03.
of RANTES gene polymorphisms in PMR patients. Further studies will be required to investigate this polymorphism in other populations and functional studies are underway to investigate importance of this polymorphism in RANTES gene transcription.

References