

NPSR1 Gene Is Associated with Reduced Risk of Rheumatoid Arthritis

GEMA ROBLEDO, MIGUEL ANGEL GONZÁLEZ-GAY, BENJAMÍN FERNÁNDEZ-GUTIÉRREZ, JOSÉ RAMÓN LAMAS, ALEJANDRO BALSAL, DORA PASCUAL-SALCEDO, SANTOS CASTAÑEDA, RICARDO BLANCO, ISIDORO GONZÁLEZ-ALVARO, ANTONIO GARCÍA, ENRIQUE RAYA, CARMEN GÓMEZ-VAQUERO, MARIO DELGADO, and JAVIER MARTÍN

ABSTRACT. *Objective.* Neuropeptide S receptor 1 (*NPSR1*) is a G protein-coupled receptor involved in immune response and is associated with several inflammatory diseases. We investigated the possible contribution of several polymorphisms in the intronic region of *NPSR1* to rheumatoid arthritis (RA).

Methods. Genotyping of 7 single-nucleotide polymorphisms (SNP) was performed in a total of 1232 patients with RA and 983 healthy controls of Spanish white origin by real-time polymerase chain reaction technology, using the TaqMan 5'-allele discrimination assay.

Results. One out of the 7 SNP analyzed (rs740347) was associated with RA [*p* after Bonferroni correction ($p_{\text{BNF}} = 1.2 \times 10^{-3}$, OR 0.73)]. An association was also observed with rheumatoid factor-positive and shared epitope-positive RA ($p_{\text{BNF}} = 0.011$, OR 0.73; $p_{\text{BNF}} = 0.037$, OR 0.75, respectively).

Conclusion. Our results show that variations in the *NPSR1* intronic region are associated with low risk in patients with RA, supporting other evidence that this locus represents a common genetic factor in inflammatory diseases. (J Rheumatol First Release May 1 2012; doi:10.3899/jrheum.111205)

Key Indexing Terms:

RHEUMATOID ARTHRITIS *NPSR1* RHEUMATOID FACTOR SHARED EPITOPE
ANTICITRULLINATED PROTEIN ANTIBODIES SINGLE-NUCLEOTIDE POLYMORPHISM

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease, characterized by inflammation of synovial joints and pain affecting approximately 1% of the population worldwide.

From the Instituto de Parasitología y Biomedicina López-Neyra, CSIC, Granada; Department of Rheumatology, Hospital Universitario Marqués de Valdecilla, IFIMAV, Santander; Department of Rheumatology, Hospital Clínico San Carlos, Madrid; Department of Rheumatology, Hospital Universitario La Paz, Madrid; Department of Rheumatology, Hospital Universitario La Princesa, Madrid; Department of Rheumatology, Hospital Virgen de las Nieves, Granada; Department of Rheumatology, Hospital Universitario Clínico San Cecilio, Granada; and Department of Rheumatology, Hospital Universitario de Bellvitge, IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain.

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G. Robledo, PhD; M. Delgado, PhD; J. Martín, MD, PhD, Instituto de Parasitología y Biomedicina López-Neyra, CSIC; M.A. González-Gay, MD, PhD; R. Blanco, MD, PhD, Department of Rheumatology, Hospital Universitario Marqués de Valdecilla, IFIMAV; B. Fernández-Gutiérrez, MD, PhD; J.R. Lamas, PhD, Department of Rheumatology, Hospital Clínico San Carlos; A. Balsa, MD, PhD; D. Pascual-Salcedo, PhD, Department of Rheumatology, Hospital Universitario La Paz; S. Castañeda, MD, PhD; I. González-Alvaro, MD, PhD, Department of Rheumatology, Hospital Universitario La Princesa; A. García, MD, PhD, Department of Rheumatology, Hospital Virgen de las Nieves; E. Raya, MD, PhD, Department of Rheumatology, Hospital Universitario Clínico San Cecilio; C. Gómez-Vaquero, MD, PhD, Department of Rheumatology, Hospital Universitario de Bellvitge, IDIBELL, L'Hospitalet del Llobregat.

Address correspondence to Dr. J. Martín, Instituto de Parasitología y Biomedicina López-Neyra, CSIC, Parque Tecnológico de Ciencias de la Salud, Avenida del Conocimiento s/n 18100, Armilla, Granada, Spain. E-mail: martin@ipb.csic.es

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Both genetic and environmental factors seem to be involved in its complex etiology, although a strong genetic component has been described^{1,2}.

Neuropeptide S receptor 1 (*NPSR1*), located in chromosome 7p, is a G-protein coupled receptor gene involved in leukocyte immune response³. Several studies have shown that *NPSR1* expression is upregulated in inflammatory processes such as asthma, inflammatory bowel disease (IBD), and intestinal and cutaneous inflammatory diseases^{4,5,6}, suggesting a main role of this gene in inflammation.

We analyzed 7 variations along the *NPSR1* gene in RA patients and controls from Spain. Five of them (rs323917, rs323922, rs324377, rs324396, and rs740347) are located at the region between exons 2 and 3 of the *NPSR1* gene and have been previously published as conferring genetic risk for diseases that present chronic inflammation including asthma^{4,7,8} and IBD^{5,9}. Further, 2 additional single-nucleotide polymorphisms (SNP) (rs324987 and rs10263447) located in introns 3 and 4, respectively, were included in this study. Recently, these variations were reported to be associated with anticitrullinated protein antibody (ACPA)-negative RA and with disease activity in a Swedish population¹⁰.

MATERIALS AND METHODS

Patients. Our case-control study consisted of 1232 Spanish white patients with RA who met the American College of Rheumatology revised criteria for RA¹¹ and 983 blood donors. All samples were matched by age and sex.

In our cohort 73.1% of the samples were characterized for rheumatoid factor (RF), 65.4% for shared epitope (SE), and 63.2% for ACPA. Of these

patients, 67.6% were positive for RF, 65.4% encoded the SE, and 59.3% were seropositive for ACPA. All patients and controls were enrolled with local ethics committee approval and provided their informed consent.

NPSRI SNP selection and genotyping. For genotyping, genomic DNA from patients and controls was isolated from peripheral white blood cells, using standard procedures. Seven SNP (dbSNP rs323917, rs323922, rs324377, rs324396, rs740347, rs324987, and rs10263447) localized in the *NPSRI* gene were selected for investigation. Samples were genotyped using TaqMan® 5' allelic discrimination Assay-By-Design method. The genotype call rate for every SNP was > 94.5% in controls and > 93.9% in patients.

Statistical analysis. All statistical analyses of the allele frequencies were carried out using the Linux software PLINK v1.07 (<http://pngu.mgh.harvard.edu/purcell/plink/>). To test for association, 2×2 contingency tables and chi-square and/or Fisher's exact tests, when appropriate, were used to calculate *p* values, OR, and 95% CI. Allelic combinations were analyzed using Haploview (V.4.2) and Unphased.

RESULTS

We performed single-point association analysis of 7 representative SNP, along the intronic region of *NPSRI* (for details, see Appendix). All the SNP showed allele frequencies similar to those previously reported in European populations^{4,5}. In a first step, we analyzed the possible implication of these SNP in RA susceptibility (Table 1, upper rows). We found an association for rs740347. The minor allele (C) was significantly decreased in RA patients compared to healthy controls [*p* after Bonferroni correction ($p_{\text{BNF}} = 1.2\text{E-}03$; OR 0.73)].

To assess the possible implications of these 7 SNP for RA severity, we performed a stratification analysis for the presence of RF, SE alleles, and ACPA, when available (Table 1, lower rows). We found an association when comparing patients positive for RF or SE with controls for the *NPSRI* rs740347: RF ($p_{\text{BNF}} = 0.011$; OR 0.73), SE ($p_{\text{BNF}} = 0.037$; OR 0.75). When we compared patients negative for RF, SE, or ACPA with controls, no association was observed for any of the 7 SNP (data not shown). Additionally, we compared RA patients positive for RF, SE, and ACPA with RA patients negative for each (Table 2). No association was detected for any of the analyzed SNP. This suggests that rs740347 is not associated with a clinical phenotype but with the global disease.

Table 3 shows allelic combination tests performed to analyze the possible interaction between the SNP of this genomic region. We found 3 different allelic combinations associated with RA susceptibility (CGCCGGC and CGCTGTG) or protection (CCACCGC).

DISCUSSION

NPSRI gene seems to play a role in asthma^{7,8}, IBD^{5,9}, and RA¹⁰ in independent studies with ethnically different populations.

One of the 7 SNP analyzed in this study, rs740347, showed strong evidence of association with RA after correction for multiple comparisons. The rs740347 C allele conferred a protective effect on the global disease. On the other hand, the allelic combination analysis of *NPSRI* variants suggests that the genomic region of this gene plays an important role in RA association.

The presence of positive RF, ACPA, and HLA-DRB1 alleles encoding a conserved sequence at amino acid positions 70–74, commonly known as the SE, has been associated with susceptibility and severity of RA^{12,13}. We performed a stratification comparing patients with RA who were positive and negative for SE, RF, and ACPA with each other and with controls. We found an association for rs740347 comparing RF- and SE-positive patients with controls but not with RF- and SE-negative patients. It may be speculated that rs740347 is linked to the global disease and not to a subtype. The minor allele (C) of this SNP was previously seen to confer susceptibility to asthma in German and Italian populations^{7,14}, but showed a low risk for susceptibility in a Colombian cohort¹⁵, similar to our results. Additionally, D'Amato, *et al* analyzed 19 *NPSRI* polymorphisms in a RA cohort from Sweden¹⁰. However, none of the SNP analyzed showed association with the global disease. Population genetic variability between the 2 cohorts or interactions with other genes may be responsible for the lack of coincidence in the results. This group showed that rs324987 and rs10263447 were associated with ACPA-negative RA and disease activity. In our population these 2 SNP showed no evidence of association with the disease or with any of the subgroups analyzed.

Intronic position of the studied SNP suggests a possible involvement of associated polymorphism in regulation of splicing and protein isoform production. Variations in rs740347 may downregulate the function of *NPSRI* in immunomodulation.

Our results provide evidence of involvement of *NPSRI* in the genetic contribution for RA association in a Spanish population. Other replication studies and functional analyses are necessary to better elucidate its role in chronic inflammatory diseases.

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Table 1. Genotype and minor allele frequencies of SNP located in the *NPSRI* gene in patients with rheumatoid arthritis and healthy controls from Spain, according to specific characteristics of the disease. Values shown in bold type indicate statistical significance.

| SNP | 1/2 | Subgroup (N) | Genotype, n (%) | | | MAF (%) | p * | p _{Bonf} ** | Allele Test | |
|------------|-----|--------------------|-----------------|-------------|-------------|---------|----------------|----------------------|------------------|--|
| | | | 1/1 | 1/2 | 2/2 | | | | OR (CI 95%]**** | |
| rs323917 | C/G | Controls (n = 945) | 859 (90.90) | 78 (8.25) | 8 (0.85) | 4.97 | | | | |
| | | RA (n = 1166) | 1041 (89.28) | 116 (9.97) | 9 (0.75) | 5.75 | 0.269 | 0.999 | 1.17 (0.88-1.55) | |
| | | RF+ (n = 584) | 512 (87.67) | 67 (11.47) | 5 (0.86) | 6.59 | 0.057 | 0.399 | 1.36 (0.99-1.86) | |
| | | SE+ (n = 508) | 453 (89.17) | 50 (9.84) | 5 (0.98) | 5.91 | 0.275 | 0.999 | 1.21 (0.86-1.69) | |
| | | ACPA+ (n = 453) | 404 (89.18) | 45 (9.93) | 4 (0.88) | 5.85 | 0.320 | 0.999 | 1.20 (0.84-1.70) | |
| rs323922 | G/C | Controls (n = 929) | 306 (32.94) | 504 (54.25) | 109 (11.73) | 39.86 | | | | |
| | | RA (n = 1157) | 436 (37.68) | 573 (49.52) | 148 (12.80) | 37.55 | 0.158 | 0.999 | 0.91 (0.80-1.04) | |
| | | RF+ (n = 582) | 213 (36.60) | 296 (50.86) | 73 (12.54) | 37.97 | 0.323 | 0.999 | 0.93 (0.79-1.08) | |
| | | SE+ (n = 504) | 181 (35.91) | 252 (50.00) | 71 (14.09) | 39.09 | 0.712 | 0.999 | 0.97 (0.83-1.14) | |
| | | ACPA+ (n = 443) | 164 (37.02) | 219 (49.44) | 60 (13.54) | 38.26 | 0.445 | 0.999 | 0.94 (0.79-1.11) | |
| rs324377 | C/A | Controls (n = 945) | 332 (35.13) | 452 (47.83) | 161 (17.04) | 40.95 | | | | |
| | | RA (n = 1182) | 411 (34.77) | 575 (48.65) | 196 (16.58) | 40.90 | 0.976 | 0.999 | 1.00 (0.88-1.13) | |
| | | RF+ (n = 595) | 204 (34.29) | 292 (49.08) | 99 (16.64) | 41.18 | 0.909 | 0.999 | 1.01 (0.87-1.17) | |
| | | SE+ (n = 516) | 168 (32.56) | 259 (50.19) | 89 (17.25) | 42.34 | 0.475 | 0.999 | 1.06 (0.91-1.24) | |
| | | ACPA+ (n = 460) | 159 (34.57) | 218 (47.39) | 83 (18.04) | 41.74 | 0.699 | 0.999 | 1.03 (0.88-1.21) | |
| rs324396 | C/T | Controls (n = 951) | 439 (46.16) | 399 (41.96) | 113 (11.88) | 32.86 | | | | |
| | | RA (n = 1157) | 547 (47.28) | 480 (41.49) | 130 (11.23) | 32.00 | 0.585 | 0.999 | 0.96 (0.84-1.10) | |
| | | RF+ (n = 581) | 274 (47.16) | 240 (41.31) | 67 (11.53) | 32.19 | 0.710 | 0.999 | 0.97 (0.83-1.14) | |
| | | SE+ (n = 503) | 243 (48.31) | 206 (40.95) | 54 (10.74) | 31.21 | 0.377 | 0.999 | 0.93 (0.79-1.10) | |
| | | ACPA+ (n = 445) | 214 (48.09) | 185 (41.57) | 46 (10.34) | 31.12 | 0.370 | 0.999 | 0.92 (0.78-1.10) | |
| rs740347 | G/C | Controls (n = 952) | 614 (64.50) | 311 (32.67) | 27 (2.83) | 19.17 | | | | |
| | | RA (n = 1166) | 848 (72.73) | 290 (24.87) | 28 (2.40) | 14.84 | 1.7E-04 | 1.2E-03 | 0.73 (0.62-0.86) | |
| | | RF+ (n = 609) | 446 (73.23) | 146 (23.97) | 17 (2.79) | 14.78 | 1.6E-03 | 0.011 | 0.73 (0.60-0.89) | |
| | | SE+ (n = 527) | 381 (72.30) | 133 (25.24) | 13 (2.47) | 15.09 | 5.3E-03 | 0.037 | 0.75 (0.61-0.92) | |
| | | ACPA+ (n = 462) | 326 (70.56) | 120 (25.97) | 16 (3.46) | 16.45 | 0.079 | 0.553 | 0.83 (0.68-1.02) | |
| rs324987 | T/C | Controls (n = 943) | 334 (35.42) | 452 (47.93) | 157 (16.65) | 40.62 | | | | |
| | | RA (n = 1161) | 420 (36.18) | 539 (46.42) | 202 (17.40) | 40.61 | 0.996 | 0.999 | 1.00 (0.88-1.14) | |
| | | RF+ (n = 591) | 214 (36.21) | 276 (46.70) | 101 (17.09) | 40.44 | 0.929 | 0.999 | 0.99 (0.85-1.15) | |
| | | SE+ (n = 518) | 176 (33.98) | 255 (49.23) | 87 (16.80) | 41.41 | 0.676 | 0.999 | 1.03 (0.88-1.21) | |
| | | ACPA+ (n = 459) | 154 (33.55) | 228 (49.67) | 77 (16.78) | 41.61 | 0.615 | 0.999 | 1.04 (0.89-1.23) | |
| rs10263447 | G/C | Controls (n = 955) | 719 (75.29) | 214 (22.41) | 22 (2.30) | 13.51 | | | | |
| | | RA (n = 1167) | 860 (73.69) | 279 (23.91) | 28 (2.40) | 14.35 | 0.451 | 0.999 | 1.07 (0.89-1.29) | |
| | | RF+ (n = 593) | 438 (73.86) | 141 (23.78) | 14 (2.36) | 14.25 | 0.583 | 0.999 | 1.06 (0.86-1.31) | |
| | | SE+ (n = 520) | 387 (74.42) | 120 (23.08) | 13 (2.50) | 14.04 | 0.710 | 0.999 | 1.04 (0.84-1.30) | |
| | | ACPA+ (n = 460) | 344 (74.78) | 106 (23.04) | 10 (2.17) | 13.70 | 0.911 | 0.999 | 1.01 (0.80-1.28) | |

*All p values have been calculated for the allelic model; **p-values after Bonferroni correction for 7 tests; ***Odds ratio for the minor allele. RF+/-: positive/negative rheumatoid factor; SE+/-: positive/negative shared epitope; ACPA+/-: positive/negative anticitrullinated protein antibody. MAF: minor allele frequency.

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Table 2. Genotype and minor allele frequencies of SNP located in the *NPSRI* gene, comparing positive versus negative rheumatoid factor, positive versus negative shared epitope, and positive versus negative anticitrullinated peptide antibody in patients with rheumatoid arthritis from Spain.

| SNP | 1/2 | Subgroup (N) | Genotype, n (%) | | | MAF (%) | p* | Allele Test | |
|------------|-----|-----------------|-----------------|-------------|-------------|---------|---------|---------------------|------------------|
| | | | 1/1 | 1/2 | 2/2 | | | p _{BNF} ** | OR (CI 95%)*** |
| rs323917 | C/G | RF- (n = 290) | 262 (90.34) | 26 (8.97) | 2 (0.69) | 5.17 | | | |
| | | RF+ (n = 584) | 512 (87.67) | 67 (11.47) | 5 (0.86) | 6.59 | 0.244 | 0.999 | 1.29 (0.84-2.00) |
| | | SE- (n = 276) | 242 (87.68) | 31 (11.23) | 3 (1.09) | 6.7 | | | |
| | | SE+ (n = 508) | 453 (89.17) | 50 (9.84) | 5 (0.98) | 5.9 | 0.531 | 0.999 | 0.87 (0.57-1.33) |
| | | ACPA- (n = 307) | 270 (87.95) | 36 (11.73) | 1 (0.33) | 6.18 | | | |
| | | ACPA+ (n = 453) | 404 (89.18) | 45 (9.93) | 4 (0.88) | 5.85 | 0.785 | 0.999 | 0.94 (0.61-1.45) |
| rs323922 | G/C | RF- (n = 290) | 114 (39.31) | 138 (47.59) | 38 (13.10) | 36.9 | | | |
| | | RF+ (n = 582) | 213 (36.60) | 296 (50.86) | 73 (12.54) | 37.97 | 0.662 | 0.999 | 1.05 (0.85-1.29) |
| | | SE- (n = 279) | 115 (41.22) | 132 (47.31) | 32 (11.47) | 35.13 | | | |
| | | SE+ (n = 504) | 181 (35.91) | 252 (50.00) | 71 (14.09) | 39.09 | 0.121 | 0.847 | 1.19 (0.96-1.47) |
| | | ACPA- (n = 311) | 120 (38.59) | 157 (50.48) | 34 (10.93) | 36.17 | | | |
| | | ACPA+ (n = 443) | 164 (37.02) | 219 (49.44) | 60 (13.54) | 38.26 | 0.409 | 0.999 | 1.09 (0.88-1.35) |
| rs324377 | C/A | RF- (n = 294) | 99 (33.67) | 146 (49.66) | 49 (16.67) | 41.5 | | | |
| | | RF+ (n = 595) | 204 (34.29) | 292 (49.08) | 99 (16.64) | 41.18 | 0.897 | 0.999 | 0.99 (0.81-1.21) |
| | | SE- (n = 277) | 105 (37.91) | 124 (44.77) | 48 (17.33) | 39.71 | | | |
| | | SE+ (n = 516) | 168 (32.56) | 259 (50.19) | 89 (17.25) | 42.34 | 0.310 | 0.999 | 1.12 (0.90-1.38) |
| | | ACPA- (n = 316) | 116 (36.71) | 151 (47.78) | 49 (15.51) | 39.4 | | | |
| | | ACPA+ (n = 460) | 159 (34.57) | 218 (47.39) | 83 (18.04) | 41.74 | 0.357 | 0.999 | 1.10 (0.90-1.36) |
| rs324396 | T/C | RF- (n = 293) | 137 (46.76) | 123 (41.98) | 33 (11.26) | 32.25 | | | |
| | | RF+ (n = 581) | 274 (47.16) | 240 (41.31) | 67 (11.53) | 32.19 | 0.978 | 0.999 | 1.00 (0.81-1.23) |
| | | SE- (n = 274) | 125 (45.62) | 118 (43.07) | 31 (11.31) | 32.85 | | | |
| | | SE+ (n = 503) | 243 (48.31) | 206 (40.95) | 54 (10.74) | 31.21 | 0.509 | 0.999 | 0.93 (0.74-1.16) |
| | | ACPA- (n = 310) | 135 (43.55) | 135 (43.55) | 40 (12.90) | 34.68 | | | |
| | | ACPA+ (n = 445) | 214 (48.09) | 185 (41.57) | 46 (10.34) | 31.12 | 0.147 | 0.999 | 0.85 (0.68-1.06) |
| rs740347 | G/C | RF- (n = 292) | 203 (69.52) | 80 (27.40) | 9 (3.08) | 16.78 | | | |
| | | RF+ (n = 609) | 446 (73.23) | 146 (23.97) | 17 (2.79) | 14.78 | 0.271 | 0.999 | 0.86 (0.66-1.13) |
| | | SE- (n = 279) | 192 (68.82) | 79 (28.32) | 8 (2.87) | 17.03 | | | |
| | | SE+ (n = 527) | 381 (72.30) | 133 (25.24) | 13 (2.47) | 15.09 | 0.309 | 0.999 | 0.87 (0.66-1.14) |
| | | ACPA- (n = 317) | 228 (71.92) | 85 (26.81) | 4 (1.26) | 14.67 | | | |
| | | ACPA+ (n = 462) | 326 (70.56) | 120 (25.97) | 16 (3.46) | 16.45 | 0.343 | 0.999 | 1.15 (0.87-1.52) |
| rs324987 | T/C | RF- (n = 280) | 100 (35.71) | 134 (47.86) | 46 (16.43) | 40.36 | | | |
| | | RF+ (n = 591) | 214 (36.21) | 276 (46.70) | 101 (17.09) | 40.44 | 0.974 | 0.999 | 1.00 (0.82-1.23) |
| | | SE- (n = 264) | 108 (40.91) | 113 (42.80) | 43 (16.29) | 37.69 | | | |
| | | SE+ (n = 518) | 176 (33.98) | 255 (49.23) | 87 (16.80) | 41.41 | 0.156 | 0.999 | 1.17 (0.94-1.45) |
| | | ACPA- (n = 296) | 120 (40.54) | 132 (44.59) | 44 (14.86) | 37.16 | | | |
| | | ACPA+ (n = 459) | 154 (33.55) | 228 (49.67) | 77 (16.78) | 41.61 | 0.08467 | 0.999 | 1.21 (0.97-1.49) |
| rs10263447 | G/C | RF- (n = 283) | 206 (72.79) | 71 (25.09) | 6 (2.12) | 14.66 | | | |
| | | RF+ (n = 593) | 438 (73.86) | 141 (23.78) | 14 (2.36) | 14.25 | 0.817 | 0.999 | 0.97 (0.73-1.29) |
| | | SE- (n = 266) | 203 (76.32) | 60 (22.56) | 3 (1.13) | 12.41 | | | |
| | | SE+ (n = 520) | 387 (74.42) | 120 (23.08) | 13 (2.50) | 14.04 | 0.370 | 0.999 | 1.15 (0.84-1.58) |
| | | ACPA- (n = 299) | 229 (76.59) | 67 (22.41) | 3 (1.00) | 12.21 | | | |
| | | ACPA+ (n = 460) | 344 (74.78) | 106 (23.04) | 10 (2.17) | 13.7 | 0.401 | 0.999 | 1.14 (0.84-1.55) |

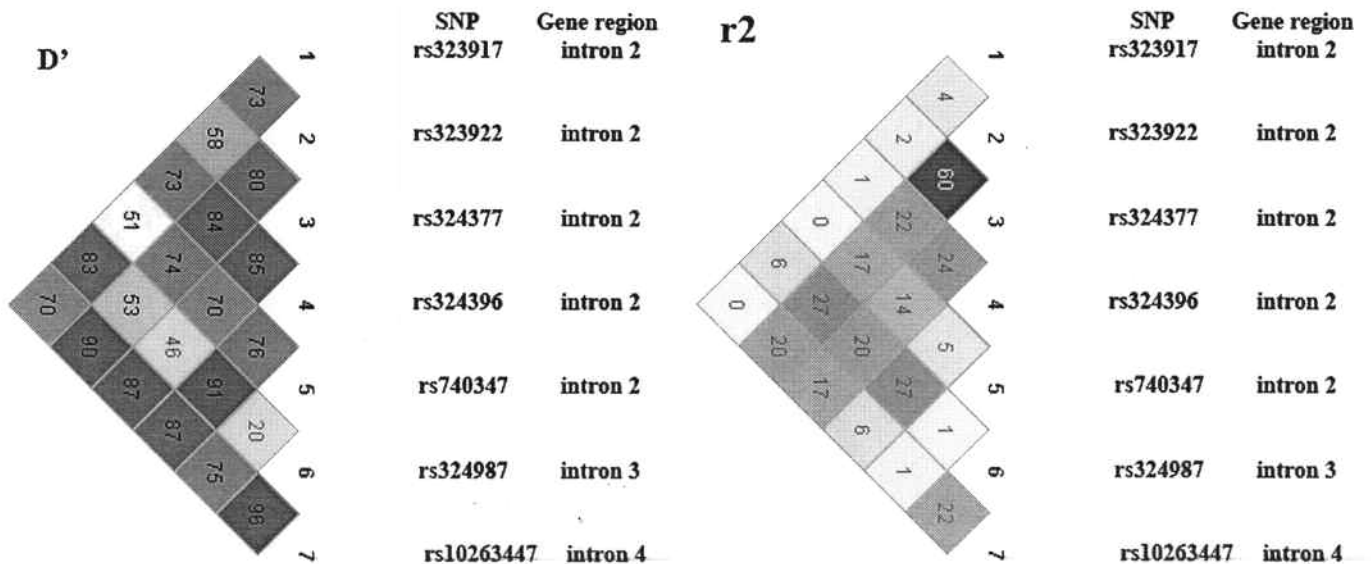
*All p values have been calculated for the allelic model; **p values after Bonferroni correction; ***Odds ratio for the minor allele. RF+/-: positive/negative rheumatoid factor; SE+/-: positive/negative shared epitope; ACPA+/-: positive/negative anticitrullinated protein antibody; MAF: minor allele frequency.

Table 3. Analysis of *NPSR1* gene single nucleotide polymorphisms (SNP) for allelic combinations in Spanish patients with rheumatoid arthritis (RA) and healthy controls. Bold face values indicate statistical significance.

| Allelic Combination | Controls, n (%) | RA, n (%) | p | OR (95% CI) |
|---------------------|-----------------|------------|--------------|------------------|
| CCACCCG | 105 (10.4) | 87 (6.9) | 0.003 | 0.64 (0.48–0.87) |
| CCACCTG | 77 (7.6) | 90 (7.2) | 0.670 | 0.93 (0.68–1.29) |
| CCACGCC | 167 (16.6) | 188 (15.0) | 0.300 | 0.89 (0.71–1.12) |
| CGCCGCG | 120 (11.9) | 186 (14.8) | 0.044 | 1.28 (1.00–1.67) |
| CGCCGTG | 185 (18.4) | 211 (17.0) | 0.336 | 0.90 (0.72–1.12) |
| CGCTGTG | 352 (35.0) | 491 (38.7) | 0.044 | 1.28 (1.00–1.64) |

Order of the SNP: rs323917, rs323922, rs324377, rs324396, rs740347, rs324987, rs10263447. Allelic combinations with a frequency lower than 5% in control groups were excluded from analysis.

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APPENDIX

Linkage disequilibrium maps and location on the *NPSR1* gene of the 7 studied variations as analyzed in Haploview software. Left panel: D' values; right panel: r² values; right side of each panel: position of single-nucleotide polymorphism (SNP) in the gene.