ARTHRITIS & RHEUMATISM
Vol. 50, No. 6, June 2004, pp 1770–1773
DOI 10.1002/art.20280

Association of the PD-1.3A Allele of the *PDCD1* Gene in Patients With Rheumatoid Arthritis Negative for Rheumatoid Factor and the Shared Epitope

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Objective. **To study the frequency of allele A of polymorphism PD-1.3 of the *PDCD1* gene in patients with rheumatoid arthritis (RA) and its subsets, based on the presence of rheumatoid factor (RF) and the shared epitope (SE) alleles.**

Methods. **A total of 1,175 patients with RA and 3,404 controls were genotyped for the PD-1.3 A/G polymorphism, which previously was identified as being involved in susceptibility to systemic lupus erythematosus (SLE) in patients of European descent.**

Results. **We first detected a trend for association of allele A of the single-nucleotide polymorphism PD-1.3 with RA ($P = 0.053$, odds ratio [OR] 1.18, 95% confidence interval [95% CI] 0.99–1.41). To further clarify the nature of this association, patients with RA were divided into 4 groups according to the presence of RF and the SE alleles. Association was found only in the group of patients negative for both RF and the SE alleles ($P = 0.0054$ [corrected $P = 0.015$], OR 1.75, 95% CI 1.15–2.65).**

Conclusion. **Patients negative for both RF and the SE alleles showed association with the same allele that we previously identified as being involved in susceptibility to SLE. These results provide the first evidence of the involvement of the human *PDCD1* gene in arthritis.**

Rheumatoid arthritis (RA) is a complex disease affecting 0.2–2% of the population worldwide (1). Many genetic and environmental factors have been implicated in or suggested to play a role in the development of RA (2). Patients with RA may be differentiated based on the presence or absence of rheumatoid factor (RF). Patients who are RF positive show more severe joint destruction and have a worse disease prognosis compared with individuals who are negative for this serologic marker (3).

The first association of RA with the major histocompatibility complex (MHC) was described in 1978 by Stastny (4,5). Later, it was recognized that several alleles of the *DRB1* gene had a similar motif known as the shared epitope (SE), which confers an increased risk of RA (5). For RA, the calculated risk ratio for siblings (λ_s, an indirect measure of the genetic contribution to the phenotype) is roughly estimated to be 2–10, compared with a risk ratio of at least 10–20 for other autoimmune diseases (6). A significant part of this risk ratio (λ_s 1.7 [~20–85% of the total risk ratio]) can be accounted for by the MHC locus, but still, the MHC can only partly explain the genetic component of the disease, and the SE alleles can only partly explain the impact of MHC on the disease. This means that other genes within and/or outside the MHC are involved in the pathogenesis of RA.

Some of the main difficulties in the identification of genes involved in susceptibility to RA are related to the heterogeneity of the disease, as well as to differences attributable to ethnicity. Studies on the whole genome have shown that the contribution of genes outside the MHC may be small, resulting in the need for large sets of patients, preferably from homogeneous popula-

Supported by the Swedish Research Council, the Swedish Association Against Rheumatism, the King Gustaf V 80th Birthday Jubilee Foundation, the Clas Groschinski Memorial Foundation, the Beijer Foundation, the Swedish Heart & Lung Foundation, and the Wallenberg Foundation.

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Submitted for publication October 27, 2003; accepted in revised form February 12, 2004.

tions (7), and demonstrating that appropriate subphenotyping of patients is important.

The *PDCD1* gene located on chromosome 2q37 encodes for the programmed cell death 1 (PD-1) molecule. We recently described a regulatory single-nucleotide polymorphism (SNP) named PD-1.3, with a G-to-A change at nucleotide 7809 (GenBank accession no. AF363458), located in an enhancer within the fourth intron of the *PDCD1* gene. Allele A of SNP PD-1.3 disrupts binding of the Runx1 transcription factor to the enhancer and thereby alters the regulation of gene expression. This A allele was shown to be associated with systemic lupus erythematosus (SLE) and lupus nephritis, primarily in Europeans (8,9). Later, the same allele was also shown to be associated with diabetes (10).

SLE and RA have many features in common, among these are increased B cell activation and some systemic manifestations, although in general, the arthritis features are different. Patients with SLE do not experience erosion and destruction of cartilage or pannus formation, but do experience synovial joint inflammation and pain.

To test whether SNP PD-1.3 is involved in susceptibility to RA, we studied 1,175 patients with RA and 3,404 controls from the Swedish population. After having detected a trend for association for the whole group of patients with RA ($P = 0.053$), we stratified 876 patients for whom information was available into 4 groups according to the presence/absence of RF and the SE alleles. In patients with RA who were both RF negative and SE negative, the frequency of the A allele of PD-1.3 was 12.1%. This frequency was significantly different from that among controls ($P = 0.0054$ [corrected $P = 0.015$]) and the remaining groups of RA patients ($P = 0.023$), and was similar to the frequency observed in patients with sporadic SLE (11.5%) (8,9). These results may indicate that different autoimmune diseases, such as SLE and RA, could share similar disease mechanisms.

PATIENTS AND METHODS

Patients and controls. Approval for the study was obtained from the Karolinska Institutet ethics committee. The patient group comprised 1,175 individuals who were examined at various rheumatology units around Sweden as a part of a large study on genes and environment in the etiology of RA (the Epidemiological Investigation of Rheumatoid Arthritis study) (11). In all patients, RA was diagnosed according to American College of Rheumatology (ACR; formerly, the American Rheumatism Association) revised criteria (12), and patients were recruited to this study within 1–3 years from the time of onset of disease. All patients fulfilled the ACR revised criteria for RA.

For 876 patients (629 men and 247 women), information on RF and on the SE alleles was available, and this group of patients was used for a subgroup analysis. Control subjects consisted of 3 groups of individuals. In the first group (n = 658), controls were identified through the Swedish general population registry after matching with the RA patients for sex, age, and place of residence, as previously described (11). The second control group was composed of 1,082 individuals with first-time myocardial infarction (MI) and 1,335 individuals without MI from the Stockholm Heart Epidemiology Program (SHEEP) study (Stockholm City Council, 1992–1994) (13). In these 2 subgroups, the frequencies of PD-1.3A did not differ between those with and those without MI (7.6% and 6%, respectively). Thus, the combined SHEEP set of 2,417 individuals was used as the second control group; in this group, the average frequency of PD-1.3A was 6.9%. The third control group comprised 329 blood donors at the Uppsala Academic Hospital, as previously reported (8). The 3 control groups represented a total of 3,404 individuals.

Genotyping of PD-1.3. Peripheral blood samples were obtained from all participants, after receiving informed consent. DNA was prepared according to standard procedures. The SNP PD-1.3 was genotyped as described previously (8).

Determination of RF. Detection of RF was performed by routine validated agglutination methods.

Genotyping of the SE alleles. An analysis of HLA–DRB1 genotypes was performed using the sequence-specific primer–polymerase chain reaction method (DR Low Resolution kit; Olerup SSP, Saltsjöbaden, Sweden) as previously described (14,15). Interpretation of the bands was done according to the manufacturer's instructions. Among the HLA–DRB1 genes, the DRB1*01, DRB1*04, and DRB1*10 alleles were defined as the shared epitope alleles/genes. A part of the material was subtyped for identification of HLA–DRB1*01 and *04 alleles.

Statistical analysis. Statistical significance was evaluated by chi-square test (2×2 contingency tables). Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. Genotype frequencies were in Hardy-Weinberg equilibrium. Bonferroni adjustments for multiple comparisons were made.

RESULTS

Among controls, men and women were first analyzed separately, and because frequencies of allele A did not differ (data not shown) the data were combined. The frequencies of allele A in the different control groups were as follows: for group 1, 8.6%; for group 2, 6.9%; and for group 3, 7.8%. These were combined for the final analysis, giving an overall frequency of 7.3%.

The frequency of PD-1.3A was compared between controls (n = 3,404) and all patients with RA (n = 1,175), and a trend for association was detected ($P = 0.053$, OR 1.18, 95% CI 0.99–1.41). To further clarify the nature of this association, 876 patients were divided into

Table 1. Characteristics of the RA patient groups and the controls*

	No. (%) of individuals	% women/ % men	Mean age, years
RA patients			
RF+/SE+	449 (51)	70/30	51.3
RF+/SE−	124 (14)	72/27	51.4
RF−/SE+	183 (21)	73/27	50.9
RF−/SE−	120 (14)	72/28	49.5
Total	876 (100)	71/29	51.0
Controls	3,404 (100)	40/60	56.5

* Of all rheumatoid arthritis (RA) patients, 65% were positive for rheumatoid factor (RF), and 72% were positive for the shared epitope (SE).

4 groups according to presence/absence of the SE alleles and RF.

Table 1 shows characteristics of the entire control group and 4 subgroups of patients (categorized according to their RF and SE status). Controls were, on average, 5.5 years older than patients (mean age 56.5 years versus mean age 51.0 years). The ratio of controls to patients studied was 3.9 to 1 (3,404 controls and 876 patients). Among patients, the ratio of women to men was 2.5 to 1 (71% women and 29% men), with an excess of females that was similar in all of the stratification groups. Among controls, the female-to-male ratio was 1 to 1.5 (40% women and 60% men). Among all patients, 65% were RF positive and 72% were positive for the SE alleles. The majority of patients (51.0%) were positive for both RF and the SE alleles; 14% were negative for both RF and the SE alleles. The frequencies of RF and the SE alleles did not differ between men and women.

In all patient subgroups, frequencies of allele A in men and women were first analyzed separately. The frequencies were found to be comparable, and therefore all patients were analyzed together as one group in the subsequent analysis (Table 2). The frequency of allele A was similar in the control group and all patients groups except for the group that was negative for both RF and the SE alleles, in which the frequency of allele A was 12.1% (Table 3). When the group that was negative for

Table 2. Frequency of the PD-1.3A allele in the RA patient groups*

RA group	No. of A alleles/no. of chromosomes (%)		
	Females	Males	Joint group
RF+/SE+	47/632 (7.4)	21/266 (7.9)	68/898 (7.6)
RF+/SE−	11/184 (6.0)	8/64 (12.5)	19/248 (7.7)
RF−/SE+	24/270 (8.9)	6/96 (6.3)	30/366 (8.2)
RF−/SE−	21/172 (12.2)	8/68 (11.8)	29/240 (12.1)

* RA = rheumatoid arthritis; RF = rheumatoid factor; SE = shared epitope.

Table 3. Association analysis in the RA patient groups and the controls*

Group	No. of subjects	Genotype			No. of chromosomes (frequency of PD1.3A)
		AA	AG	GG	
RA					
Total	1,175	10	180	985	200 (8.5)†
RF+/SE+	449	3	62	384	68 (7.5)
RF+/SE−	124	2	15	107	19 (7.7)
RF−/SE+	183	2	26	155	30 (8.2)
RF−/SE−	120	1	27	92	29 (12.1)‡
Controls	3,404	19	458	2,927	496 (7.3)

* RA = rheumatoid arthritis; RF = rheumatoid factor; SE = shared epitope.
† $\chi^2 = 3.73$, $P = 0.053$, odds ratio (OR) 1.18, 95% confidence interval (95% CI) 0.99–1.4.
‡ $\chi^2 = 7.74$, $P = 0.0054$, OR 1.75, 95% CI 1.15–2.65 versus controls, and $\chi^2 = 5.15$, $P = 0.023$ versus all other patients with RA. The frequency of PD-1.3A in this group (12.1%) was similar to that in 656 European patients with systemic lupus erythematosus (11.5%) (see ref. 9).

both RF and the SE alleles was compared with the control group, the difference was clearly significant ($P = 0.0054$ [corrected $P = 0.015$], $\chi^2 = 7.74$, OR 1.75, 95% CI 1.15–2.65). The difference was significant ($P = 0.023$) even when the group that was RF negative and SE alleles negative was compared with all other patients with RA (12.1% versus ~7.7 %).

DISCUSSION

The main finding in the present study is that the functionally important polymorphism in the *PDCD1* gene is associated with a subset of RA. Because the polymorphism in this gene has previously been shown to be associated with SLE (8), these new data provide some of the first evidence at the gene level of common genetically defined mechanisms being involved in RA and SLE.

A basis for genetic analysis of RA is the observation that the relative risk for siblings (λ_s) is estimated to be between 2 and 10, and that a major part of this value can be attributed to the effects of MHC genes. In our analysis, the OR for the SE was 2.3 (95% CI 1.86–2.85, $P = 4 \times 10^{-15}$) (data not shown). Thus, even if genetic factors other than the MHC contribute to the disease, the chances of finding them are small. In order to study such genetic factors in case–control studies, relatively large groups of patients and controls are needed, and great caution must be taken to select patients and controls from the same population, which should preferably be homogeneous. In the present study, we were able to use a large sample size of both patients (n = 1,175) and controls (n = 3,404) from the relatively

homogeneous population of Sweden. This allowed us to detect a trend for association of allele A of SNP PD-1.3 with RA ($P = 0.053$, OR = 1.18). Further analysis of 876 patients stratified according to their RF and SE allele status showed that the observed association was solely present in the group of patients who were negative for both RF and the SE alleles ($P = 0.0054$, OR 1.75) as compared with controls.

PD-1 (a 50–55-kd type 1 transmembrane receptor) is an immunoreceptor tyrosine-based inhibitory motif–containing molecule expressed on lymphocytes in the thymus and in activated T cells (16,17). Knockout mice lacking the *Pd1* gene show, among other features, lupus-related manifestations and arthritis. Moreover, it has recently been demonstrated that the synovial fluid of patients with RA contains a population of CD4+PD-1+ cells that present anergic features (18). How may the *PDCD1* PD-1.3 polymorphism be involved in disease susceptibility? The polymorphism disrupts the binding of transcription factor Runx1, a member of the Runt domain family of transcription factors (8). Our own data suggest that the presence of this allele may affect the expression of PD-1 (data not shown). Whether the presence of the anergic population in RA synovial fluid (18) and the presence of the PD-1.3 polymorphism are related events is not clear at this point and will require further study.

In conclusion, using the largest set of Caucasian patients with RA and controls ever examined in an association study, we were able to demonstrate an association between RA patients negative for both rheumatoid factor and the shared epitope and allele A of the PD-1.3 polymorphism of *PDCD1*. We have thus demonstrated that PD-1.3A is a susceptibility factor for SLE (8), for lupus nephritis (9), and for a subgroup of RA patients negative for RF and the SE.

ACKNOWLEDGMENTS

This study was made possible through contributions from the collaborators of the Epidemiological Investigation of Rheumatoid Arthritis project, who sampled blood (for DNA) from the RA patients and provided clinical data. We gratefully acknowledge Camilla Silva for collecting the patient data and Eva Jemseby for collecting blood samples in the EIRA study.

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