Genetic studies in rheumatoid arthritis

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Chapter 3

Replication of the *Tumor Necrosis Factor Receptor Associated Factor 1/Complement Component* 5 Region as a Susceptibility Locus for Rheumatoid Arthritis in a European Family-Based Study

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Abstract

Objective

We recently showed, using a candidate gene approach in a case-control association study, that a 65-kb block encompassing *tumor necrosis factor receptor associated factor 1* (*TRAF1*) and Complement component 5 (*C5)* is strongly associated with rheumatoid arthritis (RA). Compared with case-control association studies, family-based studies have the added advantage of controlling potential differences in population structure and are not likely to be hampered by variation in population allele frequencies, as is seen for many genetic polymorphisms, including the *TRAF1/C5* locus. The aim of this study was to confirm this association in populations of European origin by using a family-based approach.

Methods

A total of 1,356 western European white individuals from 452 "trio" families were genotyped for the rs10818488 polymorphism, using the TaqMan allelic discrimination assay.

Results

We observed evidence for association, demonstrating departure from Mendel's law, with an overtransmission of the rs10818488 A allele (A = 55%; P = 0.036). By taking into consideration parental phenotypes, we also observed an increased A allele frequency in affected versus unaffected parents (A = 64%; combined *P* = 0.015). Individuals carrying the A allele had a 1.2-fold increased risk of developing RA (allelic odds ratio 1.24, 95% confidence interval 1.04–1.50).

Conclusion

Using a family-based study that is robust against population stratification, we provide evidence for the association of the *TRAF1/C5* rs10818488 A allele and RA in populations of European descent, further substantiating our previous findings. Future functional studies should yield insight into the biologic relevance of this locus to the pathways involved in RA.

Introduction

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, affecting ~1% of the population worldwide. Environmental as well as genetic factors are thought to play an important role in both the onset and the progression of the disease (1). Because the genetic contribution to RA has been estimated to be 50-60%, the identification of genes contributing to the disease is important for the understanding of underlying biologic mechanisms (2).

In addition to HLA, the first identified genetic risk factor (3), and 3 other replicated regions including the protein tyrosine phosphatase N22 gene (4), the 6q23 locus near the *tumor necrosis factor (TNF)α induced protein 3* gene (5,6), and the STAT4 gene (7,8), we recently reported a new genetic locus associated with RA (9). This region encompasses the *TNF receptor associated factor 1 gene (TRAF1*) as well as the *complement component factor 5 (C5)* gene, both of which are immune regulators and potential perpetuators of inflammation. We identified 1 single-nucleotide polymorphism (SNP), rs10818488, located within a 65kb haplotype block, explaining most of the association signal in this region in several populations, including Dutch, Swedish, and US sample sets consisting of 2,719 patients with RA and 1,999 control subjects (odds ratio [OR] 1.28, 95% confidence interval [95% CI] 1.17-1.39, $P = 1.40 \times 10^{-8}$). Interestingly, the same genetic association was described in a recent whole-genome association study (10).

Our data revealed that although the case-control allele frequency increase in different sample sets ranged from 4% to 9%, the population frequency ranged from 38% to 46% in populations of European ancestry. Given that association studies compare the frequencies in patients versus healthy individuals, unknown biases in control frequencies may lead to spurious associations. Thus, family-based association studies remain important to definitively establish association, especially when small effect sizes as well as variability in allele frequency in different populations are observed. Therefore, with the aim to further substantiate the association at the *TRAF1/C5* locus, we took advantage of one of the largest reported European family resources dedicated to RA family-based studies.

Patients & Methods

Study population

DNA was available from 452 white trio families from western Europe, through the European Consortium on Rheumatoid Arthritis Families. Each family consisted of 1 patient with RA and both of his or her parents. Ethnicity was determined by the origin of the grandparents. At the time of inclusion in the study, all patients with RA fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria (11). All individuals provided written informed consent, and the study was approved by the ethics committees in each country.

For each patient, the characteristics collected were sex, age at the onset of RA, disease duration (years), presence of bone erosions on radiographs, presence of rheumatoid nodules, and seropositivity for rheumatoid factor (RF). RF status was not available for 9 of the patients with RA. Because the anti-citrullinated protein antibody (ACPA) status was available for only a small proportion of the patients (n 197), we did not perform further analyses for ACPAs. The 452 families included 313 families from France, 53 from Italy, 37 from Spain, 22 from Belgium, 13 from The Netherlands, and 14 from Portugal. The characteristics of the French and European sample sets are summarized in Table 1. None of the cases reported in this study overlap with our previous case-control study (9).

Genotyping

All DNA samples were genotyped using the TaqMan allelic discrimination assay according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). At least 2 positive controls and 1 negative control were performed in each plate, and no inconsistencies were detected. Furthermore, the concordance rate between 10% random samples genotyped in duplicate was 99%. The genotyping success rate in the 452 trio families was 100% (parents and probands included).

Statistical analysis

The family-based analysis was performed using the basic transmission disequilibrium test (TDT) combined with parental phenotype information (parenTDT), as implemented in Haploview 4.0 (12). The basic TDT compares, for a given allele, the transmission of that allele from heterozygous parents to a child with RA, to the expected 50% transmission according to Mendel's law. The parental discordance test is based on counting the number of alleles in affected versus unaffected parents, treating each nuclear family parental pair as a matched pair. These counts combined with the transmitted and untransmitted counts of the basic TDT give a combined test statistic (13).

The current data set included 42 families with 1 affected parent. The control genotypes were derived from the untransmitted parental chromosomes, using Unphased version 3.0.10 (14). By combining the case-control data from the probands with parental data, genotypic OR were calculated using a conditional logistic regression model stratifying on matched pairs (each proband-pseudocontrol as a matched pair; each affected and unaffected parent as a matched pair). Robust standard errors were computed, taking into account the dependency between pairs from the same family. These analyses were performed using Stata version 9.0 software (www.stata.com).

Table 1. Characteristics of the patients with rheumatoid arthritis (RA)

Characteristic	All (n 452)	French (n 313)	European (n 139)
Female sex, no. (%)	393 (86.9)	275 (87.8)	118 (84.9)
Age at onset of RA, mean SD years	30.8 9.4	30.7 9.4	30.9 9.4
Disease duration, mean SD years	10.3 7.9	12.0 8.1	6.6 5.9
Bone erosions, no. (%)	340 (75.2)	246 (78.6)	94 (67.6)
Rheumatoid factor positivity, no. (%)	321 (71.0)	221 (70.6)	100 (71.9)
Nodules, no. (%)	72 (15.9)	55 (17.6)	17 (12.2)

Results

A total of 1,356 European individuals from 452 trio families (1 patient with RA and both parents) were analyzed. Three hundred thirteen families were of French origin, and 139 were from other continental western European countries (Table 1). No deviation from Hardy-Weinberg equilibrium was detected in parental genotypes (n = 904; P = 0.548). We observed deviation from Mendel's first law, with a 55% overtransmission of the A allele to the patients in the 452 families (P = 0.036) (Table 2) along with an increased prevalence of the A allele in affected parents (64% increase versus unaffected parents). By applying a parenTDT test that takes into consideration both the transmission from parents to patients and the occurrence of alleles in discordant parents (discordant for both disease status and genotype), we obtained a combined statistic (P = 0.015). There were no differences between families of French origin and those from other continental western European countries (55% and 56% overtransmission, respectively) as well as no differences between paternal and maternal transmissions (56% and 57% overtransmission, respectively).

One of the advantages of family trio data is that such data provide perfectly matched control subjects for each patient investigated. Each patient chromosome transmitted by a given parent is perfectly matched for the population of origin with the untransmitted chromosome of each heterozygous parent. We observed an rs10818488 A allele frequency of 36% in control subjects, increasing to 41% in the patients with RA (data not shown). Using conditional logistic regression for the combined data set of case-control and parental discordant pairs, we observed an allelic OR of 1.24 (95% CI 1.04-1.50) (Table 3).

Table 2. Family-based association of the TRAF1/C5 region with rheumatoid arthritis (RA)

Sample set	Trio families	rs10818488 A allele		Transmission†	*P*‡	Discordant parents§	*P*¶
		Transmitted	Untransmitted				
All	452	231	188	55	0.036	64	0.015
RF+	335	175	136	56	0.027	67	0.0099
RF−	108	47	49	49	0.92	40	0.84

* *TRAF1* = tumor necrosis factor receptor–associated factor 1; RF = rheumatoid factor.
† Percent transmission of the rs10818488 A allele from heterozygous parents.
‡ By standard transmission disequilibrium test (TDT), as implemented in Haploview 4.0.
§ Percent of the rs10818488 A allele from the affected parents in discordant parent pairs.
¶ Combined TDT with parental phenotype information statistic, as described by Purcell et al (13).

Table 3. Case–control association of *TRAF1/C5* rs10818488 with RA

Sample set	AA†	AG†	Allelic‡
All	1.48 (0.87–2.51)	1.37 (1.01–1.86)	1.24 (1.04–1.50)
RF+	1.93 (1.01–3.69)	1.47 (1.03–2.10)	1.31 (1.06–1.63)
RF−	0.56 (0.18–1.69)	1.16 (0.63–2.12)	1.00 (0.69–1.45)

* *TRAF1* _ tumor necrosis factor receptor–associated factor 1; RA = rheumatoid arthritis; RF = rheumatoid factor.
† Genotype-specific odds ratios (ORs) (95% confidence intervals [95% CIs]), using GG as the referent.
‡ Allelic ORs (95% CIs), using the G allele as the referent.

Because RA is a heterogeneous disease, and distinct subsets of patients are characterized by the presence of autoantibodies such as RF and ACPAs, we also performed a stratified analysis for the presence or absence of RF (only limited data were available for ACPAs [see Patients and Methods]). In concordance with our previous findings, we observed an overtransmission (A transmitted 56%) as well as a higher prevalence of the A allele in affected parents (A 67%; P = 0.0099, by parenTDT) in the 335 RF-positive families (Table 2).

Among RF-positive individuals, harbouring 1 copy of the risk allele yielded an OR of 1.47 (95% CI 1.03-2.10), and homozygous individuals had an almost 2-fold increased risk of developing RA (OR 1.93, 95% CI 1.01–3.69) (Table 3). Interestingly, this effect was not detected in the RF-negative subgroup, as reflected by transmission of 49% and an A allele frequency in affected parents of 40% in the 108 RF-negative families (*P* = 0.84) (Table 2), resulting in an allelic OR of 1.00 (95% CI 0.69–1.45) (Table 3).

Discussion

In the current family-based study, we observed evidence of association between RA and the *TRAF1/C5* rs10818488 A variant in a western European sample set, replicating our initial findings. One of the advantages of the family trio design is that it provides accurate estimations of matched control subjects for each patient, an approach that is robust against population stratification. In this set of perfectly matched cases and controls, we observed a 5% increase in the A allele frequency in the overall sample set and a 6% increase in the group of RF-positive patients of western European descent. Because these differences are well within the previously observed effect in the Dutch (9%), Swedish (4%), and American (7%) populations, these data together indicate that the contribution of the *TRAF1/C5* locus to RA is not likely caused by underlying stratification in populations of European descent, and that the effect size is modest (OR 1.3).

Although we observed association in the overall sample set as well as in the RF-positive subset of patients with RA, we did not observe any overtransmissions in the RF-negative subset of patients with RA (49% versus the expected 50%), indicating that the effect of this genetic risk factor in the overall sample set is likely to be attributable to the RF-positive subset of patients with RA. Given the recent finding of this locus by a genomewide SNP association study in ACPA-positive patients with RA, the current evidence further substantiates the role of the *TRAF1/C5* region in the autoantibodypositive subset of patients with RA. However, whether this association extends to the autoantibody-negative subset of patients remains to be determined, because our RF-negative sample set possesses only 26% power to detect small effect sizes (OR 1.3) at a significance level less than 0.05. Therefore, our data do not allow a conclusion regarding the (lack of) contribution of the *TRAF1/C5* locus to autoantibody-negative RA.

The currently identified polymorphism lies in an intergenic region between *TRAF1* and C5. Because *TRAF1* is involved in TNF-mediated signaling and C5 generates C5a, the most potent chemoattractant involved in inflammation, both genes possess characteristics relevant to the pathogenesis of RA. Linkage disequilibrium patterns have so far revealed that the haplotype block surrounding this polymorphism encompasses the *TRAF1* gene as well as the 3region of C5. Interestingly, our group recently observed an association of this SNP with the polyarticular form of juvenile idiopathic arthritis (15). It is therefore plausible that the association of the *TRAF1/C5* variant may not be restricted to RA as such but may be relevant for other diseases. Furthermore, because current HapMap phase II data suggest that the frequency of the rs10818488 A allele is high in Chinese, Japanese, and Yoruban populations (44±69%), it would be interesting to investigate whether this genetic risk factor is also relevant across various ethnic populations.

In conclusion, this study provides evidence of the association of the *TRAF1/C5* locus as one of the widely confirmed genetic risk factors for RA in white individuals of European descent. Endeavors to characterize the functional relevance of this polymorphism and/or others highly linked to it will yield insights into the biologic effects of this locus and will generate further crucial information on the pathways underlying disease.

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