Genetic studies in rheumatoid arthritis

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Chapter 4

The *TRAF1/C5* region is a risk factor for polyarthritis in juvenile idiopathic arthritis

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Abstract

Objective

Juvenile idiopathic arthritis (JIA) is a chronic disorder in which both genetic and environmental factors are involved. Recently, we identified the *TRAF1/C5* region (located on chromosome 9q33–34) as a risk factor for rheumatoid arthritis (RA) ($p_{combined}=1.4x10^{-8}$). In the present study the association of the *TRAF1/C5* region with the susceptibility to JIA was investigated.

Methods

A case-control association study was performed in 338 Caucasian patients with JIA and 511 healthy individuals. We genotyped the single nucleotide polymorphism rs10818488 as a marker for the *TRAF1/C5* region.

Results

The A allele was associated with the susceptibility to rheumatoid factor-negative polyarthritis with an 11% increase in allele frequency (OR 1.54, 95% CI 1.09 to 2.18; p=0.012). This association was stronger when combining subtypes with a polyarticular phenotype (OR 1.46, 95% CI 1.12 to 1.90; p=0.004). In addition, we observed a trend towards an increase in A allele frequency in patients with extended oligoarthritis versus persistent oligoarthritis (49%, 38% respectively); p=0.055.

Conclusions

Apart from being a well replicated riskfactor for RA, *TRAF1/C5* also appears to be a risk factor for the rheumatoid factor-negative polyarthritis subtype of JIA and, more generally, seems to be associated with subtypes of JIA characterised by a polyarticular course.

Introduction

Juvenile idiopathic arthritis (JIA) is defined as arthritis of unknown aetiology that persists for at least 6 weeks and begins before the age of 16 years. It is the most common chronic inflammatory rheumatic disease in childhood (1). In 1997 the International League of Associations for Rheumatology formulated criteria for the classification of seven different subtypes of JIA based on clinical and laboratory features (2, 3).

Although the pathogenesis and aetiology are still poorly understood, JIA is thought to be an autoimmune disease in which genetic and environmental factors play a part. Evidence for the importance of a genetic component includes the ethnic variability in the incidence of different subtypes of JIA, a female preponderance, an increased sibling recurrence rate (λs) of 15 and the association with HLA and non-HLA genes (eg, PTPN22)(3–5). Still little is known about which genetic factors are involved in the susceptibility to JIA and the severity of JIA. Identification of these genetic factors could help to understand the pathogenesis of JIA and could be of use to identify patients with an unfavourable prognosis in an early stage.

Recently we identified the *TRAF1/C5* region (located on chromosome 9q33–34) as a genetic risk factor for RA, using a candidate gene approach (6). A similar finding was made in a genome-wide study in RA (7). *TRAF1* is encoding the *tumour necrosis factor (TNF)-receptor-associated factor 1* and *C5* is encoding the *complement component 5*. Inspired by these results we have studied whether variability in the *TRAF1/C5* region also affects the susceptibility to JIA.

Materials & Methods

A case-control association study was performed in 338 Caucasian patients with JIA from paediatric rheumatology centres in the Netherlands (54%), Belgium (24%) and Germany (22%). Genotype frequencies of 511 healthy unrelated Dutch adult controls were available of the 524 controls previously described by Kurreeman et al due to random genotyping failure (6). All patients with JIA (72% female, 28% male) were diagnosed according to the revised International League of Associations for Rheumatology criteria(2). The inclusion of patients focused on persistent (39%) and extended (14%) oligoarthritis and rheumatoid factor (RF)-negative (22%) and RF-positive (5%) polyarthritis because of their relative homogeneous phenotypes. Patients who were included in the study had a follow-up of at least 2 years. Informed consent from all patients and/or parents and approval from each institutional review board were obtained. DNA was isolated from blood samples (20%) or mouthswabs (80%).

One tagging single nucleotide polymorphism (rs10818488) was genotyped as it revealed the strongest association in RA and none of the other tagging single nucleotide polymorphisms in the 65 kb block provided additional information.6 Rs10818488 is highly linked with rs3761847 (r^2=1, data from HAPMAP) and rs2900180 (r^2=0.66), which were associated with RA as well (7). Rs10818488 was genotyped by the polymerase chain reaction–restriction fragment length polymorphism method as described (6). Each 96-well plate contained two positive and two negative controls. Eight per cent of the samples were run in duplicate and we observed a concordance rate >98%.

Differences in genotype frequencies between cases and controls were assessed using the Cochran–Armitage trend test. Allelic odds ratios (OR) with 95% confidence interval (CI) as well as the genotype specific odds ratios were computed. Case and control genotype frequencies were in Hardy–Weinberg equilibrium. Statistical analysis was performed with SPSS 14.0. p<0.05 was considered statistically significant.

Results

JIA is a heterogeneous disease consisting of several subtypes. As it is best to investigate genetic risk factors in well-defined phenotypic groups, we have analysed the genotypes of the rs10818488 single nucleotide polymorphism in the different subtypes of JIA as well as in the overall group of patients with JIA as shown in Table 1.

Frequencies in patients with persistent oligoarthritis, systemic JIA and in the overall patient group with JIA did not differ significantly fromthose in controls. In extended oligoarthritis and RF-positive polyarthritis (the equivalent of RA) a trend towards an increased A allele frequency was observed (49%, 50% respectively vs 41% in controls). However, although we do observe an 8-9% increase in the A allele, we possess limited power to detect significant differences. In RF-negative polyarthritis patients we found a significant increase in the A allele by 11% (allelic OR 1.54, 95% CI 1.09 to 2.18) when compared with controls. Homozygotes for the susceptibility allele (AA) had an OR of 2.51 (95%CI 1.23 to 5.14) compared with the homozygotes of the protective allele (GG), whereas heterozygotes had an OR of 1.50 (95% CI 0.81 to 2.77). Gender was not a significant covariate when performing a regression analysis (p=0.124).

Table 1 Genotype and allele frequencies in different subtypes of JIA versus controls

		Allele frequency	Genotype frequency				Allelic OR	Genotypic OR* (95% CI)	
Diagnosis†	n	A	AA	AG	GG	p Value‡	(95% CI)	AA	AG
Controls	511	0.41	79 (0.16)	265 (0.52)	167 (0.33)				
Persistent oligoarthritis	133	0.38	18 (0.14)	65 (0.49)	50 (0.38)	0.297	0.87 (0.66 to 1.14)	0.76 (0.42 to 1.39)	0.82 (0.54 to 1.24)
Extended oligoarthritis	48	0.49	10 (0.21)	27 (0.56)	11 (0.23)	0.136	1.36 (0.89 to 2.06)	1.92 (0.78 to 4.71)	1.55 (0.75 to 3.20)
RF-negative polyarthritis	73	0.52	19 (0.27)	38 (0.51)	16 (0.22)	0.012§	1.54 (1.09 to 2.18)	2.51 (1.23 to 5.14)	1.50 (0.81 to 2.77)
RF-positive polyarthritis	18	0.50	5 (0.28)	8 (0.44)	5 (0.28)	0.288	1.42 (0.73 to 2.75)	2.11 (0.60 to 7.51)	1.01 (0.32 to 3.13)
Systemic JIA	41	0.37	3 (0.07)	24 (0.59)	14 (0.34)	0.375	0.82 (0.51 to 1.30)	0.45 (0.13 to 1.62)	1.1 (0.54 to 2.1)
All patients with JIA	338	0.44	59 (0.17)	179 (0.53)	100 (0.30)	0.281	1.11 (0.91 to 1.35)	1.25 (0.82 to 1.90)	1.13 (0.83 to 1.54)

JIA, juvenile idiopathic arthritis; RF, rheumatoid factor.
*GG as reference genotype.
†Diagnosis according to the revision International League of Associations for Rheumatology classification.[2]
‡p Value of the Cochran–Armitage trend test.
§p Value of <0.05 is considered statistically significant.

As extended oligoarthritis, RF-negative polyarthritis and RFpositive polyarthritis have a considerable phenotypic overlap and share a polyarticular course of disease, we also analysed these subtypes grouped together to determine whether the *TRAF1/C5* region predisposes to a polyarticular disease course. The A allele was significantly increased in this combined group (51% in cases, 41% in controls), with an allelic OR 1.46 (95% CI 1.12 to 1.90), a genotypic OR (AA vs GG) of 2.25 (95% CI 1.29–3.90) and a p value (Cochran–Armitage trend test) of 0.004, which remains significant after Bonferroni correction ($p<0.013$).

As persistent and extended oligoarthritis are clinically similar at disease onset, but differ in their course and outcome, we tested the hypothesis that these two subtypes would also differ in their genetic predisposition. Intriguingly, we detected a borderline significant difference between these two subtypes when compared directly with each other (allelic OR 1.57, 95% CI 0.98 to 2.51; $p=0.055$).

Together, these data indicate that the A allele predisposes predominantly to subtypes of JIA characterised by a polyarticular course, indicating that this allele does not associate with JIA as such, but rather with a particular phenotype of JIA.

Discussion

This is one of the first studies to report an association between the *TRAF1/C5* region and JIA. The A allele of rs10818488 was significantly associated with the susceptibility to RF-negative polyarthritis. However, patients with RF-negative polyarthritis have a considerable phenotypic overlap with patients with extended oligoarthritis and RF-positive polyarthritis, in having a polyarticular course of disease. Intriguingly, rs10818488 seems to be associated with this polyarticular phenotype and this difference remained significant after Bonferroni correction for multiple tests ($p<0.013$).

Although we cannot formally exclude the possibility that population stratification may play a role in this study, we did not observe any statistically significant differences in the minor allele frequencies of the patients from the Netherlands, Belgium and Germany. Additionally, analysing the Dutch population independently did not alter the results (eg, RF-negative polyarthritis versus controls: allelic OR 1.66 (95% CI 1.10 to 2.51), genotypic OR (AA versus GG) 3.05 (95% CI 1.25 to 7.44).

At the clinical level, it is also important to make a distinction between persistent oligoarthritis and extended oligoarthritis in order to predict the probability of the development of a polyarticular disease course and adjust the medical treatment accordingly. Comparison of patients with persistent and extended oligoarthritis revealed a borderline significant difference in A allele frequency. This may indicate that the *TRAF1/C5* region may eventually be helpful in predicting the development of an extended course in patients with oligoarthritis. Extended studies of this polymorphism may confirm its relevance as a predictive marker.

It is presently unclear how the association between the *TRAF1/C5* region and disease susceptibility can be explained biologically. The associated polymorphism is highly linked to the TRAF1 gene as well as the 3' untranslated region of the C5 gene. Activated complement component 5 acts as a strong chemoattractant for neutrophils, and a deregulated activity may contribute to the perpetuation of inflammation. In JIA, complement activation is occasionally observed, especially in active polyarthritis (8). On the other hand, TRAF1 plays an essential role in the intracellular TNF-signalling pathway and is possibly a negative regulator of TNF signalling (9). Evidence for the importance of TNF in JIA is suggested by the effectiveness of treatment directed against TNFα, especially in subtypes with a polyarticular course. However, future research will be necessary to confirm the genetic association and investigate functional consequences of the associated allele and could reveal further insight in the pathogenesis of polyarticular disease in JIA. Apart from being a well replicated risk factor for RA, *TRAF1/C5* also appears to be a risk factor for the RF-negative polyarthritis subtype of JIA and, more generally, seems to be associated with subtypes of JIA characterised by a polyarticular course.

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