Genetic studies in rheumatoid arthritis

Kurreeman, F.

Chapter 13

Association of the Autoimmunity Locus 4q27 With Juvenile Idiopathic Arthritis

H.M. Albers
F.A.S. Kurreeman
G. Stoeken-Rijsbergen
D.M.C. Brinkman
S.S.M. Kamphuis
M.A.J. van Rossum
H.J. Girschick
C. Wouters
R.K. Saurenmann
E. Hoppenreijs
P. Slagboom
J.J. Houwing-Duistermaat
W. Verduijn
T.W.J. Huizinga
R. ten Cate
R.E.M. Toes
M.W. Schilham

Arthritis Rheum. 60, 901-4 (2009).

Abstract

Objective

Juvenile idiopathic arthritis (JIA) is characterized by chronic arthritis and an autoimmune etiology. In several autoimmune diseases, including rheumatoid arthritis (RA), an association with the 4q27 locus has been reported. We undertook this study to investigate the possible role of the 4q27 locus in JIA.

Methods

A case-control association study was conducted, with a total of 655 Caucasian JIA patients and 791 healthy controls divided into 2 independent sample sets. The rs6822844 marker in the 4q27 locus was genotyped.

Results

In the first and larger sample set, a 5% decrease in T allele frequency was observed in patients compared with controls (allelic odds ratio [OR] 0.72 [95% confidence interval 0.55–0.95], *P* =0.019), and in the second set, a 3% decrease was observed (allelic OR 0.81 [95% confidence interval 0.61–1.09], *P* =0.169). The combined data set generated an OR of 0.76 (95% confidence interval 0.62–0.93, $P=7.08 \times 10^{-3}$). When the different JIA subtypes were analyzed individually, significant decreases were seen in the subtypes with a polyarticular course of disease (extended oligoarthritis [*P* = 0.019] and rheumatoid factor–negative polyarthritis [*P* = 0.038]).

Conclusion

Our findings suggest that the 4q27 locus, previously reported to be associated with RA, type 1 diabetes mellitus, celiac disease, and psoriatic arthritis, is also associated with susceptibility to JIA.

Introduction

Juvenile idiopathic arthritis (JIA) is a group of heterogeneous disorders characterized by chronic arthritis diagnosed in children younger than 16 years of age (1). Seven different subtypes of JIA can be distinguished according to the International League of Associations for Rheumatology (ILAR) classification (2). The subtypes oligoarthritis (both persistent and extended) and rheumatoid factor (RF)-negative polyarthritis are considered the most homogeneous subtypes, with shared phenotypic features. Systemic JIA has a more distinct phenotype resembling an autoinflammatory syndrome. Although the precise etiology is still unknown, JIA is considered to be an autoimmune disease.

Genetic studies in autoimmune diseases have revealed the presence of shared common autoimmune susceptibility loci (3). In JIA, associations with the major histocompatibility complex locus, PTPN22, and TRAF1/C5 have been described (4–6). The 4q27 locus, a region of strong linkage disequilibrium that includes IL2 and IL21 (the genes encoding interleukin-2 [IL-2] and IL-21, respectively), has been associated with celiac disease, rheumatoid arthritis (RA), type 1 diabetes mellitus (type 1 DM), and psoriatic arthritis (7–9). To determine whether the 4q27 locus is also associated with JIA, we genotyped rs6822844 (which can be used as a proxy for the 4q27 haplotype that is associated with autoimmune disease [8]) in JIA patients and controls.

Patients & Methods

Patient population

A case-control association study was conducted in 2 independent sample sets. These sample sets consisted of Caucasian JIA patients (recruited from pediatric rheumatology centers in The Netherlands [n =327], Belgium [n= 96], Germany [n=95], and Switzerland [n =137]) and healthy Dutch adult controls, who were part of the DNA panel of the Immunogenetics and Transplantation Immunology section at Leiden University Medical Center and consisted of randomly selected unrelated individuals and blood donors.

The first sample set consisted of 328 JIA patients and 465 healthy controls, and the second sample set consisted of 327 JIA patients and 326 controls. In all patients (69% female and 31% male), JIA was diagnosed according to the ILAR revised classification criteria (2). The patients with JIA who were selected for inclusion in the study were primarily those with the oligoarthritis (both persistent and extended) or RF-negative polyarthritis subtype, because of their homogeneous phenotypes.

Forty-four percent of the patients in the overall JIA patient group had persistent oligoarticular disease, 13% had extended oligoarticular disease, 24% had RF-negative polyarticular disease, 3% had RF-positive polyarticular disease, 11% had systemic JIA, and 5% had other JIA subtypes. Because of the small sample size of patients with RF-positive polyarticular JIA and patients with types of JIA categorized as "other," these groups were excluded from the subtype analysis described below. All patients were of self- or parent-reported European Caucasian ethnicity. Written informed consent was obtained from all patients and/or their parents, and the institutional review boards from all participating centers approved the study.

DNA and Genotyping

To test for an association of the 4q27 region with JIA, rs6822844 was typed in both sample sets. DNA was collected through either a blood sample (12% of JIA patients and all controls) or a mouth swab (88% of JIA patients). Genotyping was performed using high-throughput MassArray matrix-assisted laser desorption ionization time of flight mass spectrometry, according to the protocols recommended by the manufacturer (Sequenom, San Diego, CA).

Each of the 384-well plates contained 8 positive controls (DNA obtained from the Centre d'Etude du Polymorphisme Humain, Paris, France), 8 negative controls, and 10% duplicates. The error rate was <1%. Random genotyping failure occurred in 3% of JIA patients and 1% of controls, decreasing the total number of subjects in the analyses to 635 patients and 783 controls.

Statistical analysis

Because of the adherence to an additive model and the lack of evidence for a recessive model, we compared cases and controls using an allelic odds ratio (OR) and 95% confidence interval (95% CI). The common OR of the 2 independent sample sets combined was calculated using the Mantel-Haenszel test. There was no heterogeneity between the 2 sample sets (P >0.05 by Breslow-Day test). Case and control genotype frequencies did not deviate from Hardy-Weinberg equilibrium. All statistical analyses were performed with SPSS, version 14.0 (SPSS, Chicago, IL). P values less than 0.05 were considered significant.

Results

In the first and larger sample set, the T allele frequency was significantly decreased, from 20% in controls to 15% in JIA patients (allelic OR 0.72 [95% CI 0.55–0.95], $P = 0.019$) (Table 1). A similar decrease in T allele frequency (from 18% in controls to 15% in patients) was observed in the second sample set, although it did not reach statistical significance (allelic OR 0.81 [95% CI 0.61–1.09], $P = 0.169$). The common OR in sample sets 1 and 2 combined demonstrated a positive association with JIA ($P = 7.08 \times 10^{-3}$).

Table 1. Genotype and allele frequencies (rs6822844) in JIA patients and controls in 2 independent sample sets*

	Genotype frequency, no. (%)			T allele frequency, no. (%)	Allelic OR (95% CI)	*P*
	GG	GT	TT			
Set 1						
Controls (n = 460)	293 (63.7)	152 (33.0)	15 (3.3)	182 (19.8)	–	–
Patients (n = 311)	224 (72.0)	80 (25.7)	7 (2.3)	94 (15.1)	0.72 (0.55–0.95)	0.019
Set 2						
Controls (n = 323)	218 (67.5)	95 (29.4)	10 (3.1)	115 (17.8)	–	–
Patients (n = 324)	233 (71.9)	85 (26.2)	6 (1.9)	97 (15.0)	0.81 (0.61–1.09)	0.169
Combined data sets						
Controls (n = 783)	511 (65.3)	247 (31.5)	25 (3.2)	297 (19.0)	–	–
Patients (n = 635)	457 (72.0)	165 (26.0)	13 (2.0)	191 (15.0)	0.76 (0.62–0.93)†	7.08×10^{-3}

* JIA = juvenile idiopathic arthritis; 95% CI = 95% confidence interval.
† Mantel-Haenszel odds ratio (OR).

Because of the importance of investigating genetic risk factors in homogeneous, well-defined phenotypic groups, we also analyzed association in the selected JIA subtypes (Table 2). Although a trend toward a decreased T allele frequency was observed among patients with persistent oligoarthritis and systemic JIA, only the subtypes with a polyarticular course of disease (extended oligoarthritis and RF-negative polyarthritis) showed a significant decrease in T allele frequency ($P = 0.019$ and $P = 0.038$, respectively).

Table 2. Analysis of allele frequencies in patients with selected subtypes of JIA and in controls*

	T allele	Allelic OR (95% CI)	*P*
JIA subtype†			
Persistent oligoarthritis (n = 275)	0.165	0.85 (0.65–1.10)	0.207
Extended oligoarthritis (n = 83)	0.114	0.55 (0.34–0.91)	0.019
RF-negative polyarthritis (n = 151)	0.139	0.69 (0.49–0.98)	0.038
Systemic JIA (n = 69)	0.152	0.77 (0.47–1.24)	0.280
Control (n = 783)	0.190	–	–

* RF = rheumatoid factor (see Table 1 for other definitions).
† Diagnosed according to the International League of Associations for Rheumatology revised classification criteria (2).

Discussion

This study demonstrates, for the first time, a positive association of the 4q27 locus (rs6822844) with JIA; a JIA-protective effect of the T allele was observed. When the different JIA subtypes were tested individually, a decrease in T allele frequency was observed in all subtypes. Interestingly, this decrease was significant only in the JIA subtypes that share a polyarticular phenotype. Similar observations have been made regarding the recently identified association with the *TRAF1/C5* region (4), indicating a common genetic constitution underlying the polyarticular JIA phenotype.

In studies of patients originating from different European countries, population stratification cannot be ruled out completely. However, no significant variance in allele frequency in control populations from Western Europe has been reported (7,8), and the allele frequency observed in our control population was very similar to previously reported frequencies. In addition, when allele frequencies in patients originating from the various European countries were compared, no significant difference was found (P = 0.77). Moreover, the degree of underrepresentation of the minor T allele and the 4q27 effect size were similar to those described in studies of other autoimmune diseases, such as RA and type 1 DM (8).

The 4q27 locus consists of a large region of strong linkage disequilibrium encoding the genes *KIAA1109*, *TENR*, *IL2,* and *IL21*. Both *IL2* and *IL21* are likely candidates for association with susceptibility to JIA, since both cytokines are involved in immune activation and regulation pathways.

The IL-2 pathway, in which the interaction between IL2 and IL2 receptor α is central, is involved in T cell proliferation and regulation (10). In mice deficient in *IL2,* T cells have impaired proliferation and effector function in vitro, and lethal autoimmunity develops (11). In addition to the 4q27 locus, the *IL2RA* locus has been associated with several autoimmune diseases (12,13), indicating an important role of the IL2 pathway in immune regulation and maintenance of self-tolerance.

IL21 is also involved in a wide range of immunologic processes. It appears to play a role in autoimmunity by influencing the cellular immune response through both inhibition of suppression by CD4+ regulatory T cells and generation of Th17 cells, as well as by influencing the humoral response (14).

Further analysis of the immunologic pathways involved in JIA may be helpful in identifying the causal gene in this locus. Moreover, sequencing, fine-mapping, and extensive testing of variants of this region will be required to narrow down the region of association and identify the associated gene. Functional testing of all the linked variants associated with disease is also needed.

In conclusion, like RA, type 1 DM, celiac disease, and psoriatic arthritis, JIA is associated with the 4q27 locus. The identification of the 4q27 locus as a risk factor for JIA contributes to the accumulated evidence that one of the genes in this region plays a role in autoimmune diseases in general. In addition, our data indicate that the 4q27 locus contributes to genetic susceptibility to JIA, warranting further research into the biologic pathways that would help to explain this association.

References

(1) Ravelli A, Martini A. Juvenile idiopathic arthritis. Lancet 2007;369:767–78.

(2) Petty RE, Southwood TR, Manners P, Baum J, Glass DN,Goldenberg J, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 2004;31:390–2.

(3) Phelan JD, Thompson SD, Glass DN. Susceptibility to JRA/JIA: complementing general autoimmune and arthritis traits. Genes Immun 2006;7:1–10.

(4) Albers HM, Kurreeman FA, Houwing-Duistermaat JJ, Brinkman DM, Kamphuis SS, Girschick HJ, et al. The TRAF1/C5 region is a risk factor for polyarthritis in juvenile idiopathic arthritis. Ann Rheum Dis 2008;67:1578–80.

(5) Hinks A, Worthington J, Thomson W. The association of PTPN22 with rheumatoid arthritis and juvenile idiopathic arthritis. Rheumatology (Oxford) 2006;45:365–8.

(6) Thompson SD, Moroldo MB, Guyer L, Ryan M, Tombragel EM, Shear ES, et al. A genome-wide scan for juvenile rheumatoid arthritis in affected sibpair families provides evidence of linkage. Arthritis Rheum 2004;50:2920–30.

(7) Van Heel DA, Franke L, Hunt KA, Gwilliam R, Zhernakova A, Inouye M, et al. A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. Nat Genet 2007;39:827–9.

(8) Zhernakova A, Alizadeh BZ, Bevova M, van Leeuwen MA, Coenen MJ, Franke B, et al. Novel association in chromosome 4q27 region with rheumatoid arthritis and confirmation of type 1 diabetes point to a general risk locus for autoimmune diseases. Am J Hum Genet 2007;81:1284-8.

(9) Liu Y, Helms C, Liao W, Zaba LC, Duan S, Gardner J, et al. A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. PLoS Genet 2008;4:e1000041.

(10) Malek TR. The biology of interleukin-2. Annu Rev Immunol 2008;26:453–79.

(11) Sadlack B, Lohler J, Schorle H, Klebb G, Haber H, Sickel E, et al. Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of CD4_ T cells. Eur J Immunol 1995;25:3053–9.

(12) Brand OJ, Lowe CE, Heward JM, Franklyn JA, Cooper JD, Todd JA, et al. Association of the interleukin-2 receptor _ (IL-2R_)/CD25 gene region with Graves' disease using a multilocus test and tag SNPs. Clin Endocrinol (Oxf) 2007;66:508–12.

(13) Vella A, Cooper JD, Lowe CE, Walker N, Nutland S, Widmer B, et al. Localization of a type 1 diabetes locus in the IL2RA/CD25 region by use of tag single-nucleotide polymorphisms. Am J Hum Genet 2005;76:773–9.

(14) Ettinger R, Kuchen S, Lipsky PE. The role of IL-21 in regulating B-cell function in health and disease. Immunol Rev 2008;223: 60–86.