Genetics in juvenile idiopathic arthritis

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CHAPTER 3

THE AUTOIMMUNE 4Q27 LOCUS IS ASSOCIATED WITH JUVENILE IDIOPATHIC ARTHRITIS

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ABSTRACT

Objective

Juvenile Idiopathic Arthritis (JIA) is characterized by chronic arthritis and an autoimmune etiology. In several autoimmune diseases, amongst which rheumatoid arthritis, an association has been found with the 4q27 locus. In this study the possible role of the 4q27 locus in JIA has been investigated.

Materials & Methods

A case-control association study was performed in a total of 655 Caucasian JIA patients and 791 healthy controls in two independent sample sets. The rs6822844 marker in the 4q27 locus was genotyped.

Results

In the first and largest sample set a 5% decrease in T-allele frequency was observed in patients compared to controls (allelic OR 0.72, 95% CI 0.55- 0.95; p= 0.019), together with a decrease of 2.5% in T-allele frequency in the second sample set (allelic OR 0.81, 95% CI 0.61- 1.09; p=0.169). The combined dataset generated an OR of 0.76 (95%CI 0.62- 0.93; p= $7.08*10^{-3}$). When analyzing the different JIA subtypes individually, a significant decrease was seen in the subtypes with a polyarticular course of disease (extended oligoarthritis; p= 0,019 and RF negative polyarthritis; p=0,038).

Conclusion

The 4q27 locus, previously reported to be associated with rheumatoid arthritis, type 1 diabetes, celiac disease and psoriatic arthritis, is also associated with susceptibility to juvenile idiopathic arthritis.

INTRODUCTION

Juvenile Idiopathic Arthritis (JIA) is a group of heterogeneous disorders characterized by chronic arthritis diagnosed in children less than 16 years of age.[1] Seven different subtypes can be distinguished according to the ILAR classification.[2] The subtypes oligoarthritis (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 have been described with the *MHC* locus, *PTPN22* and *TRAF1/C5*.[4-6] The 4q27 locus, a region of strong linkage disequilibrium (LD) including the genes encoding interleukin 2 (*IL2*) and interleukin 21 (*IL21*), has been associated with celiac disease, rheumatoid arthritis, type 1 diabetes and psoriatic arthritis.[7-9] To answer the question 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.

MATERIAL & METHODS

Patient population

A case-control association study was performed in two independent sample sets consecutively. 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 randomly selected by the section Immunogenetics and transplantation Immunology of the Leiden University Medical Center, the Netherlands. The first sample set contained 328 JIA patients and 465 healthy controls, and the second sample set consisted of 327 JIA patients and 326 controls. All JIA patients (69% female, 31% male) were diagnosed according to the revised ILAR classification.[2] The inclusion of patients focused on the oligoarthritis (persistent and extended) and rheumatoid factor (RF) negative polyarthritis subtypes, because of their homogeneous phenotypes. The overall JIA patient group included 44% persistent oligoarthritis, 13% extended oligoarthritis, 24% RF negative polyarthritis, 3% RF positive polyarthritis, 11% systemic JIA patients and 5% patients with other JIA subtypes. Because of the small sample size in the RF positive

polyarthritis patients and JIA patients with "other" subtypes, these groups were excluded from further subanalysis. All patients had a self-reported European Caucasian ethnicity. Written informed consent was obtained from all patients and/or parents and the institutional review boards from all participating centers approved the study.

DNA and genotyping

DNA was collected by means of a blood sample (12% of cases and all controls) or a mouthswab (88% of cases). Genotyping was performed using MassArray matrix-assisted laser desorption ionization time-of-flight mass spectrometry, according to the protocols recommended by the manufacturer (Sequenom, San Diego, California, USA). Each 384-well plates contained 8 positive controls (CEPH DNA), 8 negative controls and 10% duplicates. The error rate was less than 1%. Random genotyping failure occurred in 3% cases and in 1% 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 allelic odds ratios (OR) with 95% confidence interval (CI). The common OR of the two independent sample sets combined was calculated using the Mantel-Haenszel test. There was no heterogeneity between the two sample sets (Breslow-Day test $p<0.05$). Case and control genotype frequencies did not deviate from Hardy Weinberg equilibrium. All statistical analysis was performed with SPSS 14.0. A p-value of <0.05 was considered statistically significant.

RESULTS

To test for association of the 4q27 region with JIA, rs6822844 was typed in two independent sample sets of patients and controls, consecutively. In the first and largest sample set the T-allele frequency was significantly decreased from 20% in controls to 15% in patients (allelic OR 0.72, 95%CI 0.55- 0.95; p=0.019). The same trend in decrease in T-allele frequency from 18% to 15% was observed in the second sample set, although did not reach statistical significance (allelic OR 0.81, 95% CI 0.61- 1.09; p=0.169). The common OR of sample set 1 and 2 combined showed a positive association with JIA ($p=7.08x10^{-3}$) (Table 1).

As it is important to investigate genetic risk factors in homogeneous, well-defined phenotypic groups, we also analyzed association in the different JIA subtypes (Table 2). Although a trend towards a decreased T-allele frequency was observed in persistent oligoarthritis and systemic JIA, only the subtypes with a polyarticular course of

disease (extended oligoarthritis; p= 0,019 and RF negative polyarthritis; p=0,038) showed a significant decrease in T allele frequency.

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

		n	Genotype frequency n (%)			Allele frequency n (%)	Allelic OR (95% CI)°	p
			GG	GT	TT	T		
Set 1	Controls	460	293 (63.7)	152 (33.0)	15 (3.3)	182 (19.8)		
	JIA patients	311	224 (72.0)	80 (25.7)	7 (2.3)	94 (15.1)	0.72 (0.55- 0.95)	0.019*
Set 2	Controls	323	218 (67.5)	95 (29.4)	10 (3.1)	115 (17.8)		
	JIA patients	324	233 (71.9)	85 (26.2)	6 (1.9)	97 (15.0)	0.81 (0.61- 1.09)	0.169
Combined data sets							0.76 (0.62- 0.93)	7.08 $x10^{-3}$ *

* p-value <0.05 considered significant
° in the combined dataset the Mantel-Haenszel OR is used.

Table 2. Analysis of allele-frequencies in the different subtypes of JIA in the overall cohort of cases and controls

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

* p-value <0.05 considered significant
• Diagnosis according to the revised ILAR classification (2)

DISCUSSION

This study shows for the first time a positive association of the 4q27 locus (rs6822844) in JIA, where a protective effect of the T-allele was observed. When testing the different JIA subtypes individually, a decrease in T-allele frequency was observed in all subtypes. Interestingly, only in the JIA subtypes that share a polyarticular phenotype this decrease was significant. A similar observation was made for the recently identified association with the *TRAF1/C5* region indicating a common genetic constitution underlying the polyarticular phenotype.[4]

With patients originating from different European countries, population stratification cannot completely be ruled out. 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 comparing the allele-frequencies in the cases originating from the different European countries, no significant difference was found (p=0,77). Moreover, the underrepresentation of the minor T-allele and the 4q27 effect size is similar as described in other autoimmune diseases such as RA and T1D.[8]

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

The IL2 pathway, in which the interaction between IL2 and the IL2-receptor-alpha (IL2RA) is central, is involved in T-cell proliferation and regulation.[10] Mice deficient in *Il2*, have T-cells with impaired proliferation and effector function in vitro and develop lethal autoimmunity.[11] Not only the 4q27 locus, but also 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 involved in a wide range of immunological processes. IL21 appears to play a role in autoimmunity by both influencing the cellular immune response through inhibition of suppression by CD4+ regulatory T- cells and generating 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 as well as functional testing of all the linked variants associated with disease.

In conclusion, like rheumatoid arthritis, type 1 diabetes, celiac disease and psoriatic arthritis, also JIA is associated with the 4q27 locus. The identification of the 4q27 locus as a risk factor for JIA contributes to the collected 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 the genetic susceptibility to JIA and warrants further research into the biological pathways explaining this association.

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