Concise Report

Lack of association between Tenascin-C gene and spondyloarthritis

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Objectives. We previously identified a new susceptibility region linked to SpA in 9q31–34. Tenascin-C (TNC) appears as one of the best positional and functional candidate genes lying within this SPA2 locus. The objectives of the present study were to identify TNC polymorphisms, and to examine their putative association with SpA.

Methods. We first performed variants screening in 20 independent SpA patients from families with high linkage score to the SPA2 locus, and three unrelated controls: TNCs coding regions (28 exons), intron-exon boundaries and 5′ and 3′-flank regions were fully re-sequenced to identify polymorphisms. Then we genotyped selected variants in 183 independent trios, and assessed their intrafamilial association with SpA by transmission disequilibrium test.

Results. Variants screening allowed us to identify 26 polymorphisms, 7 of which were selected for further study, in addition to an intronic polymorphism previously reported as associated with Achilles tendon injuries. In intrafamilial association test, none of the variants showed significant transmission disequilibrium. Results from analysis restricted to AS were not different from those obtained on the whole SpA group.

Conclusions. TNC was one of the best positional and functional candidate genes within the SPA2 locus. Nevertheless, we found no association between polymorphisms in this gene and SpA. However, we cannot exclude that variants located in intronic regions or in the vicinity of TNC, which were not tested in the present study, could be implicated in the predisposition to SpA.

KEY WORDS: Ankylosing spondylitis, Spondyloarthritis, Association study, Tenascin-C, Genetics.

Introduction

SpA is a frequent inflammatory rheumatic disorder with an estimated prevalence of 0.3% in western European adult population [1]. It refers to a spectrum of manifestations sharing anatomical grounds, and most notably enthesis, i.e. inflammation at the insertion of tendons, ligaments and capsules to the bone [2]. Besides axial skeleton involvement, which is a hallmark of SpA, other frequent manifestations consist of peripheral arthritis, and enthesis, dactylitis and extra-articular symptoms (anterior uveitis, psoriasis and IBD). According to its most salient presentation feature, SpA is classically split into subtypes: AS, which requires advanced radiographic sacroiliitis as a diagnostic criterion; PsA; ReA; IBD-associated arthritis (AIBD); and in the absence of any distinctive feature, uSpA. All these subsets share genetic predisposition, as shown by their tendency to familial aggregation and also by their association with HLA-B27 [3]. The HLA-B27 allele is the first genetic factor found to be associated with SpA and it is likely the most important one [2]. Nevertheless, its contribution to the overall genetic predisposition has been estimated to only 20–30%, and that of the MHC to 40–50% [2]. Thus, non-MHC genes should contribute half of the genetic susceptibility to SpA [4].

We previously performed a genome-wide linkage study in SpA multiplex families, with the objective to identify new susceptibility loci [5]. The multipoint non-parametric linkage (NPL) analysis evidenced five regions of interest (P ≤ 0.01), including the MHC. A replication study of non-MHC regions was then performed on microsatellite D9S1776, at 121.62 cM from p-telomere. One of the two genes mapped to the vicinity of this marker is Tenascin-Cytotactin (TNC). It appeared as one of the most attractive candidate genes located within the SPA2 locus. Indeed, it codes for an extracellular matrix glycoprotein expressed in entheses, which is a major target tissue in SpA [7]. Besides, this gene has been reported as associated with Achilles tendinopathies and ruptures [8]. In the present study, we wished to examine if variations in TNC gene were associated with SpA.

Patients and methods

Patients

French SpA family trios, patients from multiplex families and unrelated healthy controls were recruited as previously described [3]. The study was approved by the Ethics Committee of Cochin Hospital and informed consent was obtained from participants. The diagnosis of SpA was made according to the classification criteria proposed by Amor [9] and/or the European Spondylarthropathy Study Group [10]. AS was diagnosed according to the modified New York criteria [11]. The diagnosis of psoriasis required typical lesions and/or a diagnosis established by a dermatologist. Anterior uveitis was retained after examination by an ophthalmologist. IBD diagnosis was based on endoscopic and histological examination of the gut. ReA was diagnosed according to the criteria published by Willkens [12]. Finally, uSpA was retained when SpA criteria were fulfilled, without AS, PsA, ReA or AIBD.

Variants screening by re-sequencing was performed on a panel of 20 independent patients selected from multiplex families with high linkage score to the SPA2 locus (NPL ≥ 1.34) [5] and three healthy controls. For family-based association study of polymorphisms, 183 independent trios (one SpA patient and his two parents) were analysed. Characteristics of the patients are shown in Table 1.
DNA isolation and variation screening

Genomic DNA was extracted from peripheral blood using standard methods. Coding regions, intron–exon boundaries and 5′- and 3′-flank regions of the TNC gene (28 exons) were amplified and then sequenced in 3′ and 5′ directions with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Primers used for PCR reaction and re-sequencing are described in Supplementary Table 2 (see supplementary data available at Rheumatology Online). DNA sequences were analysed using SeqScape software (Applied Biosystems).

Genotyping of polymorphisms

Five single nucleotide polymorphisms (SNPs) (rs13321, rs1330361, rs2104772, rs1757095 and rs3748166) were genotyped by mean of customized Illumina BeadChip (San Diego, CA, USA) using the GoldenGate assay according to the manufacturer’s recommendation, at the French National Genotyping Center (Evry, France). The rs45602433 polymorphism was genotyped using melting curve analyses (LightCycler System, Roche, Meylan, France). Ensembl database (http://www.ensembl.org/index.html) was used for the polymorphisms annotation and positioning. Primers and hybridization probes used for PCR amplification are described in Supplementary Table 2 (see supplementary data available at Rheumatology Online).

The CA-repeat containing regions (microsatellites 17 and 19) were first amplified by PCR [sequences of the primers shown in Supplementary Table 2 (see supplementary data available at Rheumatology Online)]. For analysis of repeat allele length and relative ratios, instrumentation and reagents from Applied Biosystems were used (ABI PRISM 3100 Genetic Analyzer and Gene Mapper software).

Statistical analysis

All genotypes were screened for Mendelian inheritance using FBAT (version 2.0.0c) package and PedCheck version 1.1. The departure from Hardy–Weinberg equilibrium in unrelated individuals was assessed using the PEDSTATS version 0.6.11.

Family-based association analysis was carried out using the FBAT software. We used the additive model of FBAT to compute the statistic test, and gave P-value corresponding to this test statistic. Two affection traits were considered successively: SpA and AS. A P < 0.05 was considered as statistically significant.

Results

Variants screening

We started this study by re-sequencing the 28 exons, the intron–exon boundaries and the 5′- and 3′-flank regions of the TNC gene spanning 97.63 kb, in 20 independent SpA patients from families with high linkage score to the SPA2 locus [5], and three controls. Overall, we sequenced 12 462 base pairs (bp) per individual, of which 6606 bp were located in coding regions. We identified 26 polymorphisms which included 4 non-synonymous coding SNPs, 8 synonymous coding SNPs, 11 intronic SNPs and 3 intronic dinucleotide repeats. All these variants are annotated in public databases, such as Ensembl or UCSC (http://genome.ucsc.edu/cgi-bin/hgGateway). The average rate of polymorphisms was 1.82/kb of DNA in the coding regions, and 2.39/kb in the non-coding regions. Most of these polymorphisms were distributed among patients as well as controls.

Seven polymorphisms that we considered as the most relevant were selected, in order to be genotyped in the trios: the four non-synonymous coding SNPs (rs13321, rs2104772, rs45602433 and rs1757095), two intronic tag-SNPs (rs1330361 and rs3748166) and the CA-repeat polymorphism located in the beginning of the 19th intron (possibly implicated in splicing modifying). Besides, we decided to explore whether another CA-repeat polymorphism located in the middle of the 17th intron, and identified through the literature search [8], was associated with the disease.

Association study

We genotyped 549 individuals composing 183 trios for the eight selected polymorphisms. None of the genotype distributions in the founder’s subjects differed significantly from those expected from Hardy–Weinberg equilibrium. Two affection traits were considered for association analyses: SpA and AS (101 out of 183 affected children presented with AS phenotype). Results of the single locus association tests under FBAT allelic additive model between each polymorphism and SpA and AS are shown in Table 2. None of the variants showed statistically significant transmission disequilibrium P-value neither with the SpA phenotype nor with the AS one. Despite two different phenotypes being analysed herein, allelic frequencies and association P-values were almost identical for SpA and AS trait. Finally, haplotype analyses performed using the hbat option of FBAT did not show any significant association either (data not shown).

Discussion

By using a genome-wide linkage approach, we have recently identified a new region of susceptibility to SpA in 9q31–34, which we called SPA2 [5]. According to our prediction, it should contain major susceptibility factor, accounting for 20–25% of the non-MHC genetic predisposition to SpA [2]. This region spans 17.44 Mb, to which about 120 genes, and a number of predicted coding sequences, have been mapped. Most interestingly, it is also one of the three regions paralogous to the MHC, which bears the most important genetic susceptibility load for SpA. Thus, one or more gene(s) in the SPA2 locus having paralogous counterpart in the MHC could be implicated in the genetic susceptibility to SpA.

One of our strategies for identifying genetic factor implicated in the predisposition to SpA at this new locus was a positional and functional candidate-gene approach. Here, we explored the implication of TNC in the predisposition to SpA and AS. Regarding location, the maximum NPL score in our study [5] was centred on marker D9S1776, in 9q33.1. TNC which is one of the two genes lying within 200 kb of this marker was one of the most attractive functional candidate gene for several reasons. First, TNC (or hexabrachion), one of five members of the tenasin...
family of genes, codes for 190–240 kDa extracellular matrix protein with a very restricted expression in tendons, ligaments, perichondrium and periostium, where it is specially induced during tissue repair [13, 14]. Most interestingly, TNC expression has been shown in fibrocartilage, and more specifically in the entheses, which is a major target tissue in SpA [7]. It is increased in synovitis [15], and other conditions or tissues related to the spectrum of SpA, such as IBD, and psoriatic skin [16, 17]. In all, 7 of 26 polymorphisms detected through re-sequencing were 2.39, we can indicate a protective effect. eFBAT TDT indicates an increased risk, whereas a negative Z indicates a protective effect. *FBAT TDT P-value.

In conclusion, albeit TNC was one of the most attractive candidate genes in the SPA2 locus, we failed to identify any association between polymorphisms of this gene and SpA. However, we cannot exclude that some other variants located in intronic or intergenic regions, which were not tested herein could still be implicated in predisposition to this disease. A systematic approach using tag-SNPs covering the whole SPA2 region is under way to explore such possibility (Zinovieva et al., manuscript in preparation).

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**Supplementary data**
Supplementary data are available at *Rheumatology* Online.

**References**