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Original article

A genetic association study of the *CLEC12A* gene in rheumatoid arthritis

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ABSTRACT

Objective: The *CLEC12A* gene codes for an immune inhibitory receptor that maps to *12p13.2*. Since an increase in *CLEC12A* mRNA correlates with rheumatoid factor values greater than 40 IU/ml in rheumatoid fibroblast-like synovial cells, this study assessed the potential of an association between *CLEC12A* and rheumatoid arthritis (RA) using a phenotype-based approach.
Methods: A discovery cohort of Western European ethnicity was genotyped for eight tag single nucleotide polymorphisms. Statistical analyses relied on the transmission disequilibrium test, relative risk and 95% confidence interval (CI) calculations. Observed haplotype frequencies were compared to expected frequencies using a family-based association test. Statistically significant associations were further tested in a second cohort of unrelated West-European RA patients.
Results: An overtransmission of the *C* allele of the rs1323461 tag single nucleotide polymorphism was observed (56.6% of allele *C* transmission, $P = 0.046$) in the discovery cohort. The relative risk of the *AC* and *CC* genotypes when compared to the *AA* genotype was high (relative risk = 4.08; 95% CI: 1.52–10.95, uncorrected $P = 2.1 \times 10^{-3}$), particularly in the subgroup of erosive RA (relative risk = 5.27; 95% CI: 1.53–18.19, uncorrected $P = 2.1 \times 10^{-3}$), both remaining statistically significant after conservative Bonferroni's correction. The *CGAGCCGA* haplotype was observed more frequently than expected ($P = 0.013$). In the second cohort, the *C* allele had a tendency to be more frequent in RA patients (82.4%) than controls (79.2%) ($P = 0.069$).
Conclusion: We report a potential genetic association of *CLEC12A* with RA. Since *CLEC12A* encodes for the myeloid inhibitory C-type lectin-like receptor that modulates cytokine synthesis, this receptor may contribute to the pathogenesis of RA.

1. Introduction

Rheumatoid arthritis (RA) is a disabling autoimmune disorder that affects peripheral joints leading to cartilage destruction and bone erosions [1]. Complex interactions between environmental factors (e.g. smoking) and several genetic determinants lead to the development of RA [2]. The key genetic factor that predisposes to RA, the *HLA* locus, represents about 30% of the genetic component of this disorder. In search of the remaining genetic determinants of RA, over 30 additional genetic loci have been identified by genome-wide linkage or association studies during the past 7 years [3–5].

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Several lines of evidence indicate that genes within the natural killer gene complex (NKC) are linked to autoimmune disorders including RA [6,7]. The NKC maps to chromosome 12p13.1 in humans and the syntenic region on chromosome 6 in mice [8]. It encompasses several multi-gene families that encode for C-type lectin receptors expressed either by NK or myeloid cells [9]. The C-type lectin receptors expressed by myeloid cells are distributed in two clusters, namely, the dectin-1 family and the dendritic cell immunoreceptor (DCIR) family that is located centromeric to the NKC. The *CLEC4A* gene in the DCIR cluster was recently reported to be associated with susceptibility to anti-cyclic citrullinated peptide (CCP) negative RA [10]. The *CLEC4A* gene encodes for DCIR, an inhibitory receptor with an immunoreceptor tyrosine inhibitory motif in its cytoplasmic domain [9]. Corroborating the genetic association between *CLEC4A* and RA, *CLEC4A* knock-out mice are more susceptible to collagen-induced arthritis than their wild-type litter mates [11]. DCIR is expressed by monocytes, macrophages, neutrophils, dendritic cells and B lymphocytes and is involved in multiple processes, from the inhibition of IFN-α production in plasmacytoid dendritic cells and of Ca^{2+} mobilization in B cells to antigen presentation [12].

Similar to *CLEC4A*, the *CLEC12A* gene also maps to the NKC and codes for a C-type lectin with an immunoreceptor tyrosine-based inhibitory motif in its cytoplasmic domain known as the myeloid inhibitory C-type lectin-like receptor (MICL) [13–15]. MICL is also expressed by cells of the myeloid lineage such as macrophages and dendritic cells and modulates cytokine synthesis [16,17]. The cross-linking of MICL during the stimulation of monocyte-derived dendritic cells with LPS, for instance, suppresses LPS-induced TNF-α, IL-12p40 and IL-12p70 production. Since (i) MICL shares structural and functional characteristics with DCIR and (ii) an increased gene expression of *CLEC12A* in rheumatoid fibroblast-like synovial cells was previously reported to correlate with elevated rheumatoid factor (RF) values [18], we sought to determine whether a genetic association exists between the *CLEC12A* gene and RA by combining a candidate gene study and phenotype-based approach.

2. Methods

The present study was approved by the Ethics Committee of Hôpital Kremlin-Bicêtre (Paris, France) and all individuals provided an informed consent before participating in the study.

2.1. Patients and families

The discovery cohort comprising 384 trio families was previously reported in detail in the literature [19,20]. Trio families are composed of one RA patient and both parents, all of Western European ethnicity as determined by grandparental origin. The families were recruited through the European Consortium on Rheumatoid Arthritis Families in France, Italy, Portugal, Spain, Belgium and The Netherlands. Index cases of trio families have a phenotype that fulfilled the American College of Rheumatology 1987 criteria for RA [21] according to the rheumatologist in charge of the patient and the information in the patient's clinical record [19,20]. Presence of RF was determined by a positive result by Latex fixation, Waaler Rose assay, Laser Nephelometry, or IgM RF by ELISA method (Quanta, Lite RFIgM, INOVA diagnostics, San Diego, CA, USA). The anti-CCP status was provided by an anti-CCP antibody ELISA (Immunoscan RA, Euro-Diagnostic, Malmö, Sweden). Presence of erosions at baseline was defined by the presence of at least one rheumatoid erosion at X-ray examination. Families with an additional affected sibling and RA patients under 18 years of age were excluded from the study.

A second cohort consisting of a case-only sample of 747 unrelated RA patients from Western European ethnicity, fulfilling the same 1987 RA classification criteria as the discovery cohort, was also available to further investigate statistically significant associations identified in the discovery cohort.

2.2. TagSNP selection and genotyping

TagSNPs were selected from the HAPMAP database (http://www.hapmap.org/) in a 15.6 kb region encompassing the *CLEC12A* gene (HapMap Data Phase II/Rel#2, Nov08, on NCBI B36 assembly, dbSNP b126; chr12:10014572..10030169, accessed on the 10th of June 2010). We selected TagSNPs with a minor allele frequency greater or equal to 0.05 and r^2 at 0.8 for the Caucasian European Union population with the aggressive Tagger program. An additional SNP located in exon 6, rs479499, that introduces a non-synonymous amino-acid substitution (Lys211Gln) that can have potential functional consequences was also added to this selection. A second additional SNP located downstream of the *CLEC12A* gene, rs770738, was also included in this study since it was reported to exhibit a moderate association with RA [22]. This SNP was recently merged to rs679982. Genotyping of TagSNPs relied on the TaqMan allelic discrimination assay and was performed by KBiosciences (Hertfordshire, UK). Centre d'étude du polymorphisme kumain DNA samples were co-genotyped with all our samples, with no inconsistencies detected. The genotyping success rate was 98.5% in the discovery sample and 97.5% in the second cohort.

2.3. Statistical analysis

For each TagSNP, Hardy-Weinberg equilibrium was assessed with a conformity Chi-square test in the virtual control group consisting of both parental chromosomes, one from the mother and one from the father, which were not transmitted to the affected offspring. A transmission disequilibrium test (TDT) allowed the comparison of the transmission of a given parental allele from a heterozygous parent to the RA affected patient with an expected transmission of 50% (Mendel's law) [23]. TDT analysis SNP by SNP was performed with Genehunter V2.0β and with the FBAT program. The distribution of genotypes between RA patients and virtual controls was analyzed using Fisher's exact test only for SNP which provided significant transmission disequilibrium. The genotype relative risk (RR) according to Haldane was calculated as well as the 95% confidence interval (95% CI) [24,25]. Pairwise linkage disequilibrium of the eight SNPs was obtained with the FBAT program [26]. Haplotypes consisting of the rs2760953, rs770750, rs770749, rs650368, rs1323461, rs479499, rs588272 and rs679982 SNPs were estimated with the FBAT program [26]. For all haplotypes with an overall frequency greater than 2%, observed haplotype frequencies were compared to expected frequencies. Similar statistical analyses were performed on three subgroups of the global sample of 384 trio families for the rs132346 SNP. In one of the subgroups (345 trio families), the index cases are positive for RF, in a second subgroup (168 trio families), the index cases are positive for anti-CCP antibodies and in the third subgroup (356 trio families), the index cases have erosive RA. Conservative Bonferroni's correction was applied for multiple testing in the discovery cohort and uncorrected $P < 6.25 \times 10^{-3}$ (0.05/8) was considered statistically significant in the single SNP analysis, and uncorrected $P < 5.55 \times 10^{-3}$ (0.05/9) was considered statistically significant in the haplotype analysis. The power of our sample of 384 trio families to provide an association with an OR of 1.6 is about 80% considering the hypothesis of one gene following an additive model of