

Concise Report

Genetic variation in proteins of the cryopyrin inflammasome influences susceptibility and severity of rheumatoid arthritis (The Swedish TIRA project)

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Objectives. The genetic background to RA is incompletely understood. As new cytokine-targeted therapies emerge, early predictors of disease severity are becoming increasingly important. The inflammasomes are essential regulators of cytokine production. We investigated whether two polymorphisms in the genes encoding cryopyrin (*CIAS1*) and TUCAN (*CARD8*) influence susceptibility and disease course in RA.

Methods. Genotype frequencies were assessed in 174 Swedish patients with early RA and 360 population-based controls without rheumatic disease. Genotypes were categorized according to the presence (+) or absence (–) of two wild-type alleles and compared between patients and controls. In the RA patients, antibodies towards cyclic citrullinated peptides (anti-CCP) and the ‘shared epitope’ (SE) were assessed, and medication and measures of disease activity were monitored regularly during 3 yrs.

Results. The combination of *CIAS1/TUCAN* –/–, as compared with *CIAS1/TUCAN* +/+, was significantly more common among patients than in controls [odds ratio (OR) 2.2, 95% CI 1.03–4.6]. This association was strengthened when patients were divided into anti-CCP+ [OR 2.8 (1.1–6.7)] or presence of ≥ 1 SE copy [OR 2.8 (1.3–6.2)]. At most time-points during the 3-yr follow-up, patients with *CIAS1/TUCAN* –/– showed significantly higher disease activity. Furthermore, *CIAS1/TUCAN* –/– patients proved to be much more likely to receive TNF-blocking therapy [relative risk 20 (2.6–149)].

Conclusions. Compound polymorphisms in *CIAS1* and *TUCAN* associate with RA susceptibility and severity. The cryopyrin inflammasome needs further attention regarding a possible aetiopathogenetic connection with RA.

KEY WORDS: Disease course, Genetics, Inflammasome, Rheumatoid arthritis.

Introduction

Apart from a few genetic connections, e.g. HLA-DRB1/‘shared epitope’ (SE) and *PTPN22*, most of the complex hereditary background to RA remains unidentified. Cytokine imbalances play central roles in chronic inflammatory conditions, and targeting of TNF or IL-1 is a well-documented treatment modality in RA. Overall, TNF-blockers have superior anti-inflammatory efficacy compared with IL-1 receptor antagonist (anakinra) [1]. Nevertheless, IL-1 is considered a key mediator of cartilage and bone injury [2], and distinct cytokine patterns may relate to different therapy responsiveness in different subgroups [3].

Formation of biologically active IL-1 β depends on cleavage of its precursor by caspase-1. Recently, cytosolic protein complexes called ‘inflammasomes’ were shown to activate caspase-1 and regulate IL-1 β formation [4, 5]. Formation of the cryopyrin inflammasome by assembly of NALP3 (cryopyrin), ‘apoptosis-associated speck-like protein’ (ASC) and TUCAN, activates two caspase-1 molecules ultimately resulting in IL-1 β production [5].

Mutations in the cryopyrin-encoding gene *CIAS1* are strongly associated with rare auto-inflammatory conditions characterized by excessive IL-1 production, e.g. Muckle–Wells syndrome (MWS) and familial cold-induced auto-inflammatory syndrome (FCAS), which respond favourably to anakinra [6, 7]. In a preceding study at our departments, genes encoding the proteins of the cryopyrin inflammasome were scanned for polymorphisms

in a young man presenting with sacroiliitis and severe periodic fever, and who responded dramatically to anakinra. Although his condition did not resemble MWS or FCAS, he was heterozygous regarding two non-synonymous polymorphisms; Q705K in *CIAS1* and C10X in *TUCAN* [8]. The Q705K polymorphism of *CIAS1* has been described previously (alternatively numbered Q703K) and proposed to be a low-penetrance mutation in FCAS [9]. The C10X polymorphism of *TUCAN* (rs2043211) prematurely terminates this normally 643 amino acid protein and is most likely of functional importance. This single-nucleotide polymorphism (SNP) was reported to associate with Crohn’s disease [10].

Considering the benefits of early aggressive pharmacotherapy in RA and the growing arsenal of biologics, there is an urge to identify predictors of disease course and therapy response to enable accurate individual therapy decisions. Although pro-inflammatory cytokines are hallmarks of the disease, little is known about the inflammasomes in RA. This study is the first to analyse if genotypes in proteins of the cryopyrin inflammasome influence RA susceptibility and severity.

Patients and methods

Subjects

One hundred and seventy-four RA patients (70% women, mean age 56 yrs) were recruited to an inception cohort (the Swedish TIRA project) at 10 Swedish rheumatology units during 27 months 1996–98 [11]. They fulfilled $\geq 4/7$ of the ACR criteria [12] (95% of the patients), or had morning stiffness ≥ 60 min, symmetrical arthritis and small joint arthritis. Symptom duration (joint-swelling onset) was ≤ 12 months and ≥ 6 weeks. At baseline, 63% had RF and 68% had anti-CCP. The patients were followed regularly during 3 yrs. Disease activity was assessed by CRP, ESR, physician’s global assessment (PGA) of disease activity on a 4-degree ordinal scale and disease activity score (DAS28) [13]. DMARDs, analgesics and corticosteroids were instituted as

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judged appropriate by the physician. Anti-TNF therapy became available during the study period, and was prescribed when response to conventional DMARDs including methotrexate was poor. Three hundred and sixty controls (63% women, mean age 57 yrs) were randomly selected among a population-based reference material from the same geographical area. Individuals reporting rheumatic disease were omitted. All study subjects gave written informed consent. The study protocol was approved by the regional ethics committee.

Laboratory analyses

Autoantibodies. Agglutinating RF was measured at the local laboratories of the participating rheumatology units. Anti-CCP was analysed by a commercial assay (Immunoscan RA CCP2; Eurodiagnostica, Arnhem, The Netherlands); cut-off limit ≥ 25 units.

Genotyping. The Q705K polymorphism of *CIAS1* was detected by a Megabace™ SNUpe genotyping kit (GE Healthcare, Amersham, UK). DNA from peripheral blood was amplified using the primers 5'-TCCTCTTGGCCTGGTAAAC-3' and 5'-CAAGAAGAAGCTGGCGAGGAA-3'.

TUCAN-C10X was detected, using the primers 5'-TGCTATCA TCAGGCACCTACC-3' and 5'-GAGCTTGGGAGGACACT-3'. The SNP-specific primer used was 5'-AGAGGCAGAGCC ATTATTG-3'. SE was defined and analysed as previously described [14].

Statistical analyses

Genotype frequencies were compared by the chi-square test with Yates' correction or Fisher's exact test, where appropriate. Odds ratios (ORs), relative risks (RRs) and 95% CIs were calculated. Disease activity measures were compared by the Mann-Whitney U-test. Two-sided *P*-values < 0.05 were regarded as significant.

Results

Genotype frequencies

CIAS1 and *TUCAN* genotype frequencies are detailed in Table 1. None of the polymorphisms deviated significantly from the Hardy-Weinberg equilibrium among patients or controls. Compared with *CIAS1-QQ*, genotypes with the variant allele present in *CIAS1* (*CIAS1-QK* or *CIAS1-KK*) were non-significantly more prevalent among patients than controls (OR 1.2, 95% CI 0.7–2.1). Similar trends were seen when *TUCAN-CX* and *TUCAN-XX* was compared with *TUCAN-CC* [OR 1.4, (0.9–2.1) and 1.3, (0.7–2.3), respectively]. Genotypes were then grouped according to the presence (+) or absence (–) of wild-type alleles in *CIAS1* and *TUCAN*, respectively. Compared with those homozygous for the wild-type allele in both genes (*CIAS1/TUCAN* +/+), presence of at least one variant allele in both genes (*CIAS1/TUCAN* –/–) was significantly more common among RA patients than in controls [OR 2.2 (1.03–4.6), *P* = 0.04] (Table 2). Subgroup analyses were performed according to SE and anti-CCP status. The *CIAS1/TUCAN* –/– combination did not appear to associate with SE-negative RA [OR 0.8 (0.1–3.9), *P* = 1.0], whereas in patients with ≥ 1 SE copy, *CIAS1/TUCAN* –/– was significantly overrepresented [OR 2.8 (1.3–6.2), *P* = 0.01].

TABLE 1. Genotype frequencies (%) in patients (*n* = 174) and controls (*n* = 360)

| | <i>TUCAN-CC</i> | | <i>TUCAN-CX</i> | | <i>TUCAN-XX</i> | |
|-----------------|-----------------|----------|-----------------|----------|-----------------|----------|
| | Controls | Patients | Controls | Patients | Controls | Patients |
| <i>CIAS1-QQ</i> | 130 (36) | 54 (31) | 137 (38) | 74 (42) | 41 (11) | 17 (10) |
| <i>CIAS1-QK</i> | 30 (8) | 11 (6) | 14 (4) | 10 (6) | 5 (1) | 7 (4) |
| <i>CIAS1-KK</i> | 1 (0) | 0 (0) | 1 (0) | 2 (1) | 1 (0) | 0 (0) |

Furthermore, the OR was increased in patients possessing two SE copies [3.3 (1.06–10), *P* = 0.025]. *CIAS1/TUCAN* –/– was associated with anti-CCP-positive RA [OR 2.8 (1.1–6.7), *P* = 0.025], but not with anti-CCP-negative disease [OR 1.3 (0.3–5.6), *P* = 0.7]. The most pronounced association was found among patients positive for both SE and anti-CCP [OR 3.5 (1.4–8.9), *P* = 0.005] (Table 2).

Disease progression

Disease activity and severity measures were, if not stated otherwise, compared in the same manner as genotype frequencies, i.e. *CIAS1/TUCAN* –/– vs *CIAS1/TUCAN* +/+. At inclusion, there were no differences in the proportion of patients who were prescribed DMARDs. During follow-up, DAS28, ESR, PGA and CRP were significantly higher in *CIAS1/TUCAN* –/– patients at several time-points (data not illustrated). Furthermore, a significantly smaller proportion of *CIAS1/TUCAN* –/– patients were in remission after 3 yrs compared with *CIAS1/TUCAN* +/+ (13% vs 43%, *P* = 0.035). Conventional DMARD or oral glucocorticoid therapy did not differ significantly between the groups, although there was a trend towards *CIAS1/TUCAN* –/– patients more often receiving a combination of ≥ 2 DMARDs (data not shown).

Within 5 yrs of diagnosis, 23 (13%) of the patients had been prescribed TNF-blockers (infliximab or etanercept). A case-case analysis was performed to investigate the impact of *CIAS1* and *TUCAN* genotypes on the risk of receiving TNF-blocking therapy. Among patients presenting *CIAS1/TUCAN* +/+, only one received such therapy (2%), as compared with *CIAS1/TUCAN* –/– patients where seven patients (37%) were treated with TNF-blockers. The corresponding numbers of patients not receiving TNF-blocking therapy were 52 (98%) and 12 (63%), respectively. The RR of receiving TNF-blockers was independently raised in patients carrying a risk allele in either *CIAS1* [RR 3.0 (1.4–6.2), *P* = 0.008] or *TUCAN* [RR 4.1 (1.3–13), *P* = 0.02] as compared with their respective homozygous wild-type counterparts. When analysing the genes combined, patients carrying ≥ 1 risk allele at both loci showed a greatly increased risk of receiving anti-TNF therapy [RR 20 (2.6–149), *P* < 0.001].

Discussion

We found the combined genotypes of *CIAS1* and *TUCAN* to be associated with increased RA susceptibility and a more severe disease course. As the proportions of *CIAS1/TUCAN* +/- and *CIAS1/TUCAN* –/+ were similar in patients and controls, it appears that a variant allele must be present at both loci to increase the risk of RA. Hypothetically, this may contribute to the fact that neither of these positions are clearly pointed out as susceptibility loci in genome-wide screenings of RA families [15]. The patients were followed longitudinally, revealing that *CIAS1/TUCAN* –/– cases had higher disease activity measures and were at greatly increased likelihood to receive TNF-blocking therapy. Since a majority of the patients was treated early with DMARDs, it cannot be disclosed whether the difference in disease course is attributable to therapy responsiveness or reflects distinct disease

TABLE 2. Number of individuals (%) carrying at least one variant allele (*CIAS1/TUCAN* –/–) compared with those carrying only wild-type alleles at both loci (*CIAS1/TUCAN* +/+)

| | <i>CIAS1/TUCAN</i> +/+ | <i>CIAS1/TUCAN</i> –/– | OR (95% CI) | <i>P</i> -value |
|--|------------------------|------------------------|-----------------|-----------------|
| Controls (<i>n</i> = 360) | 130 (36) | 21 (6) | Reference group | - |
| RA total (<i>n</i> = 174) | 54 (31) | 19 (11) | 2.2 (1.03, 4.6) | 0.04 |
| CCP+ RA (<i>n</i> = 94) | 27 (28) | 12 (13) | 2.8 (1.1, 6.7) | 0.03 |
| SE ≥ 1 copy (<i>n</i> = 129) | 38 (30) | 17 (13) | 2.8 (1.3, 6.2) | 0.01 |
| SE ≥ 2 copies (<i>n</i> = 50) | 13 (26) | 7 (14) | 3.3 (1.06, 10) | 0.03 |
| CCP+ SE ≥ 1 copy (<i>n</i> = 81) | 21 (26) | 12 (15) | 3.5 (1.4, 8.9) | 0.005 |

phenotypes *per se*. The fact that disease activity markers did not differ at inclusion supports the former notion.

The expression pattern of inflammasome proteins remains to be established. However, recent work reveals that most immune cells express cryopyrin in the cytoplasm, and it has been reported that RA patients express more synovial cryopyrin than OA patients [16, 17]. The polymorphisms investigated here were selected on the basis of their presence in a patient with severe periodic fever and dramatic response to anakinra therapy. Although truly functional studies of the *CIAS1* and *TUCAN* SNPs are lacking, the leucocytes of this patient displayed increased spontaneous caspase-1 activity, increased IL-1 β levels and delayed apoptosis compared with cells from age- and gender-matched controls [8]. While not conclusive of a causal relationship, the results support the hypothesis that aberrant IL-1 β regulation by the cryopyrin inflammasome explains the association with a more aggressive disease course seen in this early RA cohort.

The *CIAS1/TUCAN* compound polymorphism shows stronger association with the SE+/anti-CCP+ RA cases. Apart from contributing to a more aggressive disease course [11], this raises interesting questions regarding an aetiopathogenic importance of this SNP complex in RA. It is not known whether pro-inflammatory cytokines are involved in the loss of human self-tolerance, but in autoimmune-prone mice it was recently shown that IL-1 β can induce expansion of autoreactive T-cells and promote their survival at peripheral check-points such as CD4⁺CD25⁺FoxP3⁺ T-cells [18]. IL-1 β -promoted autoimmune responses in RA could provide an explanation to the increased risk of developing SE+/anti-CCP+ RA in *CIAS1/TUCAN* -/- individuals. As opposed to expression of citrullinated proteins, the presence of anti-citrullinated-protein autoantibodies is highly RA-specific [19], and their strong association with SE is well-known [20]. Hence, aberrant IL-1 β production could hypothetically add to the risk of initiating an antigen-driven immune response towards citrullinated proteins in SE-positive individuals.

One caveat of the present study is the relatively small number of patients, resulting in wide CIs. We believe, however, that the consistent trends of the results allow the conclusion that *CIAS1* and *TUCAN* genotypes influence RA susceptibility and outcome. The number of statistical tests performed could raise the objection that correction of *P*-values should be performed. However, since the hypotheses were *a priori* decided and combining the two SNPs is highly biologically relevant, we acknowledged uncorrected two-sided *P*-values at the standard level as statistically significant. Also, we performed gene-gene interaction analyses according to the statistical methods used by Källberg *et al.* [21], and the results were completely consistent.

An advantage in the present study is the inception cohort design, allowing analysis of the early disease course and inclusion of mild cases of RA that could otherwise be under-represented due to referral bias. However, since *CIAS1* and *TUCAN* associate with disease severity, further studies based on materials with higher numbers of patients with established RA are encouraged to confirm and establish our findings that the *CIAS1-Q705K/TUCAN-C10X* combination confers RA susceptibility. In patients carrying variant alleles in *CIAS1* and *TUCAN*, traditional DMARD treatment was clearly not sufficient to bring disease activity under control. It is an intriguing thought that these patients would have benefited from early IL-1-blocking therapy.

To conclude, in this first investigation of genetic variants of the cryopyrin inflammasome in RA, we found associations with disease susceptibility and severity. In combination with functional studies on the *CIAS1* and *TUCAN* genotypes, the results must be confirmed, preferably in patients with long-standing and severe RA. The inflammasome could bring new insight into some of the still concealed clues to RA pathogenesis and hypothetically introduces a possibility to identify RA patients responsive to IL-1-targeted therapy.

Rheumatology key message

- Inflammasome genetics may influence RA susceptibility and disease course.

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