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CASPASE-12 and rheumatoid arthritis in African-Americans

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ABSTRACT

CASPASE-12 (CASP12) has a down-regulatory function during infection, and thus may protect against inflammatory disease. We investigated the distribution of CASP12 alleles (#rs497116) in African-Americans (AA) with rheumatoid arthritis (RA). CASP12 alleles were genotyped in 953 RA patients and 342 controls. Statistical analyses comparing genotype groups were performed using Kruskal-Wallis non-parametric ANOVA with Mann-Whitney U tests and chi-square tests. There was no significant difference in the overall distribution of CASP12 genotypes within AA with RA, but CASP12 homozygous patients had lower baseline joint narrowing and total disease scores. CASP12 homozygosity appears to be a subtle protective factor for some aspects of RA in AA patients.
1. Introduction

The pathogenesis of rheumatoid arthritis (RA) is multi-factorial, with contributions from environmental factors and a strong genetic component. To date, at least 46 genes are known risk factors for the development of RA (Eyre et al. 2012; Killock 2013). We sought to determine the role of CASPASE-12 (CASP12), a well-studied gene whose murine homologue is involved in cellular stress transmitted via the endoplasmic reticulum (Rao et al. 2001), in the development of RA.

Most humans lack a functional CASP12 gene. A pseudogene (CASP12p1) resulting from a premature termination mutation is found in all Caucasians and East Asians examined, and in 80% of people of African lineage (Saleh et al. 2004). In approximately 20% of persons of recent African descent, the pseudogene is rescued by a single nucleotide polymorphism (#rs497116; A->G) that converts the premature stop codon into a functional one encoding Arg. This functional allele is a risk factor for sepsis in persons of African descent due to its ability to down-regulate inflammatory cytokines such as interleukin (IL)-1β, in responses to pro-inflammatory mediators such as bacterial lipopolysaccharide (Saleh et al. 2004). In individuals of African descent with severe sepsis, the mortality rate was 54% in individuals with the CASP12 allele, compared with 17% in individuals with only the CASP12p1 allele. Knockout mice lacking CASP12 are also less susceptible to sepsis and death than wild-type (Saleh et al. 2006). This contrasts with susceptibility to fungal septicemia, which does not correlate with CASP12 genotype (Rosentul et al. 2011).

A corollary to the observation that CASP12 is a risk factor for sepsis would be that CASP12 is protective against some inflammatory autoimmune diseases. We decided to test this hypothesis with the Consortium for Longitudinal Evaluation of African-Americans with Early Rheumatoid Arthritis (CLEAR) and the Veterans Administration Rheumatoid Arthritis (VARA) Registry of African-Americans with RA. CLEAR and VARA are multicenter chronic disease registry programs enrolling self-defined African Americans with RA as defined by the revised
American College of Rheumatology (ACR) classification criteria. Both have been described previously (Bridges et al. 2003; Mikuls et al. 2007).

2. Methods

2.1 Genotyping

Single nucleotide polymorphism genotyping of CASP12 (#rs497116; A->G) was performed in a single tube assay (Waterfall and Cobb 2001) by allele-specific polymerase chain reaction (PCR) using primers containing a mismatch introduced at the second base from the 3’ end to improve allele specificity (Pettersson et al. 2003). PCR products were analyzed on 1% agarose gels. To verify results, 200 bp from the polymorphic region of CASP12 exon 2 was amplified and subjected to DNA sequencing using a high-fidelity DNA polymerase. Sequence analysis was sensitive enough to discriminate between homozygous and heterozygous individuals.

2.2 Statistical analysis

The relationship of discrete variables to genotype was assessed with Fisher's Exact test. The relationship of continuous variables to genotype was first assessed with a Kruskal-Wallis non-parametric ANOVA. If the ANOVA was significant (p<0.05), a Mann Whitney U test was used to determine which groups were different.

3. Results

To determine if CASP12 would have an effect upon the pathologic manifestations of RA due to its anti-inflammatory functions, we genotyped the CASP12 SNP rs497116 in 953 patients and 342 controls. Clinical and demographic data on our subjects are shown in Table 1. The data in Table 2 show that frequencies of CASP12 alleles in both patients and controls were similar to each other, and to normal controls reported by others (Saleh et al. 2004). Furthermore, genotype did not affect seropositivity of rheumatoid factor ($\chi^2=4.1$, p=0.128) or anti-citrullinated peptide antibodies ($\chi^2=1$, p=0.618). As expected, while nearly 20% of the
controls were positive for RF, and only 3% were so for anti-CCP, there was no connection to CASP12 genotype in either group (Supplemental Table 1).

We then compared the distribution of CASP12 alleles in 697 CLEAR patients for whom we had additional data on the ACR diagnostic criteria for RA. We also assessed CASP12 genotype in 742 CLEAR patients for whom we had data on the presence or absence of subcutaneous or rheumatoid nodules. No significant differences were found for the clinical parameters of baseline joint tenderness or swelling scores, or elevations in the inflammatory markers C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). The results of the analysis can be seen in Supplemental Table 2.

Despite the lack of correlation between CASP12 genotype and joint swelling or pain, patients with CASP12 had decreased scores for joint pathologies as discerned upon radiographic changes. Figure 1 shows the data given as a strict genotypic distribution for all patients. While joint erosion scores were barely significant between the homozygous groups, and were not significant between the CASP12p1 homozygous and heterozygous groups (p = 0.15), baseline joint narrowing and overall disease scores were greater in those patients who were homozygous for the CASP12p1 pseudogene than those who were CASP12 homozygous. In addition, heterozygotes had significantly different lower joint narrowing and total scores than did the CASP12p1 homozygotes, but to a lesser extent than the CASP12 homozygotes.

For joint narrowing or overall scores (defined as joint erosion + narrowing scores) >0, patients homozygous for CASP12p1 had higher mean and maximum scores (p = 0.008). While only three CASP12 homozygous patients had pathologic scores (i.e., a score >0), it was striking that the maximum value in this group was 28, compared to 181 and 341 for heterozygotes and CASP12p1 homozygotes, respectively (p = 0.002). There were no significant differences between CASP12p1 homozygotes and heterozygotes in these three categories. Similar data was noted for joint narrowing, with maximum scores of 11 vs 117 vs 166 (p = 0.008). In
addition, radiologic pathology scores also appeared to be lesser in heterozygotes as compared than CASP12p1 homozygotes, although not reaching statistical significance.

4. Discussion

We have shown that while possession of the intact CASP12 has no overall effect upon the development of RA in AA, there is a subtle protective effect against the erosive pathologies of RA. The findings thus potentially point to pathways involved in the severity of joint damage. While the number of normal controls in our study are approximately one third the patient sample, the allele frequencies for controls and patients are nearly identical to those of Saleh et al (2004) who used 623 normal subjects. While the small numbers of CASP12 homozygous patients we identified are a limitation, CASP12/p1 heterozygotes also had significantly lower joint narrowing and overall scores, which complement the genotypic effect upon inflammatory cytokine production as observed by Saleh and colleagues (Saleh et al. 2004).

The study used all available data so our power was restricted by the data available. While we did not have patient numbers to have power to detect an odds ratio of 1.2, (which would require a very large sample number), there were 579 individuals with the CASPp1 homozygous genotype and 163 individuals with at least one CASP12 allele. This provided 81% power to detect an odds ratio of 1.69 (if the proportions being compared are 0.300 and 0.420). While it would be desirable to be able to detect a smaller odds ratio, there is sufficient power to indicate whether there is an association worthy of future study, as there are very few confirmed genetic markers of RA severity and this polymorphism could explain inter-ethnic differences in radiological damage.

It is unknown how CASP12 exerts these protective effects. CASP12 is proposed to bind to the inflammasome and inhibit CASP1 activity, preventing the maturation and release of IL-1β (Saleh et al. 2006; Saleh et al. 2004). Yet the published data is contradictory as to whether CASP12 actually reduces IL-1β in humans. CASP12 genotype does not influence the susceptibility to Candida sepsis in Africans, nor does it have any effect on serum cytokine
concentrations in septic candidiasis patients (Rosentul et al. 2011) or in responses to *Yersinia pestis* (Ferwerda et al. 2009). CASP12’s effects upon TNF production, which is pathogenic in RA (Boissier et al. 2012; Choy 2012), is also contradictory (Ferwerda et al. 2009; Plantinga et al. 2010; Yeretssian et al. 2009). These differences may be due to sample size, the populations tested, or the assay systems used. If CASP12 is indeed down-regulatory, it may fail to reduce serum concentrations of IL-1β and other pro-inflammatory cytokines below disease-inducing levels, but does so in the synovium or synovial fluid. It is also possible that CASP12 acts via an inflammasome-independent pathway, as CASP1 is upregulated in RA patients (Mathews et al. 2013), and the inflammasome does not appear to play an overt role in joint pathology (Kolly et al. 2010; So et al. 2013). Murine CASP12 interferes with NOD signaling by competitively competing for binding on RIP2 with TRAF6 (LeBlanc et al. 2008), and both NOD1 and TRAF6 contribute to RA pathogenesis (Plantinga et al. 2013; Zhu et al. 2012).

Examination of levels of pro-inflammatory cytokines in serum and synovial fluid from AA with RA may help discern whether CASP12 has systemic effects or within joint-invasive mononuclear cells. In addition, as data on CASP12 expression in normal or diseased human tissues is also lacking, further study of CASP12 expression may yield clues to this protein’s molecular mode of action.

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References


**Figure Legends**

**Fig. 1** *CASP12* genotype affects radiologic measures of RA pathogenesis in AA. Abbreviations are as per Table 2. A, Joint erosion score at baseline; B, Joint space narrowing score at baseline; C, Overall score at baseline (Erosion + joint space narrowing). Units for joint erosions and narrowing as determined by radiographic data have been described in detail in (Bridges et al. 2010); briefly, radiographs were categorized as not having damage (total score = 0) or having damage (total score > 0). Analyses on data were performed using non-parametric 2-tailed t tests with Mann-Whitney corrections.