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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 scores. *CASP12* homozygosity appears to be a subtle protective factor for some aspects of RA in AA patients.

Keywords

RHEUMATOID ARTHRITIS; AFRICAN-AMERICAN; CASPASE-12; INFLAMMATION; IMMUNOGENETICS

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

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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 (X2=4.1, p=0.128) or anti-citrullinated peptide antibodies (X2=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 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 scores than did the *CASP12p1* homozygotes, but to a lesser extent than the *CASP12* homozygotes.

For joint 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 11, compared to 117 and 166 for heterozygotes and *CASP12p1* homozygotes, respectively). There were no significant differences between *CASP12p1* homozygotes and heterozygotes in these categories. In addition, radiologic pathology scores also appeared to be lesser in heterozygotes as compared to *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 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 Y*ersinia 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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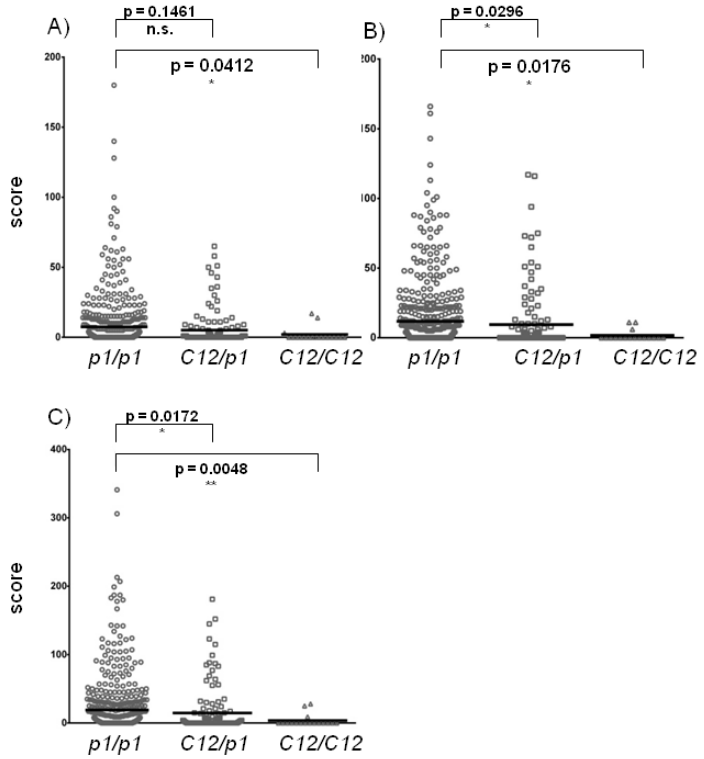

Fig. 1.

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. 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.

Table 1

Descriptors of study subjects. Numbers are percentages unless otherwise indicated, and from CLEAR patients only for whom data was available.

	Patients	Controls
Total subjects	953	342
male	323	87
female	630	255
[1]Age onset:	47.98 ± 13.2	NA
Disease duration	2.4 ± 9.1	NA
Age at screen (baseline)	56.6 ± 12.7	NA
% positive for nodules	20.0	NA
% RF positive	77.5	19.5
% CCP positive	70.7	2.95
ACR criteria		
Stiffness	78.4	NA
Joints	96.6	NA
Wrist	97.3	NA
Symmetric arthritis	96.0	NA
Rad changes	36.0	NA

[1]Mean age years (± SD) at onset of RA. ACR criteria, Stiffness, AM stiffness lasting greater than 1 hour; Joints: arthritis of three or more joint groups simultaneously; Wrist, arthritis of PIP, MCP or wrist joints; Rad changes: Radiographic changes including erosions or unequivocal periarticular osteopenia.

Table 2

Overall distribution of CASP12 genotypes.

	GENOTYPE				
	[1] ***C12/C12***	***C12p1/p1***	[2] ***p1p1***		
Patients	20 (2%)	198 (21%)	735 (77%)	χ^2=1.4	[3] p=0.502
Controls	4 (1%)	68 (20%)	270 (79%)		

[1] C12, CASP12;

[2] p1, CASP12p1;

[3] Fisher's Exact Tes