The Anti-Inflammatory CASPASE-12 Gene Does Not Influence SLE Phenotype in African-Americans

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ABSTRACT

In the vast majority of human populations, the gene encoding CASPASE-12 (CASP12) has a premature termination codon that precludes the production of protein. However, approximately 20% of persons of recent African descent have a single nucleotide polymorphism (#rs497116; A->G) that turns the stop codon into one encoding Arg. The subsequent functional allele is a risk factor for sepsis as it uniquely downregulates inflammatory cytokines in African-Americans (AA). To determine if CASP12 could be protective for systemic lupus erythematosus (SLE) in AA, we genotyped AA SLE patients and controls. There was a weak association between CASP12 genotype with the absence of anti-dsDNA autoantibodies in SLE patients. No effect was seen upon serum interleukin-1 beta levels, nor was any other protective effect noted for the CASP12 genotype, whether upon association with SLE, or any of the 11 American College of Rheumatology classification criteria. CASP12 genotype thus does not influence the phenotype of SLE in AA.

1. Introduction

In mice, CASPASE-12 (CASP12) modulates both the inflammatory process and the cellular apoptotic pathway. A truncated 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 [1]. Thus, while most humans lack a functional CASP12 gene, approximately 20% of persons of African descent have a functional pseudogene that is rescued by a single nucleotide polymorphism (#rs497116; A->G) that turns the premature stop codon into one encoding Arg [2,3]. The functional allele is a risk factor for sepsis in persons of African descent due to its genotype-dependent ability block inflammasome and CASP1 activation [4,5] which in turn down-regulates inflammatory cytokines such as interleukin-1β (IL1 β) in responses
to pro-inflammatory mediators such as bacterial lipopolysaccharide [1]. In addition, knockout mice lacking CASP12 are less susceptible to sepsis and death than wild-type mice [5].

A corollary to the observation that CASP12 is a risk factor for sepsis would be that CASP12 is protective against some inflammatory autoimmune diseases, particularly systemic lupus erythematosus (SLE), in AA populations. SLE is a serious inflammatory disease with protean manifestations that can affect any organ system. The diverse nature of SLE requires classification by the presence of no less than 4 of 11 criteria, as defined by the American College of Rheumatology [6,7]. Gender, environment, and genetic factors influence the development of SLE [8,9], with over 40 genes known to play a role in SLE pathogenesis [10]. A number of these genes are specific SLE risk factors in AA individuals [9].

AA have the highest prevalence of SLE among all ethnic groups, regardless of age, gender, or socio-economic class. They manifest the disease with greater severity and burden, and have worse prognoses and higher mortality rates [8,11,12]. AA patients tend to have more frequent organ system involvement, higher auto-antibody titers, and a more pronounced pathological course. Clinically, these patients develop organ-specific damage at a faster rate than white SLE patients [13], and are more likely to have severe disease flares [14]. We thus chose to assess the role of CASP12 in SLE using AAs selected from the Lupus Family Registry and Repository cohort [15].

2. Material and methods

2.1 Study population

The Lupus Family Registry and Repository (LFRR) is a large, multi-ethnic cohort of SLE-affected individuals, their unaffected family members, and healthy population-based controls supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases [15]. Our study group included 714 AA SLE patients and 325 healthy, population-based AA controls (demographics provided in Table 1). Study subjects were self-defined African Americans who
satisfied at least 4 of the 11 criteria for the classification of SLE [6,7]. Sera were stored at -80° C until use.

2.2 DNA genotyping

CASP12 genotyping was performed in a single tube assay [16] 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 [17]. Primers were C12-P1 (GAGTCACTATCGAGGAGGGTCTCCATG), C12-P2X (TTCTGAACCTTGACCTTTTGGAATTC), C12-P3X (TATCCAAGGTGTTCAGTAGATCTAA), and P12-P4 (CTTCACATTATTTCTGTGTTTCTCCATC). PCR parameters were an initial 60 sec of denaturation at 94° C, 5 sec of priming at 55° C, and 5 sec of extension at 72° C, for 30 cycles, using SpeedSTAR HS or Sapphire DNA polymerase (Takara, Madison, WI). PCR products were analyzed on a 1% agarose gel. To verify results, the polymorphic portion of CASP12 was subjected to DNA sequencing using a high-fidelity DNA polymerase. Sequence analysis was sensitive enough to discriminate between homozygous and heterozygous individuals.

2.3 ELISA analysis

Serum interleukin-1β (IL-1β) levels were measured using a Biolegend (San Diego, CA) ELISA-MAX Deluxe ELISA kit according to the manufacturer’s instructions.

2.4 Statistical Analyses

Pearson’s chi square statistics or Fisher’s exact p values (not adjusted for multiple testing) were generated for full model genetic analyses of CASP12 using the PLINK software program [18]. Cytokine analyses included non-parametric 2-tailed t tests with Mann-Whitney corrections. P < 0.05 was considered statistically significant.
3. Results

3.1 CASP12 has no overall effect on SLE in AAs

To determine if CASP12 is protective against SLE in AAs due to its anti-inflammatory functions, we genotyped the CASP12 SNP rs497116 in a total of 714 AA patients and 325 AA controls (all unrelated). Eight SLE patients (1.1%) were homozygous C12, 152 (21.3%) were heterozygous p1/C12, and 554 (77.6%) were homozygous p1 (Table 2). These frequencies were similar to our normal control group and to normal controls reported in other studies [1,3]. Allele frequencies were comparable: 88% for p1 and 12% for C12 alleles in cases; 87% for the p1 allele and 13% for the C12 allele in controls. There was no association with CASP12 genotype and any of the 11 ACR classification criteria for SLE (Table 2) in case-control analyses, nor was there any effect by CASP12 upon clinical criteria within patients in a case-only design, i.e., having or lacking CASP12 did not affect a patient from being susceptible to, or protected from any particular clinical presentation (Table 3).

The LFRR did not calculate Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores for their patient population. We therefore, approximated the severity of their disease at time of enrollment by defining “severe” manifestations of SLE as any case having renal disease, plus neurological manifestations or serositis. Using these criteria, we did not observe any association between CASP12 genotype and the more pathological presentations of SLE. Therefore, when evaluating SLE as a phenotype, there was no significant association observed with CASP12.

3.2 CASP12 does not affect serum IL-1β levels in AAs with SLE

With the data suggesting that CASP12 plays a protective role in one aspect of SLE, we sought to determine if the mechanism involved would be mediated by IL-1β, the cytokine best known to be influenced by CASP12 [1,5]. IL-1β production by peripheral blood mononuclear
cells (PBMCs) is reduced in lupus patients compared to normal controls [19], while serum IL-1β levels are elevated [20]. Our hypothesis was that IL-1β would be substantially reduced in a genotype-dependent manner. In testing 29 subjects (15 patients and 14 controls), there was no significant difference in IL-1β levels between patients and controls when evaluated by CASP12 genotype (Fig.1a), nor was there any difference amongst individuals, when grouped by genotype only (Fig 1b).

3.3 CASP12 protects against dsDNA antibodies of SLE in AAs.

The effect of the CASP12 genotype was also assessed for six different autoantibodies in a case-only analysis (anti-dsDNA, -Sm, -Ro, -La, -snRNP, and -cardiolipin). Of the patients for whom we have autoantibody data, a weak but significant effect was only observed for anti-dsDNA, either with a genotypic (p=0.045) or allelic (p=0.032) model (Table S1).

4. Discussion

We have found that CASP12 genotype is neither a risk factor nor protective against SLE in African-Americans. CASP12 polymorphisms do not affect any of the 11 ACR classification criteria, but have, at best, a weak protective effect against the development of at least one autoantibody.

While our data do not demonstrate the CASP12 genotype had a significant effect upon serum levels of IL-1β or any symptomology of SLE, we recognize that we evaluated a small sample. Indeed, we observe that the cases homozygous for the CASP12p1 allele have no IL-1β in their serum while three of the four controls that we evaluated have between 100-400 pg/ml. As relatively low numbers of African-Americans carry both copies of CASP12 (about 1-3% of the population [1, 3 and our data], for the number of cases evaluated, changes in even small numbers could affect the significance of the results, and therefore, replication studies
should be performed before a strong conclusion can be drawn about CASP12 genotype and SLE, especially concerning the role of IL-1β in lupus.

Close to 200 autoantibodies have been identified in SLE patients [21]. Such autoantibodies often function as biomarkers for disease activity, have pathogenic roles, and/or are part of diagnostic criteria [22-24]. Given the importance of their role in SLE, we tested whether CASP12 genotype would affect the presence or absence for six different autoantibodies (anti-dsDNA, -Sm, -Ro, -La, -snRNP, and -cardiolipin). We observed, at best, a weak but significant effect for anti-dsDNA. Yet after adjusting for multiple testing in the case-control and case-case analyses, no significant results remained, and no significant effects were seen for the other autoantibodies.

In addition to CASP12, in humans the inflammatory caspase family includes CASP1 (the best studied) [25], CASP4 and CASP5. CASP1 triggers inflammation and mediates inflammatory cell death by pyroptosis via the activation of IL-1β and IL18 from inactive pro-hormones receptors [26]. Activation of CASP1 requires assembly and activation the inflammasome complex following danger signals transmitted from pattern recognition [27]. CASP4 and 5 are also part of the inflammasome and activated by this complex [28].

What evidence is there for overt roles for the inflammatory caspases in the pathogenesis of autoimmunity? Other than CASP1 contributing to murine lupus [29], very little is known about the role of CASP4 and 5 in the pathogenesis of autoimmunity [27] although data is emerging that the latter caspases are involved in autoimmune skin [30] and bowel diseases [31], along with SLE [32].

Given the reported downregulatory effects the protein exerts upon IL1β production via inhibition of CASP1 [1,5] it was surprising that that we observed so few effects. We expected to see reduced serum IL-1β levels in our patient group, with the lowest levels in the CASP12 homozygous patients, especially given that defects in the ability of PBMCs to produce IL-1β
occur in SLE patients [19] and decreased IL-1β levels in patient serum have been described [20]. This was not the case. It should be considered, however, that nearly all studies on IL-1β production in SLE patients involve groups such as Caucasians or Asians who carry the p1/p1 genotype [33]. Therefore, CASP12’s effects may be qualitative at the cellular level rather than quantitative at the serologic level, and other inflammatory cytokines need to be examined.

If CASP12 is indeed downregulatory, it may fail to reduce serum concentrations of IL-1β or other pathogenic cytokines below disease-inducing levels, but does so at the cellular level. This notion is supported by our observation that CASP12 genotype is not protective against the development of rheumatoid arthritis (RA) in AA, but is protective against RA radiologic abnormalities [34]. Surprisingly, obese African-American children had lower serum IL6 and C-reactive protein levels, as well as less macrophage infiltration into adipose tissues, if they were CASP12-positive [35].

The key question that emerges: is CASP12 actually anti-inflammatory? Our own findings bring to mind “the dog that didn’t bark”. CASP12 genotype does not influence susceptibility or adverse events in African-Americans or black South Africans with community-acquired pneumonia [36]. In addition, susceptibility to Candida sepsis in Africans is not affected by CASP12 genotype, nor does it have any effect on serum cytokine concentrations in septic candidiasis patients [37] or in responses to Yersinia pestis [38]. CASP12’s effects upon TNF production, which is elevated in all lupus patients, especially AA [39], is also contradictory [38,40,41]. Further, while hepatitis C virus is a IL1β inducer [42], CASP12 genotype has no effect in the clearance of this pathogen [43]. Confounding the metabolic findings described above by Skeldon and co-workers, no protective effects were found in African-American adults with CASP12 when metabolic parameters or C-reactive protein levels were assessed [35].

While these differences may be due to sample size, populations tested, underlying disease states assessed, or the assay systems used, one other observation is worth noting: In
transgenic mice carrying human \textit{CASP12}, the gene is downregulated in a gender-specific manner, due to an endogenous estrogen response element [41]. Yet there is a profound skewing of the gender ratio in SLE: 90% of AA SLE patients are female [44] while male lupus patients have a more severe phenotype and worse outcomes [45,46]. A protective \textit{CASP12} gene product would be expect to yield the opposite ratios.

The down-regulatory effects of \textit{CASP12} may be subtle enough that it can increase risk for bacterial sepsis, yet not interrupt the pathogenesis of autoimmunity. It may be that in other autoimmune or autoinflammatory disorders that \textit{CASP12} has a protective role, but thus far, using \textit{CASP12} genotype to predict protection from, or disease outcomes in rheumatic diseases, appears to be problematic.

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References


Figure Legends

Fig 1. *CASP12* genotype has no effect upon serum levels of IL1β when comparing patients and controls. *p1/p1, CASP12p1* homozygous; *C12/p1, CASP12p1/CASP12* heterozygous; *C12/C12, CASP12* homozygous. Data represent means of two-well replicates assayed via ELISA. Open circles, patients. Closed circles, normal controls.