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Research Article

Osteopontin Alleles Are Associated with Clinical Characteristics in Systemic Lupus Erythematosus

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Variants of the osteopontin (OPN) gene have been associated with systemic lupus erythematosus (SLE) susceptibility and cytokine profiles in SLE patients. It is not known whether these alleles are associated with specific clinical phenotypes in SLE. We studied 252 well-characterized SLE patients from a multiethnic cohort, genotyping the rs11730582, rs28357094, rs6532040, and rs9138 SNPs in the OPN gene. Ancestry informative markers were used to control for genetic ancestry. The SLE-risk allele rs9138C in the 3′ UTR region was associated with photosensitivity in lupus patients across all ancestral backgrounds (meta-analysis OR = 3.2, 95% CI = 1.6–6.5, $P = 1.0 \times 10^{-3}$). Additionally, the promoter variant rs11730582C demonstrated suggestive evidence for association with two hematologic traits: thrombocytopenia (OR = 2.1, $P = 0.023$) and hemolytic anemia (OR = 2.6, $P = 0.036$). These clinical associations with SNPs in the promoter and 3′ UTR regions align with previously reported SLE-susceptibility SNPs in OPN and suggest potential roles for these variants in antibody-mediated cytopenias and skin inflammation in SLE.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies to nuclear antigens. The severity, prognosis, and manifestations of the disease are highly variable between patients. SLE commonly involves the hematopoietic, skin, musculoskeletal, and renal systems. Involvement of the respiratory, cardiovascular, and central nervous systems can also occur, and different patients typically exhibit different patterns of organ system involvement. Approximately ninety percent of cases are females, with the typical age of onset being in the reproductive years [1].

Genetic factors play a significant role in the pathogenesis of SLE. Familial studies have shown that 10–12% of SLE patients have an affected first-degree relative, which is a twenty- to fiftyfold increase in occurrence when compared to the general population [2]. Studies in monozygotic and dizygotic twins have shown that the disease co-occurs in monozygotic twins 24–69% of the time, while only 2–9% of the time in dizygotic twins [3]. While this data supports a strong genetic influence on SLE susceptibility, the varied concordance in different studies of monozygotic twins also implies a significant role for additional factors beyond genetic sequence variations. Genome-wide association and candidate gene studies have been successful in identifying a number of genes implicated in SLE susceptibility, including those for immunoglobulin receptors (FcγR) [4], and transcription factors such as STAT4 [5] and IRF5 [6, 7].

In order to discover mechanisms of SLE pathogenesis, candidate genes that produce cytokines are of interest [8]. Variants of the osteopontin (OPN or SPP1) gene have been associated with SLE susceptibility [9–11]. OPN is a secreted extracellular matrix cell adhesion glycoprotein. Its role in SLE may be explained through its diverse immunological functions, which include macrophage chemotaxis, T-Helper type 1 lymphocyte response, and B-cell activation [12]. High levels of OPN have been documented in biopsies of inflamed

tissues in SLE and other autoimmune diseases [13, 14]. In addition, studies have shown that an increased plasma OPN level is correlated with increased SLE disease activity [15]. In mouse models, OPN is essential for the production of interferon-alpha (IFN-α) [16]. IFN-α levels are elevated in many SLE patients [17, 18], and high IFN-α is a heritable risk factor for SLE [19]. In previous work, we have shown that the SLE risk-associated allele of OPN rs9138C was associated with higher levels of serum OPN and IFN-α in young women and men with SLE [20]. This phenomenon in which a genetic risk factor for SLE impacts cytokine profiles has been demonstrated for a number of other SLE-risk loci as well [21–23].

There is some precedent for SLE-risk loci being associated with particular SLE clinical features [24–26] although much work remains to be done in this area. While OPN genetic variants have been linked to SLE susceptibility, little is currently known about whether OPN alleles are associated with particular clinical manifestations of SLE. One study demonstrated an association between lymphadenopathy and rs7687316 in the promoter region in European ancestry individuals [10]. Another study of 81 SLE patients of European-American ancestry demonstrated an association between a synonymous change in exon 7 with avascular necrosis and renal insufficiency [11]. We hypothesized that certain clinical subphenotypes of SLE would be associated with the presence of SLE risk alleles of the OPN gene, and that some of these associations would extend across different ancestral backgrounds. By identifying clinical traits associated with OPN alleles, we hoped to gain insight into both potential mechanisms of SLE pathogenesis and the diversity of clinical presentations between patients.

2. Materials and Methods

2.1. Patients and Samples. 252 patients clinically diagnosed with SLE were recruited from the outpatient rheumatology clinic at the University of Chicago and at Rush University, including 145 African-American, 67 European-American, 23 Hispanic-American, and 11 Asian-American patients. All patients fulfilled the American College of Rheumatology (ACR) Criteria score of 4 or higher [27]. The Institutional Review Boards at the respective institutions approved the study, and all subjects provided written informed consent. Table 1 contains demographic information on the patients included in the study.

2.2. Clinical Data. A comprehensive medical history relating to lupus and other immunologic illnesses was taken at the time of enrollment. ACR classification criteria for SLE were documented for each patient using patient history and laboratory testing. The "hematologic disease" ACR criterion is composite criteria composed which can be fulfilled by the presence of leukopenia, lymphopenia, thrombocytopenia, or hemolytic anemia. We chose to study each of these hematologic criteria separately, rather than studying the composite "hematologic disease" criterion. Similarly, the "immunologic disease" criterion is a composite of anti-Smith antibodies, anti-dsDNA antibodies, and antiphospholipid antibodies, and we studied each of these individual components separately. Autoantibodies were assayed for all subjects in the University of Chicago Clinical Immunology Laboratory, using standard procedures and cutoff points for positive values. Anti-Sm and antiphospholipid antibodies were measured using ELISA, and anti-dsDNA was measured using the Crithidia luciliae method. Complete blood counts were done to assess blood cell subset counts. The prevalence of the various common clinical manifestations associated with SLE in our cohort is illustrated in Table 2.

Table 1: Demographic characteristics of the SLE population studied.

Self-reported ancestry	*N*
African American	145
European American	67
Hispanic American	23
Asian-American	11
Age	yrs.
Range	18–87
Mean	44.38
Std. deviation	13.11
Gender	*n* (%)
Males	21 (8.33%)
Females	231 (91.67%)

2.3. Genotyping. We genotyped four haplotype-tagging and/or SLE-associated SNPs within the OPN gene region. rs11730582 and rs28357094 are both in the 5′ promoter region of the OPN gene located on chromosome 4, and both have been previously associated with SLE. rs6532040 is in an intron and has not been previously associated with SLE susceptibility. rs9138 has been previously associated with SLE susceptibility and serum cytokines in SLE patients and is in the 3′ UTR region of osteopontin. Genotyping was performed using ABI Taqman assays-by-design primers for probes for each of the four SNPs on an ABI 7900HT PCR machine. Table 3 describes the genotype data at the four SNPs for patients in our study. All SNPs demonstrated the expected Hardy-Weinberg proportions when tested in each ancestral background individually (HW P value >0.01 for all), supporting the validity of the genotype data.

2.4. Statistical Analysis. The genetic and clinical databases were merged, and STATA 8.2 was used for statistical analysis to determine associations between phenotypes and the four SNPs. The four SNPs chosen were not in high-linkage disequilibrium with each other (r-squared <0.35 for each pairwise SNP combination in each ancestral background), and thus there were no strong haplotypes formed across these 4 SNPs, and each SNP was analyzed separately. Because the frequency of the SNPs of interest and the frequency of SLE manifestations both vary by race, ancestry as a confounder had to be addressed in this study. To control this potential confounding, we first analyzed each self-reported ancestral background separately. Then, we used ancestry informative

Table 2: Prevalence of clinical features in different ancestral backgrounds.

Clinical feature	African Americans	European Americans	Hispanic Americans	Asian Americans
Malar rash	0.49	0.53	0.70	0.60
Discoid rash	0.31	0.08	0.26	0.50
Photosensitivity	0.08	0.08	0.13	0.30
Oral ulcers	0.35	0.32	0.48	0.25
Arthritis	0.85	0.86	0.81	1.00
Serositis	0.34	0.35	0.43	0.20
Hemolytic anemia*	0.10	0.05	0.20	0.25
Lymphopenia*	0.83	0.76	1.00	0.75
Thrombocytopenia	0.14	0.05	0.00	0.10
Leukopenia	0.39	0.23	0.13	0.50
Neurologic symptoms	0.14	0.12	0.09	0.00
Anti-Smith antibody	0.41	0.18	0.39	0.40
Anti-DNA antibody	0.51	0.56	0.22	0.60
Antiphospholipid antibody*	0.42	0.43	1.00	0.25
ANA	0.98	0.91	0.95	0.88

* Data for these ACR criteria were only available for 130 SLE patients—84 African Americans, 37 European Americans, 5 Hispanic Americans, and 4 Asian Americans.

Table 3: Minor allele frequencies across different ancestral backgrounds.

SNP	African Americans	European Americans	Hispanic Americans	Asian Americans
rs11730582 C	0.148	0.402	0.304	0.364
rs28357094 G	0.059	0.265	0.239	0.045
rs6532040 G	0.427	0.321	0.239	0.136
rs9138 C	0.164	0.265	0.250	0.500

markers in a principal component analysis to generate quantitative values representing proportional ancestry. The full details of this analysis and graph of the first two principal components are shown in [28]. Principal components were used as a covariate in the logistic regression analyses to control for differences in proportional ancestry between SLE cases. In the case that a similar effect was observed across ancestral backgrounds, we performed a meta-analysis across groups using the same control for proportional ancestry between groups. Logistic regressions for categorical-dependent variables were performed in the STATA 8.2 program. Clinical manifestations were the dependent variables and were recorded as binary variables representing the presence or absence of each of the 11 ACR classification criteria. The four different OPN SNPs were used as the independent variables, and each SNP was modeled in an additive fashion. P values shown in the paper are uncorrected for multiple comparisons. A P value <0.0031 would withstand a Bonferroni correction for multiple comparisons, adjusting for the number of clinical variables tested in our study. Associations detected in this study which have a P value of less than 0.05 but did not meet the threshold P value of 0.0031 were described as "suggestive" or "possible" associations.

3. Results

3.1. rs9138 C Allele Is Associated with Photosensitivity. Photosensitivity was present in 8.64% (21/243) of patients in the cohort. As shown in Table 4, photosensitivity was strongly associated with the SLE risk allele rs9138 C across all ancestral backgrounds (meta-analysis OR = 3.245, P = 0.001, 95% CI = 1.609–6.542). This association would withstand a Bonferroni correction for the number of phenotypes analyzed in this study. The prevalence of photosensitivity did not differ significantly between ancestral backgrounds (7.59% in African Americans and 7.58 in European Americans, Table 2). Photosensitivity was not associated with the principal components representing genetic ancestry within each ancestral background, supporting the idea that differences in proportional ancestry or admixture were not confounding the analysis. Additionally, a similar pattern of association between rs9138 C and photosensitivity was observed in each ancestral background analyzed separately.

3.2. rs11730582 C Allele Is Associated with Thrombocytopenia and Hemolytic Anemia. The SLE risk-associated single nucleotide polymorphism rs11730582 C demonstrated a suggestive association with two different hematological manifestations (Table 4). Thrombocytopenia was associated with the rs11730582 C allele (meta-analysis OR = 2.12, P = 0.023, 95% CI = 1.11–4.04). Data for hemolytic anemia was available for 127 patients, as the samples from one of the sites did not assess this hematologic subcategory. A possible association between rs11730582 C and hemolytic anemia was observed in the patients in whom this data was available (meta-analysis OR = 2.55, P = 0.036, 95% CI = 1.06–6.13).

Table 4: Associations between clinical features and OPN SNPs.

SNP	Photosensitivity			Thrombocytopenia			Hemolytic anemia		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
rs11730582 C	1.74	0.90–3.39	0.101	**2.12**	**1.11–4.04**	**0.023**	**2.55**	**1.06–6.13**	**0.036**
rs28357094 G	0.99	0.42–2.39	0.999	0.52	0.16–1.68	0.273	0.26	0.03–2.05	0.200
rs6532040 G	0.59	0.28–1.22	0.156	1.00	0.53–1.88	0.999	1.31	0.53–3.22	0.553
rs9138 C	**3.25**	**1.61–6.54**	**0.001**	1.58	0.79–3.13	0.195	0.99	0.37–2.65	0.977

OR: odds ratio, 95% CI: 95% confidence interval of the odds ratio.

As might be expected, presence of thrombocytopenia and presence of hemolytic anemia were correlated with each other ($P = 0.0013$). In conditional analyses, each hematologic phenotype demonstrated similar suggestive evidence for association with rs11730582 C when conditioned on the other, and a higher odds ratio for association was observed for rs11730582 C in patients who exhibited both hematologic traits (OR = 3.37, $P = 0.033$).

4. Discussion

SLE is a clinically and genetically heterogeneous disease. It seems likely that the striking clinical heterogeneity is due at least in part to differences in the genetic determinants of disease between patients. We demonstrate an association between the most consistent SLE risk allele in the 3′ UTR region and photosensitivity and additionally show suggestive evidence that the promoter region of the gene is associated with hematologic phenotypes in SLE patients. This finding of two independent associations in the promoter and 3′ UTR regions has been observed previously with the OPN gene. In a case control study, this model of two independent associations was the best fit [10], and in our own previous work, we observed an antibody association with the promoter and a serum cytokine association with the 3′ UTR SNP rs9138 [20].

One limitation of this study is that a relatively small cohort with robust and detailed clinical data was analyzed. Thus, we were only able to detect associations with large-effect sizes and cannot exclude any potential moderate strength associations that we did not detect in this study. Using a Bonferroni correction for multiple comparisons, the photosensitivity association with rs9138 C would remain significant, while the two possible hematologic associations with rs11730582 C would not. The genotype frequencies in our SLE cases were similar to those previously published in large-scale case-control experiments [9].

Interestingly, rs11730582 C was associated with two distinct hematological manifestations which share some aspects of pathogenesis: hemolytic anemia and thrombocytopenia. Previous studies have established that thrombocytopenia and hemolytic anemia in lupus typically have an autoantibody-mediated pathogenesis. 90% of lupus patients with thrombocytopenia have been shown to possess antibodies directed against either the glycoprotein IIb/IIIa or thrombopoietin receptor [29]. Hemolytic anemia in SLE is frequently a Coomb's test-positive, antibody-mediated hemolytic anemia. OPN in its secreted form functions as a cytokine involved in B-cell activation and plays a role in antibody production [14]. We have previously demonstrated the rs11730582 C allele was associated with an increased prevalence of antiribonuclear protein autoantibodies (anti-RNP) [20]. These data in aggregate support a role for the promoter region in autoantibody formation in SLE.

Although the rs9138 C allele was not associated with hematological findings, it was strongly associated with photosensitivity in lupus patients. This association was particularly interesting given our previously published finding of the association of rs9138 C with increased levels of both osteopontin and IFN-α in SLE patients [20]. Numerous studies have indicated a central role of plasmacytoid dendritic cells (PDCs) and their secretion of IFN-α in SLE pathogenesis [30, 31]. Additionally, OPN is essential for IFN-α production by murine PDCs [14]. It is known that cutaneous lupus is an autoimmune process characterized by photosensitivity, ultraviolet-light-induced apoptosis of keratinocytes, and an inflammatory infiltrate in superficial and deep compartments of the skin [30]. The histopathologic accumulation of PDCs and their subsequent secretion of IFN-α have been demonstrated in these photosensitive lesions [25, 32]. In light of these findings, the statistically significant association between the OPN gene variant rs9138 C and the clinical manifestation of photosensitivity may be supported by a cytokine-mediated pathogenic mechanism.

The rs11730582 C and rs9138 C alleles of the OPN gene have been previously and independently associated with increased risk of lupus. In this study, we find that both of these alleles are independently associated with distinct clinical features in SLE patients, supporting divergent roles for these alleles in the pathogenesis of SLE. This work will inform future genetic studies of the locus and provides intriguing hypotheses regarding the molecular pathogenesis of SLE which can be followed up in mechanistic studies.

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Conflict of Interests

The authors report no financial conflict of interests.

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