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ITGAM coding variant (rs1143679) influences the risk of renal disease, discoid rash, and immunologic manifestations in lupus patients with European ancestry

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Abstract

Purpose—We hypothesized that the coding variant (R77H), rs1143679, within *ITGAM* could predict specific clinical manifestations associated with lupus.

Method—To assess genetic association, 2366 lupus cases and 2931 unaffected controls with European ancestry were analyzed. Lupus patients were coded by the presence or absence of individual ACR criteria. Logistic regression and Pearson chi-square tests were used to assess statistical significance.

Results—First, for overall case-control analysis, we detected highly significant ($p=2.22 \times 10^{-21}$, OR=1.73) association. Second, using case-only analysis we detected significant association with renal criteria ($p=0.0003$), discoid rash ($p=0.02$), and immunologic criteria ($p=0.04$). Third, we compared them with healthy controls, the association became stronger for renal ($p=4.69 \times 10^{-22}$, OR=2.15), discoid ($p=1.77 \times 10^{-14}$, OR=2.03), and immunologic ($p=3.49 \times 10^{-22}$, OR = 1.86) criteria. Risk allele frequency increased from 10.6% (controls) to 17.0% (lupus), 20.4% (renal), 18.1% (immunologic), and 19.5% (discoid).

Conclusion—These results demonstrated a strong association between the risk allele (A) at rs1143679 and renal disease, discoid rash, and immunological manifestations of lupus.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a genetically complex and clinically heterogeneous autoimmune disease. Several studies have reported differences in prevalence of disease manifestations and outcome of SLE in various ethnic groups, which have shown variations among African Americans, Hispanics, European-derived, and Asian populations. Clinical manifestations of SLE vary broadly, from relatively mild disease with long latency between the first symptom and diagnosis, to the rapid progression of severe disease. In addition, clinical manifestations vary widely from patient to patient with strong trends occurring by ethnicity and geography [1].

We recently identified a novel non-synonymous single nucleotide polymorphism (SNP), rs1143679, at exon-3 of the *ITGAM* gene associated with SLE susceptibility [2] in patients of European descent, but not in two Asian populations (Japanese and Korean) [3]. This missense coding polymorphism changes the amino acid arginine to histidine at position 77 (R77H). However, the effect of *ITGAM* on SLE was recently observed in Chinese living in Hong Kong and in a Thai population [4]. These ethnic differences in genetic association with *ITGAM* reinforce the importance of assessing genetic polymorphisms with clinical sub-phenotypes. However, the relationship between this variant and clinical sub-phenotypes have not been studied in European-derived populations. We hypothesized that rs1143679 could predict specific clinical outcomes (sub-phenotypes) of the disease. In this study, we examined the association between rs1143679 and individual ACR (American College of Rheumatology) criteria of SLE in a large cohort of patients from European ancestry.

METHODS

Study population

There were 2266 unrelated SLE cases, and 2931 unrelated, unaffected controls with self-reported European ancestry. All SLE patients met the revised ACR criteria [5]. Although all individuals were ancestry-matched, most of the cases were matched by geographic location and gender with controls. The study was approved by the Institutional Review Boards of respective institutions. Details of subject collection and characterization are described elsewhere [2,3].

Clinical Data

Clinical data were collected through review of medical records based on clinical criteria classification following guidelines set forth by the ACR. All individual ACR criteria were coded as positive, negative, or missing. Missing data were not assumed to be negative but were excluded from clinical criteria specific sub-group analyses.

Genotyping

Samples used in this study were part of a larger-scale candidate gene association study. Details of genotyping procedures have been described elsewhere [2,3,6].

Statistical analysis

SLE patients were grouped by the presence or absence of individual ACR criteria. Both logistic regression and Pearson chi-square tests were used to assess statistical significance with rs1143679 in both case-control and case-only analyses. Permutation analyses (10,000 tests) were performed to confirm significance to maximize the internal validity of these findings.

RESULTS

As expected, we confirmed that the risk allele (A) of rs1134679 was significantly associated with SLE compared to unaffected controls (Table 1). Next, we performed case-only analysis between individual ACR criteria positive SLE cases and SLE negative cases. This variant was significantly associated with renal criteria ($p=0.0003$, OR = 1.39), discoid rash ($p=0.02$, OR = 1.27), and immunologic manifestations ($p=0.04$, OR = 1.30) compared to SLE cases without these clinical manifestations. Permutation based analyses confirmed the association with renal criteria ($p=0.0007$) and discoid rash ($p=0.027$), and borderline significance with immunologic criteria ($p=0.054$). To see whether these variables are associated with each other, we performed 3 pair-wise contingency association tests. While the renal criteria was significantly associated with both immunologic manifestations ($p=6.98 \times 10^{-23}$) and discoid rash ($p=1.89 \times 10^{-5}$), the association between immunologic manifestations and discoid rash was not significant ($p=0.69$). These results were also supported by multivariate logistic regression analysis, using the renal criteria as the outcome variable and gender, discoid rash, and immunologic manifestations as covariates. Gender was not significant and was not included in final model.

To further assess the magnitude of associations between significant ACR criteria, we compared individual ACR criteria positive cases with unaffected controls. The magnitude of the effect was stronger with renal criteria versus controls ($p=4.69 \times 10^{-22}$, OR=2.15), discoid rash versus controls ($p=1.77 \times 10^{-14}$, OR=2.03), and immunologic manifestations versus controls ($p=3.49 \times 10^{-22}$, OR=1.86). Interestingly, renal criteria and immunologic manifestations were both more significant than all SLE cases together versus controls despite the relatively small sample sizes in ACR positives. The minor allele frequency (MAF) increased from 10.6% (controls) to 17.0% (SLE cases) and 20.4% (renal criteria), 18.1% (immunologic manifestations), and 19.5% (discoid rash). This increasing MAF confirms the trend of associations of this allele with renal criteria, discoid rash, and immunologic manifestations. Similarly, respective ORs were also increased from 1.73 (SLE vs. controls) to 2.15 (renal criteria vs. controls), 2.03 (discoid rash vs. controls), and 1.86 (immunologic manifestations vs. controls) (Table 1, Figure 1). Notably, the 95% CIs did not overlap between renal criteria positive cases versus controls, and renal criteria negative cases versus controls (Figure 1). For discoid rash and immunologic manifestations, the 95% CIs did overlap between positive cases versus controls, and negative cases versus controls. However, the nature of the retrospective analysis could introduce bias, especially when in which clinical data was collected by different investigators over time. To see the consistency of this association we reanalyzed our data separately as provided by the investigator. We observed the consistency of our results (Supplementary Table 1).

Autoantibody data was available for a subset of data (999 cases). Case-only analysis was performed for SLE patients positive and negative for anti-dsDNA, anti-La, anti-nRNP, anti-Ro, and anti-Sm. Significant association between rs1143679 and anti-dsDNA ($p=0.0011$, OR (95% CI)=1.65 (1.22-2.23)) and anti-nRNP ($p=0.006$, OR (95% CI)=1.89 (1.19-3.00)) were observed.

DISCUSSION

ITGAM encodes a cell surface receptor protein (integrin) that is implicated in various adhesive interactions of monocytes, macrophages, and granulocytes as well as in mediating the uptake of complement-coated particles. After identifying variants associated with disease risk, it is important to correlate genotypic data with phenotypic expression of the disease that may provide insight into disease pathogenesis and, more importantly, new therapeutic approaches. Subsetting allows us to create more homogeneous groups of patients and thus may increase power to detect associations despite reduced of sample size.

In this study we found that SLE patients often had stronger associations between SLE and rs1143679 when patients were stratified by certain clinical ACR criteria, especially renal criteria, discoid rash, and immunologic manifestations. While results confirm that rs1143679 was strongly associated with SLE in general, SLE patients with renal criteria, discoid rash, and immunologic manifestations were enriched for the risk allele (A) and risk estimates (ORs) were substantially increased, especially for renal criteria (Figure 1).

The strongest associations in individuals of European-descent were found with SLE cases positive for renal criteria compared to cases without this clinical manifestation. A similar result was recently observed among Chinese and Thai populations [4]. Renal criteria is one of the most serious manifestations of SLE and is a major predictor of poor prognosis [7] and mortality [8]. Among those patients with renal criteria, nearly 70% of patients never treated with cyclophosphamide develop an end-stage renal disease outcome [8]. As confirmed by our logistic regression analysis, diverse clinical manifestations are associated with renal criteria. It would be important to follow up this study with more clinical information and possibly information about environmental variables. Indeed, clinical and ethnic subsetting of SLE may help explain the natural history of SLE differences by ethnicity.

Our results may provide a genetic correlate into the proposed role by which *ITGAM* mediates the inflammatory processes in SLE pathogenesis. *ITGAM* (CD11b) expression was shown to increase DNA fragmentation, a prerequisite for apoptosis and fragmented DNA level is elevated in intravascular cells that induces IFN α [9] in SLE mice. CD11b also induces TNF α [10] and modulate innate immune mechanism [11] in SLE patients. This suggest that association of this SNP in *ITGAM* with molecular mechanism of immunological manifestations may be correlated. Although the molecular mechanism of discoid rash and involvement of *ITGAM* has not been assessed earlier, it could be a clinical manifestation of cell-mediated immunological reactions as well. *ITGAM* is a major surface receptor in monocytes and monocytes are involved in clearing deposits from glomeruli [12]. In the normal kidney, *ITGAM* is expressed on endothelial cells of glomerular and peritubular capillaries of Bowman's capsule, but in renal criteria, its expression is significantly increased on tubular epithelial cells and on infiltrating mononuclear cells [13]. In contrast, extensive series of studies by two groups [14] [15] suggest that C3b receptor (CR3, *ITGAM*) expression is decreased in renal criteria patients in compare to control groups. It is also possible that cellular regulatory mechanisms allow *ITGAM* to be over expressed to cope with the loss of clearance of deposits in glomeruli by monocytes. However, whatever the disease mechanisms, this SNP changes the amino acid (R77H) in the vicinity of alpha domain (metal ion binding domain or A domain) and may overall modulate the numerous ligand binding activities of *ITGAM* in monocytes and neutrophils and dendritic cells. Within this context, it is conceivable that risk allele (A) may confer its effect through mutant receptor protein with respect to ligand binding affinity, or this change may aggravate the inflammatory process by decreasing initial kidney cellular infiltration. These individuals are prone to develop renal criteria together with other manifestations like discoid rash or immunologic manifestations.

In summary, we consistently showed strong correlation between the coding variant (rs1143679) of *ITGAM* and renal, discoid, and immunological manifestation of lupus. The next challenges are to understand how this coding variant actually modulates molecular pathways leading to lupus kidney involvement or other associated clinical manifestations.

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Figure 1.

OR (95% CI) for SLE cases with ACR criteria positive cases vs. controls, and SLE cases with ACR criteria negative vs. controls for renal criteria, discoid rash, and immunologic manifestations.

Table 1

ITGAM (rs1143679) association results between SLE Cases with individual ACR criteria vs. controls

ACR criteria	ACR-		ACR +		Case-only analysis		ACR+ vs. control**	
	n (%)	MAF*	n (%)	MAF*	P	OR (95% CI)	P	OR (95% CI)
All SLE			2366	17.0			2.22E-21	1.73 (1.54-1.94)
Arthritis	236 (11.2)	16.0	1877 (88.8)	16.9	0.5934	1.07 (0.83-1.39)	4.49E-19	1.72 (1.52-1.93)
Discoid	1029 (69.4)	16.0	453(30.6)	19.5	0.0203	1.27 (1.04-1.56)	1.77E-14	2.03 (1.69-2.45)
Hematologic	554 (30.2)	16.8	1279 (69.8)	16.9	0.9551	1.01 (0.83-1.22)	2.20E-15	1.71 (1.49-1.95)
Immunologic	283 (16.8)	14.5	1405 (83.2)	18.1	0.0403	1.30 (1.01-1.68)	3.49E-22	1.86 (1.64-2.12)
Malar Rash	512 (26.4)	16.5	1428 (73.6)	17.4	0.5018	1.07 (0.88-1.29)	6.26E-19	1.78 (1.56-2.02)
Nephritis	1052 (61.4)	15.6	662 (38.6)	20.4	0.0003	1.39 (1.16-1.66)	4.69E-22	2.15 (1.84-2.52)
Neurologic	1242 (78.7)	16.4	337 (21.3)	17.9	0.3428	1.12 (0.89-1.40)	1.86E-08	1.84 (1.48-2.27)
Oral Ulcers	583 (34.1)	17.3	1125 (65.9)	15.9	0.268	0.90 (0.74-1.09)	1.19E-10	1.58 (1.38-1.82)
Photosensitivity	439 (23.2)	18.1	1452 (76.8)	16.5	0.2737	0.89 (0.73-1.09)	4.67E-15	1.67 (1.50-1.96)
Serositis	775 (45.0)	16.6	948 (55.0)	16.0	0.6196	0.95 (0.80-1.15)	4.19E-10	1.60 (1.38-1.86)

* MAF=minor allele frequency

** MAF=10.6% based on 2931 unrelated controls