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Genetic load in incomplete lupus erythematosus

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ABSTRACT

Objective Patients with incomplete lupus erythematosus (ILE) have lupus features but insufficient criteria for SLE classification. Some patients with ILE transition to SLE, but most avoid major organ involvement. This study tested whether the milder disease course in ILE is influenced by reduced SLE risk allele genetic load.

Methods We calculated the genetic load based on 99 SLE-associated risk alleles in European American patients with SLE (≥4 American College of Rheumatology (ACR) 1997 criteria, n=170), patients with ILE (3 ACR 1997 criteria, n=169), a subset of patients with ILE not meeting Systemic Lupus International Collaborating Clinics (SLICC) classification (ILE^{SLICC}, n=119) and healthy controls (n=133). Unweighted genetic loads were calculated as the total sum of risk alleles for each individual, while weighted genetic loads were defined as the sum of risk alleles multiplied by the natural logarithm of the previously published OR of each risk allele for SLE susceptibility.

Results The median unweighted and weighted SLE risk allele genetic load was significantly greater in patients with ILE (unweighted: 81, p value=0.01; weighted: 16.3, p value=0.001) and patients with SLE (80, p value=0.02; 16.29, p value=0.0006) compared with healthy controls (78, 15.76). Patients with ILE^{SLICC} trended towards an increased genetic load, although not statistically significant (unweighted: 80, p value=0.14; weighted: 16.05, p value=0.07). However, the median genetic load did not significantly differ between ILE and SLE, and genetic load did not differentiate patients with ILE and SLE (area under the curve=0.51, p=0.78) by receiver operator characteristic analysis.

Conclusions Patients with ILE and SLE have comparable genetic loads of SLE risk loci, suggesting similar genetic predispositions between these conditions. Phenotypical differences between SLE and ILE may instead be influenced by ILE-specific variants and gene–environment interactions.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Patients with incomplete lupus erythematosus (ILE) exhibit some features of SLE but not enough for SLE classification. Although some patients with ILE progress to SLE classification, most maintain a mild disease course with limited major organ involvement; however, the factors limiting disease severity in a subset of patients with ILE are unknown. In SLE, increases in the genetic load of SLE risk alleles are associated with SLE susceptibility and severity; therefore, we determined whether the genetic load of SLE-associated risk alleles was reduced in patients with ILE compared with patients with SLE, limiting disease severity.

WHAT THIS STUDY ADDS

⇒ We found that patients with ILE exhibited similar SLE risk allele genetic loads as patients with SLE, and genetic load did not affect the odds of having SLE compared with ILE. Therefore, patients with SLE and ILE exhibit a similar genetic predisposition, and SLE risk allele genetic load cannot differentiate subjects with ILE.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study found that patients with ILE and SLE have similar SLE risk allele genetic load, suggesting that a reduction in genetic susceptibility does not limit SLE transition in some patients with ILE. Therefore, disease severity may be influenced by genetic variants specific to ILE and/or gene–environment interactions. Determining the factors that limit ILE disease severity may help with risk stratification and preventative treatment.

INTRODUCTION

SLE is a complex chronic autoimmune disease with various systemic manifestations. SLE is typically diagnosed based on characteristic clinical and serological features defined by the American College of Rheumatology (ACR) or Systemic Lupus International Collaborating Clinics (SLICC).[1–3] However, a subset of patients, referred to as incomplete lupus erythematosus (ILE), exhibit some clinical symptoms or serological evidence of SLE but do not fulfil classification criteria. Approximately 20% of patients with ILE transition to classified SLE within 5 years of onset, but most experience a relatively mild disease course with no symptomatic progression and limited involvement of major organs.[4–7] The factors that limit disease severity in ILE are unknown.

Genome-wide association studies have identified over 100 genes associated with SLE classification, including variants associated with specific disease manifestations, such as nephritis.[8 9] Increases in the number of these SLE risk alleles, termed genetic load, are associated with SLE susceptibility.[10–12] Furthermore, increased genetic load correlates with more severe disease, organ damage, renal dysfunction and mortality.[13] Therefore, we hypothesise that ILE may share genetic associations with SLE but with a reduced genetic load. However, the genetic risk of ILE has not been studied.

In this study, we determined the cumulative burden of SLE variants on ILE susceptibility by comparing the genetic load of SLE risk alleles in European American patients with ILE, patients with SLE and healthy controls.

METHODS

Study population

European American patients with SLE (n=170) or ILE (n=169) and healthy controls with no self-reported lupus manifestations (n=133) were selected from existing collections in the Arthritis & Clinical Immunology Biorepository (CAP# 9418302) at the Oklahoma Medical Research Foundation. Demographic information was self-reported. Participants with SLE or ILE were characterised by a systematised medical records review for SLE classification criteria. ILE was defined as three ACR 1997 criteria and SLE as four or more ACR-1997 criteria.[2] Patients with ILE by ACR who also did not meet SLICC classification criteria[3] were considered ILE^{SLICC}. All individuals with ILE were previously enrolled in the Lupus Family Registry and Repository (LFRR) (1995–2012).[14] Healthy controls with no documented lupus manifestations were also previously enrolled in the LFRR or from the Oklahoma Immune Cohort through the Oklahoma Rheumatic Disease Research Cores Centre collections.

Genotyping, quality control and imputation

Samples were genotyped on the Infinium Global Screening Array-24 V.2.0 (Illumina, San Diego, California, USA), with 665 608 variants genotyped per sample. With consulting support from Rancho BioSciences (San Diego, California, usa), quality control was performed at the sample and variant level in PLINK V.2.0 (V.1.90) (online supplemental figure 1). Samples with call rates below 90%, extreme heterozygosity measured by Wrights inbreeding coefficient (F<−0.05||F>0.1) or discordance between genotyped and clinically recorded sex were excluded. Variants from sex and mitochondrial chromosomes and somatic variants with minor allele frequency of <0.1% were also excluded.

After quality control, 542 524 variants were available for imputation. The data were then prephased to infer underlying haplotypes with the 1000 Genomes phase III reference panel using SHAPEIT V.2.79, and whole-genome imputation was performed on the prephased haplotypes using IMPUTE V.2.3.2. To filter for variants of high imputation accuracy, only those with an information score of >0.9 were retained.

Genetic load

The genetic load was calculated for 472 subjects based on previously identified SLE-associated SNPs with genome-wide significance in the European population.[11] Of the 123 variants meeting tier 1 statistical significance ($p>5\times10^{-8}$ and $P_{FDR}<0.05$),[11] 99 met postimputation quality control and were included for genetic load calculation (online supplemental figure 1 and table 1). Unweighted genetic loads were calculated as the total sum of risk alleles for each individual. Weighted genetic loads were defined as the sum of risk alleles multiplied by the beta coefficient (the natural logarithm of the previously published OR of each risk allele for SLE susceptibility).[11] If the beta coefficient was negative, the count for the reverse coded allele and the inverse OR was used.

Statistical analysis

The genetic load was compared using Kruskal-Wallis with Dunn's post hoc test for multiple corrections. Statistical comparisons and receiver operator characteristic (ROC) analysis were performed using GraphPad Prism V.8.3.1. ORs were computed using Excel V.14.6.9, comparing individuals with a specific weighted genetic load (±2) with those within the lowest 10%. P values less than 0.05 were considered statistically significant.

RESULTS

Study population

To assess the impact of known SLE genetic associations on ILE susceptibility, we compared the genetic load of a set of 99 previously described SLE risk variants[11] (online supplemental table 1) in European American patients with ILE (n=169), patients with SLE (n=170) and unaffected controls (n=133) (online supplemental table 2). Due to the low numbers of subjects from other races in the ILE cohort and challenges with combining race-specific genetic load information, we elected not to attempt any other race-specific genetic load comparisons. A similar frequency of childhood onset was observed in patients with ILE (7.1%) and patients with SLE (3.7%) (online supplemental table 3). As expected, the total number of ACR criteria met per patient was higher in patients with SLE (mean 5.7) than in patients with ILE (mean 3, p value <0.0001) (online supplemental table 3). In addition, the frequency of patients who met malar or discoid rash, photosensitivity, oral or nasal ulcers, arthritis, serositis, renal disease, and neurological or haematological ACR criteria was significantly higher in patients with SLE compared with patients with ILE; however, the frequency who met immunological or antinuclear antibody criteria was similar between the two groups (online supplemental table 3).

Patients with ILE exhibit a similar increased SLE risk allele genetic load as patients with SLE

Consistent with previous findings,[10–12] European American patients with SLE exhibited significantly greater

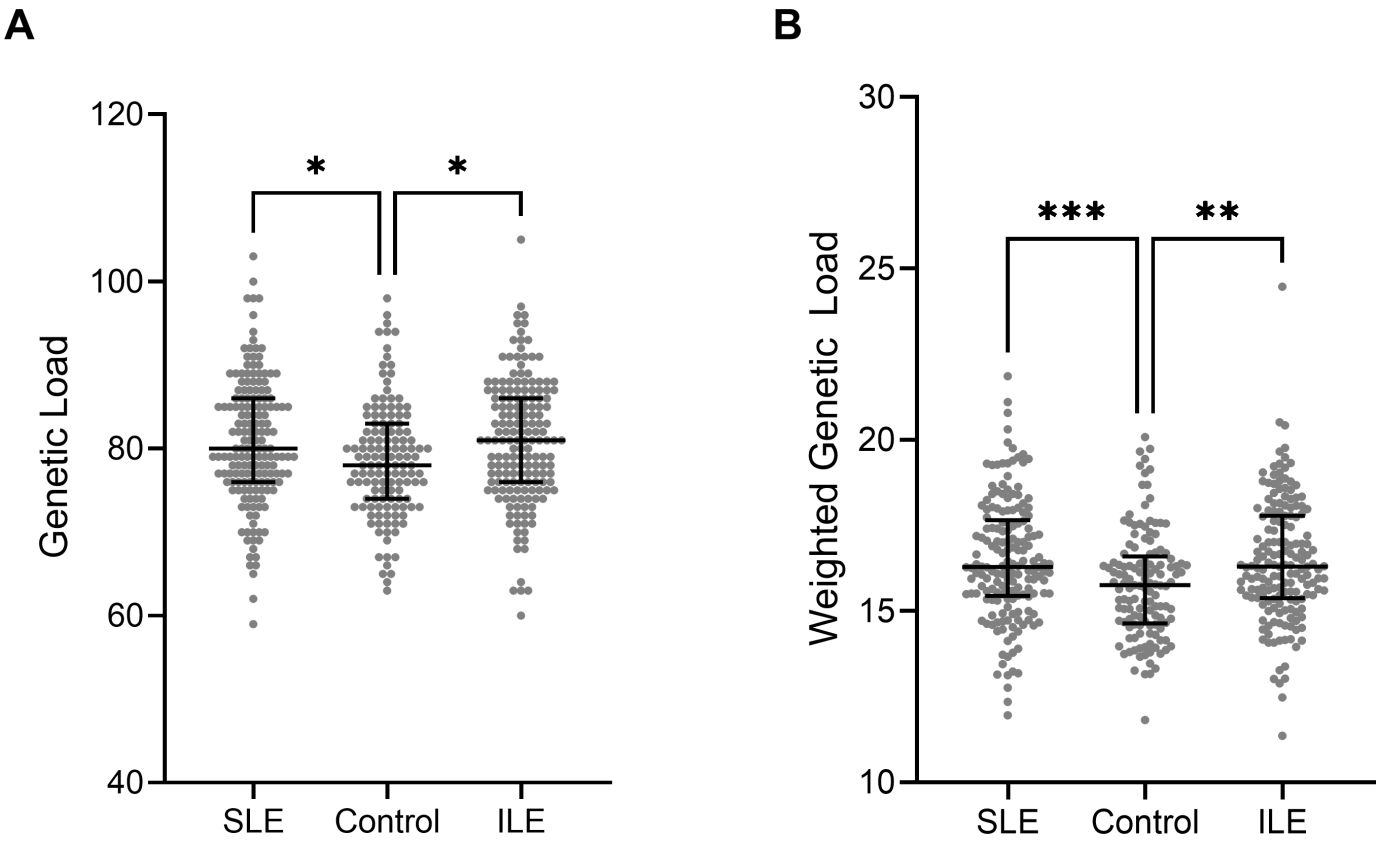

Figure 1 Patients with ILE exhibit increased genetic load of SLE risk alleles, similar to patients with SLE. (A) Unweighted and (B) weighted SLE risk allele genetic loads in European American patients with SLE (n=170), patients with ILE (n=169) and healthy controls (n=133). Graphs show the median and IQR. Statistical significance was determined using Kruskal-Wallis with Dunn's post hoc test. *P<0.05, **P<0.01, ***P<0.001. ILE, incomplete lupus erythematosus.

unweighted and weighted genetic loads compared with healthy controls (figure 1A,B, and online supplemental table 4). Unweighted and weighted genetic loads were also higher in European American patients with ILE compared with healthy controls and did not differ from patients with SLE (figure 1A,B). We next stratified the patients with ILE based on SLICC criteria, which are more sensitive compared with ACR criteria.[3 15] A similar trend was observed in ILE^{SLICC} patients (n=119) compared with patients with SLE (online supplemental figure 2A,B, and online supplemental table 5), suggesting a comparable genetic load in patients with ILE and SLE irrespective of the classification criteria used.

To understand how SLE risk allele genetic load influenced the odds of disease in an individual, we calculated ORs comparing individuals with a given weighted genetic load (±2.0) with those within the lowest 10%. The probability of disease increased with increasing weighted genetic load for patients with SLE, ILE and ILE^{SLICC} compared with healthy controls (figure 2A–C). Specifically, those with a weighted genetic load of 19 (±2.0) or higher showed greater odds of developing SLE or ILE compared with healthy controls (figure 2A–C). However, the odds of developing SLE compared with ILE did not change with increasing weighted genetic load (figure 2D). Similarly, higher genetic load differentiated patients with ILE and SLE from controls (area under the curve=0.62 for both) but not patients with ILE from patients with SLE (area under the curve=0.51, p=0.78) by ROC analysis (figure 2E).

DISCUSSION

This study is the first to determine the genetic load of SLE risk alleles and unique risk variants in ILE. Although patients with ILE exhibit a milder phenotype compared with SLE, the genetic load of SLE risk alleles in patients with ILE was indistinguishable from patients with SLE, suggesting a similar genetic predisposition. However, it is unknown if there may be unique risk or protective variants associated with a subgroup of patients with ILE who never progress to SLE classification.

Previous studies in patients with SLE found that a higher genetic load is associated with a more severe SLE disease phenotype, including a higher frequency of renal disease.[10–13 16] As the patients with SLE in our cohort met more ACR criteria, including a higher frequency of renal disease, compared with patients with ILE, it is surprising that the genetic load is similar between the two groups. However, compared with other studies, we calculated genetic load based on the largest number of European SLE risk loci, which may be more inclusive of patients with less severe disease. Genetic load also correlates with earlier disease onset in patients with SLE,

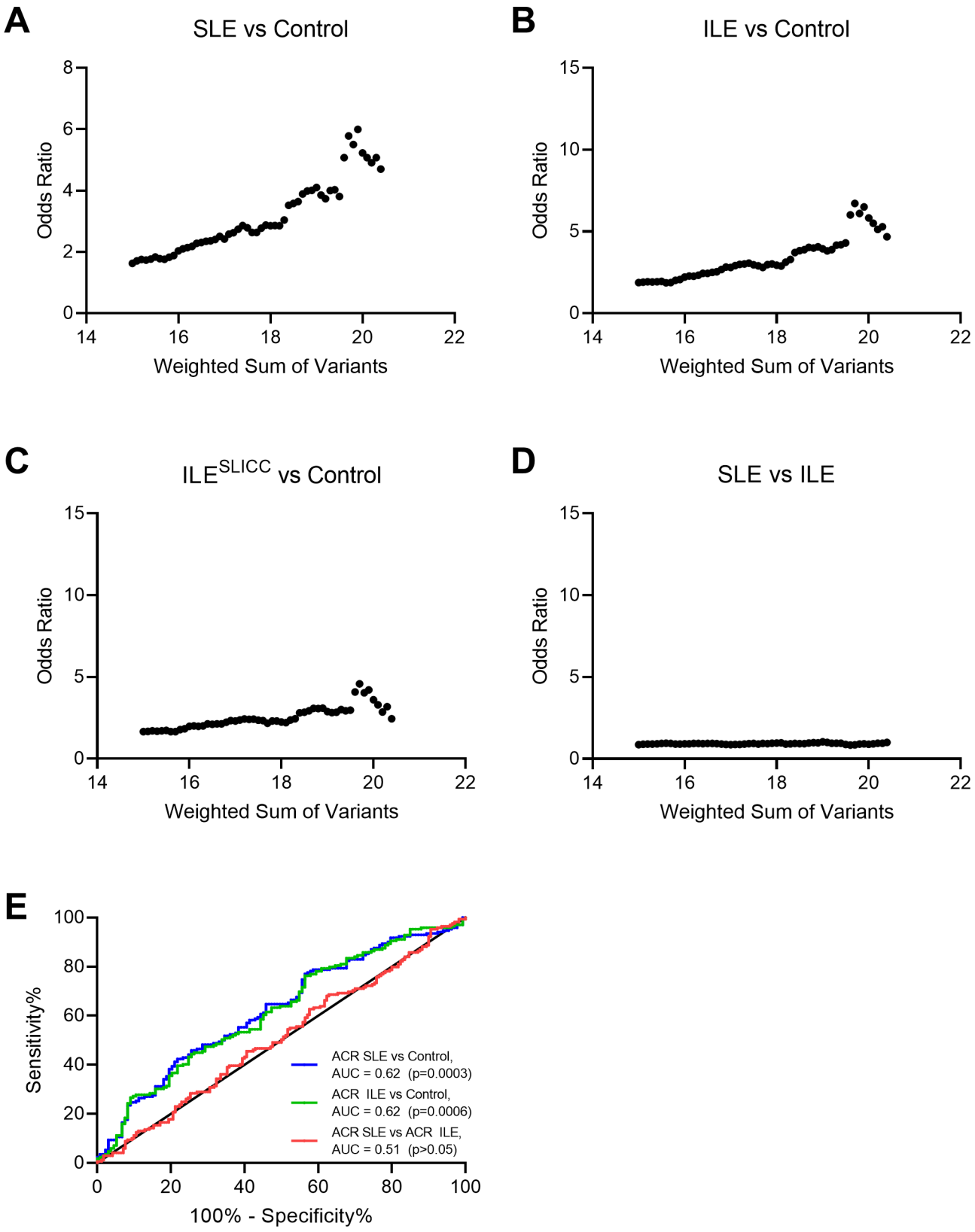

Figure 2 The genetic load of SLE risk alleles does not distinguish patients with ILE from patients with SLE. (A–D) ORs comparing individuals with a given weighted genetic load (±2.0) with those within the lowest 10% for (A) patients with SLE (n=170) and healthy controls (n=133), (B) patients with ILE (n=169) and healthy controls, (C) patients with ILE who also do not meet SLICC criteria (ILE^{SLICC}, n=117) and healthy controls, or (D) patients with SLE and ILE. (E) Receiver operating characteristic analysis to assess the prediction ability of weighted genetic load in patients with SLE, patients with ILE and healthy controls. AUC, area under the curve; ILE, incomplete lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

indicative of higher disease severity.[10–13 17 18] In our study, the frequency of childhood-onset disease was low and similar in a subset of both patients with ILE and patients with SLE, which may contribute to the similar genetic load. As patients with ILE are often older compared with patients with SLE,[7 19] patients with SLE with childhood onset may exhibit increased genetic load compared with patients with ILE.

Our study has some limitations. We were unable to examine race-specific genetic load differences between patients with SLE and ILE and healthy controls due to the low numbers of subjects in the racial subgroups. Therefore, replication in larger race-matched cohorts and subsequent transancestral meta-analysis is imperative. Furthermore, as we only had age at onset information for a subset of patients, it is unclear whether age at onset contributed to the similar genetic load.

Together, our data support an enhanced genetic predisposition towards ILE similar to SLE through aggregate genetic variants. Future studies in larger, longitudinal preclinical cohorts are needed to determine whether the phenotypical differences between SLE and ILE are governed by novel ILE genetic variants or disparate environmental or gene–environmental factors.

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Contributors JG, JAJ and MS conceived and designed the work. JAJ and TA captured, analysed and interpreted the patients' data, while JG, CM, MS, JMK, SM and WDJ helped in experimental data acquisition. JG, JAJ, MS, JMK and MS contributed to data interpretation, and MS, JAJ, CAW and JG contributed to the writing of the manuscript.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the Oklahoma Medical Research Foundation Institutional Review Board (IRB #06-12). The participants gave informed consent to participate in the study before taking part.

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