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Evidence for gene-gene epistatic interactions among susceptibility loci for systemic lupus erythematosus

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Abstract

Objective—Several confirmed genetic susceptibility loci for lupus have been described. To date, no clear evidence for genetic epistasis is established in lupus. We test for gene-gene interactions in a number of known lupus susceptibility loci.

Methods—Eighteen SNPs tagging independent and confirmed lupus susceptibility loci were genotyped in a set of 4,248 lupus patients and 3,818 normal healthy controls of European descent. Epistasis was tested using a 2-step approach utilizing both parametric and non-parametric methods. The false discovery rate (FDR) method was used to correct for multiple testing.

Results—We detected and confirmed gene-gene interactions between the HLA region and *CTLA4*, *IRF5, and ITGAM*, and between *PDCD1* and *IL21* in lupus patients. The most significant interaction detected by parametric analysis was between rs3131379 in the HLA region and rs231775 in *CTLA4* (Interaction odds ratio=1.19, z-score= 3.95, $P = 7.8\times10^{-5}$ (FDR≤0.05), $P_{MDR} = 5.9\times10^{-45}$). Importantly, our data suggest that in lupus patients the presence of the HLA lupus-risk alleles in rs1270942 and rs3131379 increases the odds of also carrying the lupus-risk allele in *IRF5* (rs2070197) by 17% and 16%, respectively ($P = 0.0028$ and 0.0047).

Conclusion—We provide evidence for gene-gene epistasis in systemic lupus erythematosus. These findings support a role for genetic interaction contributing to the complexity of lupus heritability.

Introduction

Recent candidate gene and genome-wide association studies (GWAS) led to the discovery and validation of multiple susceptibility loci for systemic lupus erythematosus (1). However, the heritability of lupus cannot be completely explained by the susceptibility loci already discovered. We suggest that the missing heritability in lupus can be explained by three potential mechanisms: A heritable epigenetic component, common and rare disease susceptibility variants yet to be discovered, and gene-gene interactions involving known and perhaps yet to be discovered genetic variants for disease susceptibility. There are very

limited and controversial data regarding gene-gene interaction (epistasis) in lupus (2–3). Consequently, it is widely accepted that the known lupus susceptibility loci operate additively rather than epistatically to increase the risk for lupus.

Herein, we sought to examine gene-gene interactions in some of the previously established and confirmed susceptibility loci for lupus, using a large set of lupus patients and controls. We discovered and confirmed 6 novel gene-gene interactions for lupus, using both parametric and non-parametric statistical methodologies.

Methods

Study participants and genotyping

A total of 4,248 lupus patients and 3,818 normal healthy controls of European descent were included in this study. Eighteen SNPs representing previously confirmed and independent autosomal lupus susceptibility loci were genotyped (Table 1). A summary for the allelic association results in these loci using the patients and controls included in this study is shown in Supplementary Table 1. We genotyped 2 tag SNPs in the HLA region. These 2 SNPs were selected as they were recently shown to have independent genetic effects using logistic regression analysis of a large number of lupus-associated SNPs in the HLA region (4). Likewise, 3 tag SNPs representing independent genetic susceptibility effects in *IRF5* were genotyped (5). All lupus patients fulfilled the ACR lupus classification criteria (6–7). Genotyping was performed using Illumina Custom Bead system on the iSCAN instrument as part of a large lupus candidate gene association study to reduce cost of genotyping and maximize sample size. We genotyped 347 ancestry informative markers (AIMs) in our samples (8–11). Individuals with a genotype success rate of <90% (361 samples) were excluded from the analysis. The remaining samples were then evaluated for duplicates or related individuals and one individual from each pair was removed (117 samples) if the proportion of alleles shared identical by descent (IBD) > 0.4. Samples were assessed for mismatches between their reported gender and their genetic data and 112 samples were removed from the analysis as they did not meet the following criteria: an assigned male was required to have chromosomal X heterozygosity ≤10% and be heterozygous at rs2557524 and an assigned female was required to have chromosomal X heterozygosity >10% and be homozygous at rs2557524. The SNP rs2557524 is mapped on a region on chromosome X and Y that is identical except for this 1 base. Because of this 1 base difference males generate a heterozygous genotype (due to the presence of both X and Y chromosomes) and females generate a homozygous genotype (due to the presence of only X chromosomes).

Next, samples with increased heterozygosity (>5 standard deviation around the mean) were removed from the analysis (5 samples). Finally, 42 genetic outliers were removed from further analysis as determined by principal components analysis. An additional 2 outlier samples identified by admixture proportions calculated using ADMIXMAP were also removed. After applying the quality control measures detailed above, samples included in our analysis consisted of 3,936 European-derived lupus patients (3,592 females, 344 males), and 3,491 European-derived normal healthy controls (2,340 females, 1,151 males).

Detection of gene-gene interaction

Testing for gene-gene interaction was performed sequentially using two independent statistical approaches. First, a parametric analysis for epistasis was applied as implemented in PLINK (12). Epistatic interactions detected using PLINK were validated using allelic 2×2 tables among lupus patients to calculate interaction odds ratios and identify the specific alleles in each SNP pair that contributed to the interaction detected. Allelic 2×2 tables (Figure 1) were obtained from 3×3 genotypic tables (Supplementary Figure 1) for each

interaction tested. The allelic 2×2 tables are based on 4N allele counts, where N is the total number of individuals, with each individual contributing a total of 4 independent alleles. Z-scores were calculated as the natural logarithm of the odds ratio divided by the square root of the variance, and associated *P* values were assigned from the z-scores for each interaction. Chi-square statistics for pair-wise interaction were calculated as were chi-square derived *P* values. Second, a pair-wise non-parametric epistasis test was applied utilizing multifactor dimensionality reduction analysis (MDR) (13–14).

The false discover rate (FDR) method as described by Benjamini and Hochberg was used to correct for multiple comparisons (15–16).

Results

To test for gene-gene interactions within the known lupus susceptibility loci examined, we performed a 2-step epistasis analysis using a parametric approach followed by a non-parametric analysis. This 2-step approach has the strength of examining and confirming epistatic interactions using 2 independent statistical methods. This is necessary as the best methodology to detect gene-gene interaction remains controversial.

We first used a case-only pair-wise epistasis analysis implemented in PLINK. The case-only analysis was selected as it was shown to be a more powerful test for epistasis compared to case-control analysis (17–18). Interactions with FDR of ≤ 0.05 were considered established, and those with FDR >0.05 and ≤ 0.25 were considered suggestive interactions that require confirmation. A high FDR was used in the initial screening for suggestive interactions to avoid excluding true gene-gene interactions from confirmatory analyses.

We discovered six gene-gene interactions using parametric analysis (Table 2). The two most significant interactions were between *CTLA4* and the two SNPs representing two independent genetic effects within the HLA region (FDR≤ 0.05). The detected epistasis signal between the risk alleles in *CTLA4* and rs3131379 (HLA region 1) and *CTLA4* and rs1270942 (HLA region 2) showed an interaction odds ratio of 1.19 and 1.18 (z-score= 3.95, $P= 7.8\times10^{-5}$, and z-score= 3.88, $P= 1.0\times10^{-4}$, respectively). These data indicate that in lupus patients, the presence of the lupus-risk allele in *CTLA4* increases the odds of carrying the risk allele in either of the HLA lupus associated loci by ~20% and vice versa (Figure 1). Four additional suggestive gene-gene interactions (FDR≤ 0.25) were found between the HLA and *IRF5*, the HLA and *ITGAM*, and *IL21* and *PDCD1* (Table 2). The presence of the risk allele in the two HLA lupus-associated loci examined (rs1270942 and rs3131379) increases the odds of carrying the lupus-risk allele in *IRF5* (rs2070197) by 17% and 16%, respectively, and vice versa (*P*= 0.0028 and 0.0047). Interestingly, our data suggest that the presence of the risk allele in *ITGAM* increases the odds of carrying the protective allele in rs3131379 (HLA) by 16% (*P*= 0.0075).

Next, and in order to confirm the two gene-gene interactions that we established using parametric tests, and to test if the other 4 suggestive gene-gene interactions can be established, we applied the multifactor dimensionality reduction test (MDR) to the interactions initially discovered using parametric analysis. MDR is a non-parametric test for non-linear epistasis. A pair-wise MDR analysis was applied to test the specific interactions discovered in stage 1. It should be noted, however, that results obtained using the MDR non-parametric analysis reflect a joint effect consisting of the main genetic association effect in the loci examined and the interaction effect. These results are presented in Table 3 (See Supplementary Table 2 and Supplementary Figure 2 for details).

Discussion

The very existence and nature of genetic epistasis in lupus has been elusive. We combined the strengths of two independent approaches to test for genetic epistasis in lupus, and identified several novel gene-gene interactions using a large European-derived sample. The most significant interaction we identified was between the HLA region and *CTLA4*. Indeed, two independent lupus-associated SNPs within the HLA region (rs3131379 and rs1270942) showed evidence for significant interaction with rs231775 in *CTLA4* (Tables 2 and 3). The HLA-*CTLA4* interaction in lupus underscores antigen presentation and T cell stimulation as an important process involved in the pathogenesis of lupus. This interaction is biologically logical as CTLA4 is upregulated on T cells following T cell activation by antigen presenting cells (19). Following T cell activation via the binding of MHC:antigen complex to the T cell receptor (signal 1), the binding of CD80/CD86 on antigen presenting cells to CD28 on the surface of T cells (signal 2) ensures T cell activation and IL-2 production (19). CTLA4 competes with CD28 to bind CD80/CD86 and provides a negative signal that suppresses T cell activation. This process is thought to be important to control T cell activation and prevent autoimmunity.

A role for antigen presenting cells in lupus is highlighted again with the HLA/*ITGAM* gene-gene interaction, though this interaction is between the risk and protective alleles in these 2 loci. *ITGAM* (integrin, alpha M) encodes for CD11b, the alpha chain in the integrin molecule CD11b/CD18 (MAC-1, CR3). It is expressed on the surface of antigen presenting cells and neutrophils, and plays a role in cell-cell adhesions, leukocyte extravasation, and in complement-mediated phagocytosis of C3bi opsonized antigens (20–21).

We also showed evidence for gene-gene interaction between the 2 independent lupus-associated SNPs within the HLA region with rs2070197 in *IRF5*. This interaction emphasizes the role of the interferon pathway in the pathogenesis of lupus.

The other gene-gene interaction we identified was between rs907715 in *IL21* and rs11568821 in *PDCD1*. This interaction is very interesting as it highlights a role for follicular helper T cells (T_{FH}) in lupus. High PDCD1 expression and IL-21 production is a hallmark of T_{FH} cells (22). T_{FH} cells promote germinal center formation, plasma cell differentiation, and antibody isotype switching (23). PDCD1 deficiency results in impaired germinal center B cell survival and diminished production of long-lived plasma cells (24). Indeed, the production of IL-21 is reduced in T_{FH} cells from *Pdcd1*−/− mice (24). IL-21 deficiency results in impaired germinal center formation, plasma cell differentiation, and isotype class switching (23), emphasizing a central role for IL-21 in T_{FH} function. Of interest, a higher fraction of circulating T_{FH} cells was detected in the peripheral blood from patients with lupus compared to normal controls (25).

In summary, we provided strong evidence that the presence of one risk allele can influence the presence or absence of other risk alleles in lupus patients across different loci. We have identified novel gene-gene epistatic interactions in lupus. Gene-gene interactions might help explain at least part of the “missing heritability” in complex diseases. Our findings argue against a simple “additive” genetic model in autoimmunity, and highlight antigen presentation and T cells activation, the interferon pathway, and follicular helper T cells as important contributors to the pathogenesis of lupus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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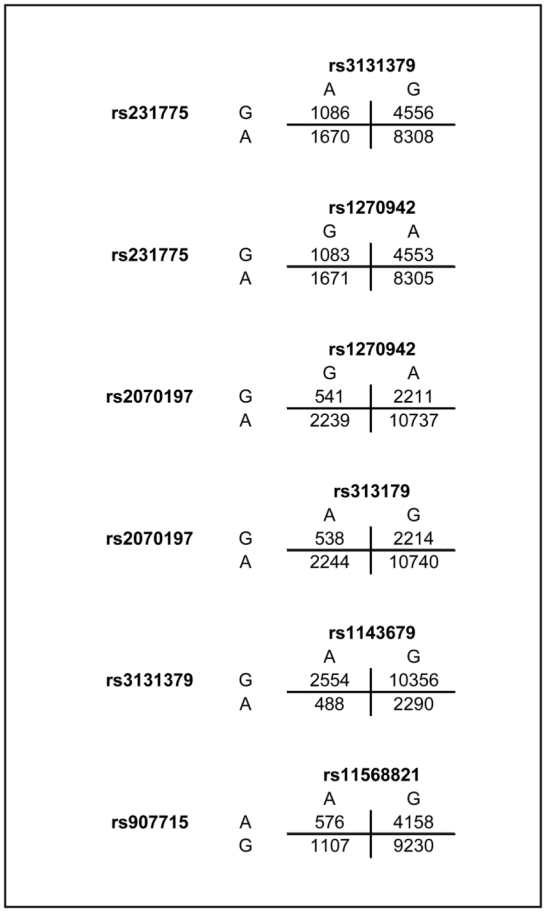

Figure 1.
Allelic 2×2 tables in lupus patients used to calculate interaction odds ratios and identify the specific alleles in each SNP pair that contributed to the interaction detected.

Table 1

Previously reported lupus susceptibility loci analyzed for gene-gene interaction in this study.

Gene/region	Chromosome	Associated SNP	Risk allele	Odds ratio*	Reference
BANK1	4q24	rs10516487	G	1.38	(26)
C8orf13-BLK	8p22–23	rs13277113	A	1.39	(27)
CTLA4	2q33	rs231775	G	1.23	(28)
FCGR2A	1q23	rs1801274	C	1.35	(29)
HLA region1	6p21.33	rs3131379	A	2.36	(30)
HLA region2	6p21.32	rs1270942	G	2.35	(30)
IL-21	4q26	rs907715	G	1.29	(31)
IRF5	7q32	rs2070197	C	1.85**	(5)
IRF5	7q32	rs729302	A	1.39**	(5)
IRF5	7q32	rs10954213	A	1.25**	(5)
ITGAM	16p11.2	rs1143679	A	1.78	(32)
KIAA1542	11p15.5	rs4963128	C	1.28	(30)
MBL	10q11	rs1800450	A	1.41	(33)
PDCD1	2q37.3	rs11568821	A	2.85	(34)
PTPN22	1p13	rs2476601	A	1.53	(35)
PXK	3p14.3	rs6445975	C	1.25	(30)
STAT4	2q32.2	rs7574865	T	1.55	(36)
TNFSF4	1q25	rs2205960	T	1.28	(37)

*SLE patients versus healthy controls as reported in previous studies referenced above

**Transmitted/untransmitted ratio reported as this was based on trio and family studies (5).

Table 2

Gene-gene interaction results in 18 known independent lupus susceptibility loci, using logistic regression analysis implemented in PLINK. Only interactions with FDR ≤0.25 are shown.

Gene	Polymorphism	Risk Allele	Interacting Alleles	Interaction Odds Ratio	Z-score	Z-score *P*	Chi^2	Chi^2*P*
CTLA4	rs231775 (A/G)	G						
HLA	rs3131379 (A/G)	A	GXA	1.19	3.95	7.8×10^{-5}	15.19	9.7×10^{-5}
CTLA4	rs231775 (A/G)	G						
HLA	rs1270942 (A/G)	G	GXG	1.18	3.88	1.0×10^{-4}	14.87	1.0×10^{-4}
IRF5	rs2070197 (A/G)	G						
HLA	rs1270942 (A/G)	G	GXG	1.17	2.99	0.0028	8.93	0.0028
IRF5	rs2070197 (A/G)	G						
HLA	rs3131379 (A/G)	A	GXA	1.16	2.83	0.0047	7.98	0.0047
HLA	rs3131379 (A/G)	A						
ITGAM	rs1143679 (A/G)	A	GXA	1.16	2.67	0.0075	6.93	0.0085
IL21	rs907715 (A/G)	G						
PDCD1	rs11568821 (A/G)	A	AXA	1.16	2.64	0.0084	6.80	0.0091

Z-scores were calculated as the natural logarithm of the odds ratio divided by the square root of the variance

Table 3

Multifactor dimensionality reduction (MDR) analysis for pair-wise interactions detected using parametric analysis in lupus patients and controls.

Interaction	Cross Validation Consistency	Balanced Accuracy	Chi^2	*P* Value
CTLA4 (rs231775) × *HLA* (rs3131379)	10/10	0.5737	208.57	5.9×10-45
CTLA4 (rs231775) × *HLA* (rs1270942)	10/10	0.5744	212.76	7.4×10-46
HLA (rs1270942) × *IRF5* (rs2070197)	10/10	0.5949	270.60	2.3×10-58
HLA (rs3131379) × *IRF5* (rs2070197)	10/10	0.5946	268.81	5.6×10-58
HLA (rs3131379) × *ITGAM* (rs1143679)	10/10	0.5985	287.71	4.6×10-62
PDCD1 (rs11568821) × *IL21* (rs907715)	10/10	0.5235	17.44	5.7×10-4

Df of 3 was used to calculate P values

Cross validation consistency reflects the number of times MDR found the same model as it divided up the data into different segments. Balanced accuracy is defined as (sensitivity+specificity)/2 where sensitivity = true positives/(true positives +false negatives) and specificity = true negatives/(false positives+true negatives). This gives an accuracy estimate that is not biased by the larger class (38).