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IRF5 SLE-Risk Haplotype is Associated with Asymptomatic Serologic Autoimmunity and Progression to Clinical Autoimmunity in Neonatal Lupus Mothers

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Abstract

Objective—Genetic variation in interferon regulatory factor 5 (IRF5) has been associated with risk of developing systemic lupus erythematosus (SLE), and this association is largely dependent upon anti-Ro autoantibodies. We studied a unique cohort of anti-Ro positive individuals with diverse diagnoses to determine if IRF5 genotype associated with maternal diagnosis or progression of autoimmunity.

Methods—We genotyped haplotype-tagging polymorphisms in IRF5 in 93 European ancestry subjects recruited to the Research Registry for Neonatal Lupus who all had high titer anti-Ro autoantibodies and a child with neonatal lupus (NL), and allele frequencies were compared to non-autoimmune controls. The mothers diagnoses included SLE, Sjogren's syndrome (SS), undifferentiated autoimmune syndrome (UAS), and asymptomatic.

Results—The SLE-risk haplotype of IRF5 was enriched in all anti-Ro positive subjects except those with SS (OR = 2.55, $p=8.8 \times 10^{-4}$). Even asymptomatic individuals with anti-Ro antibodies were enriched for the SLE-risk haplotype (OR=2.69, $p=0.019$). The same haplotype was more prevalent in subjects who were initially asymptomatic, but developed symptomatic SLE during follow up (OR=5.83, $p=0.0024$). Interestingly, SS was associated with two minor IRF5 haplotypes, and these same haplotypes were decreased in frequency in those with SLE and UAS.

Conclusions—The IRF5 SLE-risk haplotype was associated with anti-Ro antibodies in asymptomatic individuals as well as progression to SLE in asymptomatic anti-Ro positive individuals. SS in NL mothers was associated with different IRF5 haplotypes. These data suggest that IRF5 polymorphisms play a role in serologic autoimmunity in humans and may promote the progression to clinical autoimmunity.

Keywords

systemic lupus erythematosus; interferon; autoantibodies; neonatal lupus; Sjogren's syndrome

Neonatal lupus (NL) is caused by the passage of maternal autoantibodies directed to Ro (SSA) and La (SSB) ribonucleoproteins across the placenta, with subsequent tissue deposition and inflammation in the fetus. These autoantibodies are nearly universally

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present in the mother when isolated heart block is diagnosed in utero (1). Neonatal disease is not dependent upon maternal diagnosis, and mothers of NL patients may have a variety of clinical diagnoses, ranging from systemic lupus erythematosus (SLE) or Sjogren's syndrome (SS), to completely asymptomatic mothers with high titer anti-Ro antibodies. Additionally, some mothers who are initially asymptomatic can remain so for many years, or progress over time to SLE or SS (2).

Both SLE and SS are associated with high circulating type I IFN activity (3, 4). Anti-Ro autoantibodies are frequently present in patients with SLE and SS, and these autoantibodies are associated with high serum type I IFN activity in patients with SLE (4). When we studied serum IFN- α activity in NL mothers, we found that asymptomatic individuals did not have high IFN- α , despite having high titer anti-Ro antibodies (5). This suggests that background factors will also be important to the association between anti-Ro autoantibodies and high type I IFN activity, and that the association between autoantibodies and high IFN in humans in vivo is not absolute.

Some of the genetic risk loci which are shared by both SLE and SS are functional within the type I IFN pathway (6). Interferon regulatory factor 5 (IRF5) is a transcription factor that induces transcription of IFN- α and other IFN- α inducible genes. Genetic variants in IRF5 confer risk of SLE and SS in people of European ancestry (7, 8). The SLE-risk haplotype of IRF5 has been associated with increased IFN- α in SLE patients (9). Interestingly, the increase in serum IFN- α activity related to IRF5 risk haplotype was completely dependent upon the presence of autoantibodies (9, 10). In follow up, we demonstrated that the genetic association between IRF5 and SLE was largely dependent upon those subjects who had anti-Ro or anti-dsDNA autoantibodies, and much weaker evidence for association of IRF5 with SLE was observed in the anti-Ro negative, anti-dsDNA negative patient group (10).

This raises the question of whether genetic variations in IRF5 are associated primarily with autoantibody formation, or with the subset of SLE patients who have these autoantibodies. Because SLE-associated autoantibodies are not typically found in control subjects, this question cannot be effectively addressed in current case-control cohorts. To explore this question further, we examined IRF5 haplotypes in a unique cohort of mothers of NL patients who all had high titer anti-Ro autoantibodies, and had diagnoses which ranged from asymptomatic to SS or SLE. We sought to determine whether IRF5 haplotypes were associated with anti-Ro antibodies, maternal diagnosis, and/or progression of autoimmunity in this cohort.

Materials and Methods

Patients and Samples

We studied 93 European-ancestry individuals recruited to the Research Registry for Neonatal Lupus who all had high titer anti-Ro autoantibodies and a child with neonatal lupus (1). The diagnoses of these subjects were as follows: 17 healthy/asymptomatic, 30 SLE, 22 Sjogren's Syndrome (SS), and 24 undifferentiated autoimmune syndrome (UAS). SLE and SS were defined using standard diagnostic criteria (11, 12), and subjects who had symptoms of autoimmune disease which did not meet full criteria for these conditions were categorized as UAS. Several patients met criteria for both SLE and SS, and for our initial analyses these subjects were included in the SLE category, considering them as SLE patients with secondary SS. We subsequently also considered these subjects as a separate group. 117 control DNA samples were obtained from the University of Chicago TRIDOM registry, matched by ancestral background, age, and gender to the cases. Each control was individually screened for the absence of autoimmune disease by medical record review. Informed consent was obtained from all subjects.

SNP genotyping

We genotyped the rs2004640, rs3807306, rs10488631, and rs2280714 single nucleotide polymorphisms (SNPs) in IRF5 which define major European-ancestry haplotypes (7) using Taqman primer-probe sets. Haplotype frequencies observed in both NL mothers and controls were similar to published data (Figure 1) (10). All SNPs conformed to Hardy-Weinberg equilibrium (p -values >0.05), and the genotyping success rate was $>95\%$ for all SNPs.

Autoantibody ELISAs

Anti-Ro and anti-La were detected using commercial ELISAs run in the NYU/Langone Medical Center CLIA-approved immunology laboratory, employing bovine Ro60 and anti-La purified from calf and rabbit thymus respectively. ELISAs for the anti-Ro fine specificities anti-Ro 52 and anti-Ro p200 were done as outlined in (13).

Statistical Analysis

Subjects were analyzed in groups based upon their clinical diagnoses. Backward logistic regression models were used to detect associations between IRF5 haplotypes and clinical diagnoses and other parameters. Associations detected in logistic regression models were then examined in detail by comparing haplotype frequencies using a Fisher's exact test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using standard methods.

Results

IRF5 SLE risk haplotype is enriched in anti-Ro positive NL mothers with diverse diagnoses

Overall the anti-Ro positive NL mothers had a higher frequency of the SLE risk haplotype (haplotype #1) when compared to controls (Table 1). Statistically significant differences were observed between both the asymptomatic and SLE groups, and there was a non-significant similar trend in the UAS group. In contrast, the frequency of the SLE risk haplotype in NL mothers who had SS was more similar to controls (OR=1.38). In comparing all NL mothers except those with SS to controls, a strong statistically significant difference is observed (OR=2.55, 95% CI=1.42–4.53, $p=8.8 \times 10^{-4}$). Because the HLA locus has been previously associated with anti-Ro antibodies, we tested for potential gene-gene interactions between HLA-DRB1 alleles and the IRF5 SLE-risk haplotype in 39 mothers who had HLA data available. We did not detect such an interaction ($p>0.20$ for common HLA alleles), suggesting that the IRF5 association we observe is independent of HLA-DRB1 genotype (data not shown).

Minor IRF5 haplotypes are associated with Sjogren's syndrome in the NL mothers, and are significantly decreased in mothers with SLE and UAS

Because the SLE-risk haplotype was not strongly associated with SS in the NL mothers group, we explored other possible haplotype associations in this group. Two minor haplotypes (#3 and #4, -CTA) showed independent evidence for association with an SS diagnosis within the NL mothers group. Considered jointly, the -CTA haplotypes were significantly enriched in the NL mothers with SS as compared to controls (OR=2.06, Table 2). Interestingly, the same haplotypes were significantly decreased in mothers with SLE and UAS as compared to controls (OR=0.25 for SLE and 0.34 for UAS respectively). Thus, the difference in frequency between these haplotypes in NL mothers with SS vs. those with SLE or UAS was extremely large (SS vs. SLE or UAS in NL mothers OR=7.07, 95% CI=2.45–20.41, $p=2.9 \times 10^{-5}$). A number of NL mothers who met the diagnostic criteria for SLE also had secondary SS. In the analyses above, these subjects have been included in the SLE

category. The SLE-risk haplotype was present at 23.7% frequency in NL mothers with SLE and secondary SS (n=19) as compared to 27.3% in the SLE without SS category (n=11). The –CTA haplotypes (#3 and #4) were present at 5.3% frequency in SLE with SS and 3.9% in SLE without SS. Thus, IRF5 haplotype frequencies were similar in NL mothers with SLE regardless of whether they had secondary SS (Tables 1 and 2). No associations were observed between the SLE-risk or –CTA haplotypes and anti-La antibodies ($p>0.23$ for each) or with the anti-Ro p200 antibody specificity (56 mothers tested for p200, 86% were positive, $p>0.44$ for association with each IRF5 haplotype). All 93 mothers had antibodies against anti-Ro 60, and almost all mothers had anti-Ro 52 antibodies (86 out of 87 mothers tested).

IRF5 SLE-risk haplotype is associated with progression to SLE in asymptomatic individuals

Some of the NL mothers were asymptomatic at the birth of their NL child, but later progressed to SLE or SS. Five initially asymptomatic NL mothers progressed to SLE after a mean of 11 years, and three asymptomatic NL mothers progressed to SS after a mean of 9 years. The five mothers who progressed to SLE had a significantly increased frequency of the SLE-risk haplotype as compared to controls (frequency in asymptomatic progressors to SLE=0.400, compared to controls OR=5.83, $p=0.0024$). In contrast, none of the three patients who progressed to SS had the SLE-risk haplotype. In these three subjects the frequency of the –CTA haplotypes was 0.166. The –CTA haplotypes were not present in any of the patients who progressed from asymptomatic to SLE.

Discussion

We have previously shown that the IRF5 SLE-risk haplotype is associated with the presence of anti-Ro antibodies in SLE patients. Furthermore, in SLE patients with anti-Ro antibodies, the IRF5 SLE-risk haplotype was associated with greater serum IFN- α (10). This suggests that the SLE-risk haplotype results in a gain-of-function change which allows for greater IFN- α production in the presence of anti-Ro immune complexes as a consequence of Toll-like receptor ligation and subsequent signaling. While this mechanism of increasing IFN- α may explain how the risk haplotype contributes to the development of SLE, it is also possible that this same haplotype directly predisposes to the formation of anti-Ro antibodies. If so, a feed-forward mechanism would be supported, in which the IRF5 haplotype predisposes to the formation of anti-Ro antibodies, which then subsequently results in greater IFN- α production in the setting of the same IRF5 variant. Using this unique cohort of anti-Ro positive asymptomatic mothers, we tested the first portion of this hypothesis more directly. The strong enrichment of the SLE-risk haplotype in asymptomatic anti-Ro positive NL mothers supports the hypothesis that this IRF5 haplotype plays a role in autoantibody formation. Similarly, IRF5 knock-out in a lupus-like murine model greatly decreased SLE-associated autoantibody formation (14). It is possible that some of the mothers who were asymptomatic at the time of our study may later progress to SLE, although rates of progression to SLE in this group are approximately 18% over 10 years (2), and it does not seem likely that this would fully account for the two-fold enrichment of the IRF5 SLE-risk haplotype in the asymptomatic group. Due to the unique nature of the population we studied, our sample size could not be easily increased, and our results could suffer from inflation of the odds ratios related to small sample size. Future independent replication would be of interest.

Additionally, we show that the SLE-risk haplotype is associated with progression to SLE from asymptomatic status in anti-Ro positive NL mothers. This finding would support the second portion of the feed-forward loop proposed above. Anti-Ro antibodies were associated with greater serum IFN- α in SLE patients (10), and this increase in IFN- α may

be occurring in asymptomatic anti-Ro positive mothers who carry the SLE-risk haplotype, facilitating the conversion from serologic autoimmunity to SLE. A similar two-step model of SLE initiation involving autoantibodies and IFN- α has been proposed previously (15).

Surprisingly, the associations between IRF5 haplotypes and SS in the NL mothers differed dramatically from other diagnoses. Previous case-control genetic studies have reported an association between SLE-associated IRF5 variants and primary SS (8). In contrast, we found a strong association between SS in NL mothers and a different –CTA haplotype. While it is possible that these differences relate to the small number of SS patients in our study, the –CTA haplotype is particularly interesting, as it is also much less frequent in NL mothers with SLE or UAS. The molecular basis for these opposing associations observed in our anti-Ro positive cohort is unclear. An important caveat is that only a small fraction of people with SS and SLE ever have a child with NL, and the NL mothers may not be fully representative of SS and SLE in the general population.

Taken together, our data support the concept that IRF5 haplotypes are associated with both autoantibody formation and the progression to autoimmune disease in patients with serologic autoimmunity. The diversity of IRF5 haplotype associations observed would suggest that the different haplotypes confer differences in the biological function of IRF5, which then result in propensity to distinct autoimmune conditions.

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Figure 1.

IRF5 haplotype diagram and haplotype frequencies in the NL mothers. A. and B. show the pairwise correlations between SNPs as r-squared values for the NL mothers (A.) and controls (B.) respectively, with darker shading indicating a higher r-squared and thus a greater degree of correlation. C. and D. show the frequencies of the 5 major haplotypes observed in the NL mothers (C.) and controls (D.) respectively.

Table 1

Frequency of SLE risk haplotype in Ro+ NL mothers with different clinical diagnoses

Diagnostic Category	N	SLE-risk haplotype frequency	# of SLE-risk haplotype carriers (CC or CT)	OR vs. controls	p value	All Ro+ mothers except SS vs. controls
Asymptomatic	17	0.235	7	2.69	0.019	
SLE	30	0.250	13	2.92	0.0021	OR = 2.55
SS	22	0.136	6	1.38	0.44	95% CI = (1.42–4.53)
UAS	24	0.188	7	2.02	0.08	$p = 8.8 \times 10^{-4}$
Controls	117	0.103	21	X	X	

Table 2
Frequency of the -CTA minor haplotypes in Ro+ NL mothers with different clinical diagnoses

Diagnostic Category	N	-CTA haplotype frequency	OR vs. controls	p value	SS Ro+ mothers vs. SLE and UAS Ro+ mothers
Asymptomatic	17	0.176	1.11	0.62	
SLE	30	0.047	0.25	0.0067	OR = 7.07
SS	22	0.286	2.06	0.049	95% CI = (2.45–20.41)
UAS	24	0.063	0.34	0.076	p = 2.9×10 ⁻⁵
Controls	117	0.162	X	X	