Identification of Genetic Bio-markers for Systemic Lupus Erythematosus

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Summary:

Background: SLE is a complex autoimmune disease, characterized by the presence of various kinds of auto-antibodies in patients’ sera. ANAs, one of the SLE-associated auto-antibodies, are considered a diagnostic hallmark for SLE being positive in 95% or more of SLE patients. SLE is preceded by a symptom-free phase characterized by the presence of ANAs for up to several years, what suggests that ANAs could be used as a predictive biomarker for people at risk of developing SLE. However, ANAs are detectable in the sera of up to 5% of the normal healthy population and only less than 10% of those ANA-positive individuals will end up developing SLE.

Although there are many candidate-gene studies and GWASs that have been done and identified more than 35 genetic loci that contribute to the susceptibility and pathogenesis of SLE, data about the genetic polymorphisms associated with ANA-positivity in healthy individuals and their relatedness to SLE-specific genetic polymorphisms are limited.

Objectives: The aim of this study is to identify the SNPs commonly associated with ANA in apparently healthy individuals and detect whether they overlap with any of SNPs or genes known to be associated with SLE susceptibility to suggest a genetic/serologic predictive approach for SLE.

Subjects & methods: ANA was screened by IIF in 6454 serum samples of healthy blood donors at initial dilution 1:100. The ANA-positive samples were validated at different dilutions and tested for SLE-specific nuclear antigens at a dilution 1:101 by line blot immunoassay.

219 ANA-positive and 437 ANA-negative DNA samples were involved in a GWAS using Illumina Immunochip. DNA of 235 ANA-positive and 490 ANA-negative samples were genotyped for 6 SNPs [F5 (rs6027), STAT1/STAT4 (rs12469810), STAT4 (rs115862660), ATG7 (rs4684068), IL12A (rs76493595), R3HDM2 (rs7488256)] selected for the replication study from those showed a suggestive evidence for ANA-association in the discovery phase (p-value ≤ 5x10⁻⁴) using TaqMan® SNP Genotyping Assays.

The results of our present study can be summarized as follows:
The prevalence of ANA in healthy individuals at dilution 1:100 by IIF was 5.62%. The most frequent fluorescent pattern was nuclear granular (79.89%), nuclear homogeneous (13.22%), combined patterns (4.41%) and cytoplasmic Golgi complex (2.48%).
The GWAS analysis revealed 21 SNPs with suggestive evidence for ANA-association. The replication study for 6 SNPs showed that the alleles C of F5 (rs6027), G of STAT1/STAT4 (rs12469810) and G of ATG7 (rs4684068) are the minor risky alleles for the development of ANA in healthy individuals with OR 1.67, 2.88 and 1.56 respectively.

There are statistically significant associations between ANA positivity in healthy individuals and F5 (rs6027), STAT1/STAT4 (rs12469810) and ATG7 (rs4684068) SNPs when their minor risky alleles are inherited in either co-dominant, dominant or over-dominant models.

There are statistically significant associations between STAT1/STAT4 (rs12469810) and ANA-positive samples with few nuclear dots pattern in IIF and between F5 (rs6027) and ANA-positive samples with few cytoplasmic Golgi complex pattern in IIF.

There is statistically significant association between F5 (rs6027) and ANA-positive samples with anti-Sm specific antibodies detected by line blot immunoassay.

The epistasis (SNP/SNP interaction) analysis between SNP pairs and trios showed increases in the significance of associations with ANA positivity in healthy individuals more than the effect of each SNP separately.

Conclusion: We can conclude from our results and evidence from the literature that the F5 (rs6027), STAT1/STAT4 (rs12469810) and ATG7 (rs4684068) polymorphisms are novel SNPs associated with the ANA production in healthy individuals. They are overlapping with SLE susceptibility variants. Therefore, we can suggest genotyping of F5 rs6027, STAT1/STAT4 (rs12469810) and ATG7 (rs4684068) SNPs in ANA positive healthy individuals as a genetic/serologic predictive approach for the subsequent development of SLE.