Functional Polymorphisms of the Coagulation Factor II Gene (F2) and Susceptibility to Systemic Lupus Erythematosus

F. YESIM K. DEMIRCI, AMY S. DRESSEN, CANDACE M. KAMMERER, M. MICHAEL BARMADA, AMY H. KAO, ROSALIND RAMSEY-GOLDMAN, SUSAN MANZI, and M. ILYAS KAMBOH

ABSTRACT. Objective. Two F2 functional polymorphisms, rs1799963 (G20210A) and rs3136516 (A19911G), are known to be associated with elevated levels/activity of prothrombin (encoded by F2) and risk of thrombosis. Since patients with systemic lupus erythematosus (SLE) have high risk of thrombosis and accelerated atherosclerosis and also high prevalence of anti-prothrombin antibodies, we hypothesized that these two F2 polymorphisms could affect risk of SLE.

Methods. We investigated these polymorphisms in 627 women with SLE (84% Caucasian Americans, 16% African Americans) and 657 female controls (78% Caucasian Americans, 22% African Americans).

Results. While the rs1799963 A allele was almost absent in African Americans, it was present at ~2% frequency in Caucasian Americans and showed no significant association with SLE. The rs3136516 G allele frequency was significantly higher in Caucasian SLE cases than in controls (48.4% vs 43.7%, respectively) with a covariate-adjusted odds ratio (OR) of 1.22 (95% CI 1.03–1.46, p = 0.023). The association was replicated in African Americans (rs3136516 G allele frequency 65.7% in cases vs 82.2% in controls) with an adjusted OR of 1.96 (95% CI 1.08–3.58, p = 0.022). Stratification of Caucasian SLE patients based on the presence or absence of cardiac and vascular events (CVE) revealed stronger association with the CVE-positive SLE subgroup than the CVE-negative SLE subgroup (OR 1.42 vs 1.20). Prothrombin activity measurements in a subset of SLE cases demonstrated higher activity in the carriers of the rs3136516 G allele.

Conclusion. Our results suggest a potential role for prothrombin and the crosstalk between hemostatic and immune/inflammatory systems in SLE and SLE-associated cardiovascular events, which warrants further investigation in independent samples. (First Release Jan 15 2011; J Rheumatol 2011;38:652–7; doi:10.3899/jrheum.100728)

Key Indexing Terms: LUPUS PROTHROMBIN F2 POLYMORPHISM A19911G G20210A

Prothrombin is a vitamin K-dependent glycoprotein that is primarily synthesized in the liver and secreted into circulating plasma. Upon activation by the prothrombinase complex (activated factor X, factor V, calcium, and phospholipids), prothrombin (factor II) is converted to its enzymatically active form, thrombin (factor IIa). In the coagulation cascade, thrombin exerts its procoagulant activity by converting soluble fibrinogen into insoluble fibrin strands as well as by activating other coagulation factors. Thrombin acts also as an indirect anticoagulant by activating protein C on the surface of endothelial cells in the presence of thrombomodulin. Thrombin is a multifunctional protein and, in addition to its well known role in the coagulation cascade, it is involved in platelet aggregation, thrombus formation and fibrinolysis, endothelial barrier integrity, immune cell adhesion/activation, inflammation, and tissue reparative processes. Thrombin is among the key factors that mediate the extensive crosstalk between inflammation and hemostasis, the 2 major processes of defensive host response.

Systemic lupus erythematosus (SLE) is a chronic inflam-
Phenotypes. Previous studies investigated functional polymorphisms mainly for their effects on cardiovascular events, although the sample sizes were underpowered to detect the effects of such an uncommon variant. The rs1799963 (located at 3'UTR) polymorphism (located within the 13th intron, which is only 146 bp) is also functional through its effect on intronic enhancer motif by the A allele. That is, the G allele was found to cause more efficient splicing of intron 13 than the A allele (~30% higher efficiency) due to the disruption of the intronic enhancer motif by the A allele.

The extensive crosstalk between hemostasis and inflammation, multiple functions of prothrombin/thrombin that are highly relevant to SLE and SLE-associated microvascular disease and/or cardiovascular events (increased risk of thrombosis and accelerated atherosclerosis), and the high prevalence of anti-prothrombin antibodies in patients with SLE strongly support the prothrombin/thrombin gene (F2) as a plausible candidate for susceptibility to SLE and related phenotypes. Previous studies investigated functional F2 polymorphisms mainly for their effects on cardiovascular events in non-SLE individuals. Some reports examined the G20210A SNP in relation to SLE-associated cardiovascular events, although the sample sizes were underpowered to detect the effects of such an uncommon variant (1.5%-2% frequency in the general Caucasian population). To our knowledge, no study has previously examined both F2 rs3136516 (A19911G) and rs1799963 (G20210A) SNP (the 2 well known genetic determinants of plasma prothrombin levels/activity) in relation to risk for SLE, which is the focus of this study.
RESULTS
Association analyses of F2 rs3136516 and rs1799963 SNP with SLE risk in Caucasian Americans. The frequency of the rs3136516 G allele was higher in SLE patients than in controls at both Pittsburgh (47.9% vs 43.4%, respectively) and Chicago (50.0% vs 45.0%) sites. In the combined Pittsburgh + Chicago sample (Table 1), the rs3136516 G allele frequency was 48.4% in SLE cases versus 43.7% in controls (p = 0.034). The recruitment site- and age-adjusted OR for the rs3136516 G allele carriers (AA = 0, GA = 1, GG = 2) was 1.22 (95% CI 1.03–1.46, p = 0.023), indicating a modest effect. No significant association was observed for the rs1799963 SNP, which showed comparable allele frequencies between SLE cases and controls (A allele: 2.4% vs 2.0%; p = 0.593, in the combined sample). Haplotype analysis revealed 3 of the 4 expected haplotypes (GG, AG, AA); the fourth haplotype carrying the rs3136516 G and rs1799963 A alleles that are both associated with elevated prothrombin levels/activity was absent (D’ = 1, r² = 0.019). The common haplotype carrying the rs3136516 risk allele G was overrepresented in cases (GG frequency: 0.484 in cases vs 0.437 in controls) whereas the one carrying the protective allele A was overrepresented in controls (AG frequency: 0.543 in controls vs 0.493 in cases).

Next, we wanted to determine whether the association of the rs3136516 SNP with SLE risk might have been influenced by the cardiovascular status of patients with SLE. For this purpose, we stratified the Caucasian patients with SLE who had been characterized for cardiac and vascular events (CVE) into 2 subgroups based on the presence or absence of CVE and compared them separately with the controls (Table 1). Although the association trend was present in both SLE subgroups, the association of the rs3136516 SNP was stronger with the CVE-positive group (n = 100, OR 1.42) than with the CVE-negative group (n = 228, OR 1.20) as compared to the healthy controls (n = 509) and remained significant (p = 0.024 vs p = 0.114) despite more dramatically reduced sample size. The comparison between SLE patients with and those without CVE did not yield a significant result, although the numbers were relatively small (100 vs 228 patients, respectively) for meaningful analysis considering the modest effect sizes observed in Caucasians.

Association analysis of F2 rs3136516 SNP with SLE risk in African Americans. Following the observation of a significant association of the rs3136516 SNP with SLE in Caucasian Americans, we sought a similar association in African Americans. As in Caucasian Americans, the rs3136516 G allele frequency was higher in African American women with SLE than in controls at both Pittsburgh (92.9% vs 82.0%) and Chicago (89.1% vs 82.8%) sites. In the combined sample (Table 1), the rs3136516 G allele frequency was 91.2% in SLE cases versus 82.2% in controls (p = 0.003). The OR for the rs3136516 G allele carriers (AA = 0, GA = 1, GG = 2) was 1.96 (95% CI 1.08–3.58; p = 0.022) after adjustment for the effects of the recruitment site and age. Only 2 African American individuals were found to carry the rs1799963 A allele (in the heterozygous state), thus the association analysis was not feasible for this SNP in the African American sample.

Association analysis of F2 rs3136516 SNP with plasma prothrombin activity in Caucasian American SLE cases. Among Caucasian American SLE cases with available prothrombin activity measurements at the Pittsburgh site, the rs3136516 G allele was significantly associated with a modest increase in plasma prothrombin activity (p = 0.039 after adjustment for age, BMI, and warfarin use; Table 2). The effect of the rs3136516 G allele remained significant (p = 0.015) after excluding the cases carrying the rs1799963 A allele (by evaluating only the individuals with wild-type GG genotype for rs1799963).

DISCUSSION
Since first reported in 199612, the uncommon F2 variant, rs1799963 (G20210A), has been established as a risk factor for hyperprothrombinemia and venous thrombosis in Caucasian populations. The relationship between this poly-

Table 1. Allele frequencies and association statistics for F2 rs3136516 SNP in SLE women compared to control women. Only data for successfully genotyped individuals were included in the table.

<table>
<thead>
<tr>
<th>rs3136516</th>
<th>Caucasian Americans</th>
<th></th>
<th>African Americans</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alleles</td>
<td>Controls, n = 509</td>
<td>CVE Negative Cases*, n = 228</td>
<td>CVE Positive Cases*, n = 100</td>
<td>Controls, n = 143</td>
</tr>
<tr>
<td>A</td>
<td>0.563</td>
<td>0.516</td>
<td>0.515</td>
<td>0.470</td>
</tr>
<tr>
<td>G</td>
<td>0.437</td>
<td>0.484</td>
<td>0.485</td>
<td>0.530</td>
</tr>
<tr>
<td>p**</td>
<td>—</td>
<td>0.034</td>
<td>0.091</td>
<td>0.016</td>
</tr>
<tr>
<td>OR† (95% CI; p³)</td>
<td>—</td>
<td>1.22</td>
<td>1.20</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>(1.03–1.46; 0.023)</td>
<td>(0.96–1.49; 0.114)</td>
<td>(1.05–1.92; 0.024)</td>
<td>(1.08–3.58; 0.022)</td>
</tr>
</tbody>
</table>

* Caucasian SLE cases with available cardiovascular data were stratified by the occurrence of cardiac and vascular events (CVE): myocardial infarction, coronary artery bypass graft surgery, percutaneous transluminal coronary angioplasty, angina pectoris, cardiac death, stroke, transient ischemic attack, congestive heart failure, blood clots, or vascular surgery. ** Comparison of the allele frequencies between cases and controls using a standard Z-test of 2 binomial proportions. ³ Odds ratios and p values under additive genetic effect model (AA = 0, GA = 1, GG = 2), adjusted for recruitment site and age.
morphism and arterial thrombosis risk (i.e., myocardial infarction and stroke) was also evaluated, but yielded inconsistent results. More recently, a common F2 SNP (rs3136516, A19911G) was also reported to be associated with increased plasma prothrombin activity and thrombosis risk in Caucasians, although at a lesser degree. Large studies and metaanalysis suggested that the rs3136516 G allele is associated with a slight increase in prothrombin activity and thrombotic risk. Previous studies indicated that chromosomes carrying the rs1799963 A allele almost always had the rs3136516 A allele, which was also confirmed in our study.

While no significant effect of the uncommon rs1799963 variant on SLE was observed in our study, a significant association of the common rs3136516 SNP with SLE susceptibility was detected in both Caucasian and African Americans (Table 1). The rs3136516 G allele frequency was significantly higher in Caucasian American SLE cases than in controls (48.4% vs 43.7%, respectively) with a covariate-adjusted OR of 1.22 (95% CI 1.03–1.46, p = 0.023) indicating a modest effect size. The effect seemed to be stronger in the CVE-positive SLE subgroup than in the CVE-negative SLE subgroup (OR 1.42 vs 1.20), which warrants further confirmation in larger SLE samples characterized for CVE.

The role of prothrombin/thrombin in hemostasis, thrombosis, and occurrence of antiphospholipid antibodies (that are also associated with increased thrombosis risk) has long been recognized. Studies increasingly emphasize that prothrombin has actually a plethora of biological functions that...
also include an important role in inflammation and immune activation.\(^{2,3,5}\) Thrombin, the active form of prothrombin, was shown to be chemotactic for monocytes and neutrophils and can induce several inflammatory responses, including cytokine production and apoptosis.\(^{3,46}\) A number of biological pathways are being implicated in SLE pathogenesis and our study indicates that the “hemostasis and its crosstalk with immunity and inflammation” can be added to this growing list.

To our knowledge, this is the first study to evaluate the role of \(F2\) rs3136516 common SNP in relation to susceptibility for SLE. The significant and consistent association of the rs3136516 G allele with SLE risk in both Caucasians and African Americans suggests that this \(F2\) polymorphism might play a role in SLE pathogenesis. Its effect size seems to be modest, although more pronounced among SLE patients who had experienced cardiac and/or vascular events. Nevertheless, replication by independent groups is essential in establishing genetic associations with complex disorders due to various factors that may lead to false-positive associations (i.e., by chance, power issues, population stratification). Our study had more than 60% but less than 80% power to detect the odds ratios reported in our Caucasian and African American samples. Although our sample size was reasonable in Caucasians, it was relatively small in African Americans. The rs3136516 SNP was neither part of the high-density genotyping panels used by recently published genome-wide association studies of SLE\(^{47,48,49}\) nor strongly correlated with any common \(F2\) SNP included in those panels. Therefore, other groups will need to genotype this SNP in their independent large samples in order to replicate our findings, and the cardiovascular status of the participants (cases and controls) is likely to influence the results.

REFERENCES


