Genetic Risk Factors for Thrombosis in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** Thrombosis is a serious complication of systemic lupus erythematosus (SLE). We investigated whether genetic variants implicated in thrombosis pathways are associated with thrombosis among 2 ethnically diverse SLE cohorts.

Methods. Our discovery cohort consisted of 1698 patients with SLE enrolled in the University of California, San Francisco, Lupus Genetics Project and our replication cohort included 1361 patients with SLE enrolled in the PROFILE cohort. Patients fulfilled American College of Rheumatology SLE criteria, and data relevant to thrombosis were available. Thirty-three single nucleotide polymorphisms (SNP) previously shown to be associated with risk of deep venous thrombosis in the general population or implicated in thrombosis pathways were genotyped and tested for association with thrombosis in bivariate allelic analyses. SNP with $p < 0.1$ in the bivariate analyses were further tested in multivariable logistic regression models adjusted for age, sex, disease duration, antiphospholipid antibody status, smoking, nephritis, and medications.

Results. In the discovery cohort, 23% of patients with SLE experienced a thrombotic event. SNP in the following genes demonstrated association with thrombosis risk overall in the discovery or replication cohorts and were assessed using metaanalytic methods: factor V Leiden *(FVL)* rs6025 (OR 1.85, p = 0.02) and methylenetetrahydrofolate reductase (*MTHFR*) rs1801133 (OR 0.75, p = 0.04) in whites, and fibrinogen gamma (*FGG*) rs2066865 (OR 1.91, p = 0.01) in Hispanic Americans. SNP in these genes showed association with venous thrombosis risk in whites: *MTHFR* rs1801131 (OR 1.51, p = 0.01), *MTHFR* rs1801133 (OR 0.70, p = 0.04), *FVL* rs6025 (OR 2.69, p = 0.002), and *FGG* rs2066865 (OR 1.49, p = 0.02) in whites. A SNP in *FGG* rs2066865 (OR 2.19, p = 0.003) demonstrated association with arterial thrombosis risk in Hispanics.

Conclusion. Our results implicate specific genetic risk factors for thrombosis in patients with SLE and suggest that genetic risk for thrombosis differs across ethnic groups. (First Release June 15 2012; J Rheumatol 2012;39:1603–10; doi:10.3899/jrheum.111451)

Key Indexing Terms:
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Thrombosis is a serious complication of systemic lupus erythematosus (SLE). Because patients with SLE experience thrombotic events more frequently and at younger ages than the general population[1], morbidity from thrombosis is especially significant. For example, women with SLE aged 18–44 years are hospitalized with myocardial infarction (MI) or stroke almost 9 times more often than the general population[2]. In a 10-year prospective study, thrombosis was as common a cause of death as infections and high disease activity[1]. In a large Mexican SLE cohort, presence of the antiphospholipid syndrome conferred decreased survival[3]. Further, treatment with lifelong anticoagulation in this young population carries significant drawbacks, from frequent laboratory monitoring to severe bleeding complications. Better predictors of thrombosis are needed so that we can tailor treatment and prevent this potentially fatal disease complication.

Established risk factors for thrombosis in SLE include the presence of antiphospholipid antibodies (aPL)[4], smoking[5], longer disease duration, older age at SLE diagnosis[2], and disease activity[6]. These known risk factors, however, do not completely explain the thrombosis burden in SLE. For example, among the 30%–40% of patients with SLE who produce aPL, only 10% experience a thrombotic event[7]. Further, 40% of patients with SLE who have thrombosis are aPL-negative[8]. Work by Chang and colleagues suggests that thrombosis risk is highest in the first year after SLE diagnosis[9], adding a sense of urgency to predicting this SLE-related clinical manifestation.

Most known genetic risk factors for thrombosis in the general population, such as the factor V Leiden (*FVL*) and prothrombin G20210A polymorphisms, are found predominantly in whites. However, striking ethnic differences in thrombosis exist in the general population and in SLE, the genetic underpinnings of which are virtually unexplored. For example, in the general population, Asian Americans have a 3 to 5-fold lower incidence of deep venous thrombosis (DVT) and pulmonary embolism (PE) than whites[10]. African Americans have the highest rate of venous thromboembolism[11]. Mok and colleagues demonstrated in a group of Chinese, African American, and white patients with SLE that clinical factors alone could not explain the ethnic differences in incidence of thrombotic events[2]. Genetic studies of thrombosis in the general population suggest that these risk factors might also explain some of the variation in thrombosis outcomes among patients with SLE of different ethnic backgrounds[12].

We investigated single-nucleotide polymorphisms (SNP) that have been associated with thrombosis in the general white population[13] or have been implicated in thrombosis or coagulation pathways to determine whether they are associated with thrombosis in 2 multiethnic cohorts of patients with SLE. We hypothesize that these variants may increase risk of thrombosis in SLE in several ways. First, these SNP may be associated with SLE itself. Alternatively, these SNP may have the same prevalence in SLE but may be of increased importance in SLE because other risk factors for thrombosis are prevalent in SLE (e.g., aPL) and can provide the "second hit" required for a thrombotic event. Finally, these SNP may be more common in non-white ethnic groups, which are relatively overrepresented among individuals with SLE.

MATERIALS AND METHODS

Subjects. Because these SNP have not been systematically examined for a role in thrombosis in the setting of SLE, we used a discovery/replication cohort design. Patients (n = 1698) in our multiethnic SLE discovery cohort were from the University of California, San Francisco (UCSF) Lupus Genetics Project[14]. The protocol was approved by the Institutional Review Board at UCSF. Patients fulfilled American College of Rheumatology (ACR) criteria for SLE[15], completed an extensive questionnaire, gave permission for medical record review, and provided a DNA sample. Individuals were recruited for the Genetics Project from diverse sources that included tertiary care and community hospitals and clinics as well as SLE support groups in northern California and nationwide.

Baseline questionnaire data included demographic, clinical, and behavioral factors. Thrombotic events were documented on the questionnaire and were confirmed by medical record review (where other thromboses not reported on the questionnaire were also confirmed). Thromboses considered included DVT, PE, MI, cerebral vascular accident (CVA), recurrent miscarriages (at least 3 in the first trimester or 1 in the second or third trimester), and retinal vein thrombosis. Characteristics of the discovery cohort patients are shown in Table 1.

The main outcome variable was a history of at least 1 thrombotic event. Because certain genetic risk factors appear to be important in venous or arterial thrombosis, subgroup analyses were performed to evaluate these outcomes separately. Explanatory variables investigated for association with thrombosis risk included age at diagnosis and SLE disease duration. Other explanatory variables available for a majority of patients included smoking (ever vs never exposure), use of immunomodulating medications (including cyclophosphamide, azathioprine, methotrexate, and mycophenolate mofetil, ever vs never use), nephritis, and the presence of aPL [lupus anticoagulant measured by Russell's viper venom test (including confirmatory studies) and anticardiolipin (aCL) immunoglobulin G (IgG) and IgM]. The definition of aPL positivity was at least 1 laboratory test documented to be positive.

Our replication cohort consisted of 1361 patients with SLE from the PROFILE cohort[16]. PROFILE is a multiinstitutional cohort recruited from Northwestern University, Johns Hopkins University, the University of Alabama at Birmingham, the University of Texas Health Science Center at Houston, and the University of Puerto Rico. Patients meet ACR criteria, are at least 16 years of age, and have a disease duration ≤ 10 years at cohort entry. Phenotype data from these patients were obtained as part of this cohort's protocol and included age, sex, ethnicity, smoking history, nephritis, aPL status, medication use, and thrombosis outcomes of DVT, CVA, and MI (Table 1).

SNP selection and genotyping. Primary predictors included SNP associated with DVT in a large non-SLE case-control study[13]. Established and suggested genetic risk factors for thrombosis in the general population and in SLE in the literature [e.g., mannose binding lectin (MBL)][17] were also investigated. Table 2 lists all 33 SNP.

For rs6048 and rs2289252, genotyping was done by allele-specific real-time polymerase chain reaction using assays designed and validated at Celera, Alameda, CA, USA. Genotyping accuracy on this platform has been found to be > 99%[18]. For other SNP, genotyping was performed using the Luminex multiplex technology (Luminex, Austin, TX, USA) in which genotypes were determined automatically by passing the raw Luminex L-100 signal data through an unsupervised classification algorithm. About 96% to 98% of all genotypes were autocalled. A final manual review of the data was performed to assess each assay's technical performance to rescue any aberrant genotype autocalls.

In the discovery cohort, 9 patients with SLE were dropped from analysis because of failure to amplify and 11 patients were dropped because their self-reported sex did not match sex typing. We had 299 trios available among

Table 1. Patient characteristics.

Characteristics	Discovery Cohort, n = 1698, n (%)	Replication Cohort, n = 1361, n (%)
Women	1554 (92)	1248 (92)
Ethnicity		
Whites	1015 (60)	621 (46)
Hispanic	247 (15)	217 (16)
Asian/Pacific Islander	230 (13)	33 (2)
African American	206 (12)	490 (36)
Age at SLE diagnosis, yrs, mean (SD)	33 (13)	34 (13)
Duration of SLE, yrs, mean (range)	9 (8, 1–41)	13 (6, 3–43)
Ever-smoker	666 (39)	191 (14)
Nephritis	562 (33)	504 (37)
Immunomodulator therapy	957 (56)	574 (45)
Prednisone treatment	1512 (89)	952 (75)
Hydroxychloroquine	1397 (82)	991 (78)
At least 1 thrombosis		
Whites	236 (23)	72 (12)
Hispanic	61 (25)	13 (6)
Asian/Pacific Islander	40 (17)	2 (6)
African American	45 (22)	53 (11)
Total	382 (22)	140 (10)
No. thromboses		
0	1316 (78)	1221 (90)
1	272 (16)	127 (9)
2	89 (5)	12 (< 1)
3	16 (1)	1 (< 1)
4	4 (< 1)	0
5	1 (< 1)	0
Thrombosis types		
Deep venous thrombosis	119	31
Pulmonary embolism	51	NA
Cerebral vascular accident	90	91
Myocardial infarction	42	32
Retinal vein	14	NA
Miscarriage in 1st trimester (3 consecutive)	7	NA
Miscarriage late (≥ 1 in 2nd or 3rd trimester)	137	NA
Other thromboses	59	NA
Total no. thromboses	519	154
aCL* or LAC† or B2GP1** positive	589 (35)	153 (11)

* Anticardiolipin IgG or IgM; † lupus anticoagulant (Russell's viper venom test); ** Beta2 glycoprotein 1 IgG or IgM. NA: unavailable for this project; SLE: systemic lupus erythematosus.

cases in the discovery cohort that were used for quality control. Forty genotypes were set to missing after PedCheck for Mendelian errors was performed in these trios. Missingness per SNP and per subject was low (1%–2.32% and < 0.03%, respectively). No additional subjects were dropped based on these criteria among the PROFILE cohort patients. Deviations from Hardy-Weinberg equilibrium were assessed using an exact test and no deviations were observed.

Ancestry informative markers (AIM). AIM were available for a majority of the discovery cohort patients (n = 1595). These were used to identify population outliers for sensitivity analysis, using STRUCTURE (K = 5 populations) ancestry estimates. Subjects were removed if they had > 15% South Asian ancestry (the fifth population); were self-reported white, African American, or Asian but were outliers by the box test (beyond 1.5*interquartile range) of percentage white, South African, or East Asian, respectively; or were Hispanic patients with box test failure for Amerindian plus white, < 15% Amerindian, or > 15% South African. We performed subgroup analyses on the remaining patients to see if results differed after removal of outliers and adjusting for ancestry.

Statistical analysis. SNP were coded according to the presence or absence of the minor allele. SNP that were significantly associated with thrombosis in bivariate allelic analyses performed for each major ethnic subgroup ($p < 0.10$) were tested in multivariable logistic regression models to adjust for age, sex, disease duration, aPL positivity, smoking history, nephritis, and medication use. Each SNP was the primary predictor. Because the B, C, and D MBL variant alleles each have large effects on MBL concentrations, they were grouped into one O allele for analysis (A being wild-type), as is standard in the literature, and genotypes were analyzed as A/A, A/O, or O/O[19]. Other SNP did not demonstrate major linkage disequilibrium (LD), with the exception of rs13146272, which is in LD with the F11 SNP. Metaanalytic techniques using random effects (with the "meta" Stata function) were used to compare OR within ethnic groups for SNP found to be significant in logistic regression analyses in either the discovery or replication cohorts. We chose a more conservative random-effects metaanalysis approach (vs pooling) to account for differences in the cohorts. Our primary outcome was all thrombotic events but we also considered venous and arterial thrombosis separately in sensitivity analyses because data suggest that certain genes or variants contribute specif-

Table 2. Single-nucleotide polymorphisms (SNP) investigated in this study.

No.	RS No.	Gene	Type of Polymorphism	Biologic Pathway
1	rs1799963	*F2*	UTR3	Coagulation cascade/natural anticoagulation
2	rs6025	*F5 Leiden*	Silent mutation	
3	rs1800595	*F5 (R2 allele)*	Missense mutation	
4	rs4524	*F5*	Missense mutation	
5	rs6049	*F9*	Donor splice site	
6	rs6048	*F9 Malmö*	Missense mutation	
7	rs2036914	*F11*	Intron	
8	rs2289252	*F11*	Intron	
9	rs3756008	*F11*	Intergenic	
10	rs13146272*	*CYP4V2*	Missense mutation	
11	rs2227589	*SERPINC1*	Intron	
12	rs2066865	*FGG*	Intergenic	
13	rs1800450	*MBL2*	Missense mutation	Innate immunity/complement
14	rs1800451	*MBL2*	Missense mutation	
15	rs5030737	*MBL2*	Missense mutation	
16	rs1838065	*MBL2*	Intron	
17	rs7096206	*MBL2*	Intergenic	
18	rs9332245	*CYP2C9*	Intergenic	Warfarin metabolism
19	rs1799853	*CYP2C9*	Missense mutation	
20	rs9923231	*VKORC1*	Intergenic	
21	rs9934438	*VKORC1*	Intron	
22	rs1801690	*APOH*	Missense mutation	Glycoproteins
23	rs1613662	*GP6/RDH13*	Missense mutation	
24	rs1801131	*MTHFR*	Missense mutation	Homocystein metabolism
25	rs1801133	*MTHFR*	Missense mutation	
26	rs20455	*KIF6*	Missense mutation	Other
27	rs2001436	*NAT8B*	Intergenic	
28	rs1523127	*NR12*	UTR5	
29	rs2234628	*XYLB*	Intron	
30	rs1417121	*AKT3*	Intron	
31	rs2266911	*ODZ1*	Intron	
32	rs670659	*RGS7*	Intron	
33	rs2585008	*CASP8AP2*	Intron	

* This SNP is in linkage disequilibrium with the F11 SNP.

ically to venous or arterial thrombosis. We performed a sensitivity analysis in which we excluded subjects for whom miscarriage was their only thrombosis because basic science evidence suggests mechanisms for thrombosis may differ in this subgroup[20].

Multiple testing is an important issue in our analyses, because we start with 33 loci in 4 ethnicities and 3 outcomes (venous thrombosis, arterial thrombosis, any thrombosis). Because our outcomes are not independent and associations may or may not be ethnicity-specific, we believe that the primary concern for multiple testing is the 33 loci. Therefore results of the meta-analyses were adjusted for multiple testing at 33 loci using the false discovery rate (FDR) control procedures[21].

Statistical analyses were performed using the Stata SE software, version 11.0 (StataCorp, College Station, TX, USA), Haploview[22], PedCheck[23], PLINK[24], STRUCTURE (http://pritch.bsd.uchicago.edu/structure.html)[25], and R (http://www.R-project.org)[26].

RESULTS

Characteristics of the 1698 patients with SLE in the discovery cohort are shown in Table 1. Ninety-two percent of subjects were women and 60% were white (with 15% Hispanic, 13% Asian American, and 12% African American ancestry). The mean age at SLE diagnosis was 33 years and the mean disease duration was 9 years. About 23% of subjects experienced at least 1 thrombosis. Thirty-five percent of subjects were aPL-positive. Characteristics of the 1361 patients with SLE in the replication cohort (also shown in Table 1) were similar to the discovery cohort in terms of age at disease onset and proportion of women, but there were fewer whites and Asian Americans and more African Americans, consistent with the different geographic recruitment sites for this cohort. In addition, 23% of subjects in the discovery cohort experienced at least 1 thrombosis, while only 12% of subjects in the replication cohort experienced a thrombotic event. This difference in numbers of thrombotic events between the cohorts likely reflects differences in the recording of thrombotic events. Fewer types of events were recorded in the replication cohort because this cohort was not specifically designed to study the outcome of thrombosis in SLE.

Results of multivariable analyses for thrombosis outcomes (venous, arterial, or any thrombosis) for the discovery and replication cohorts are shown in metaanalyzed form in Table 3. Twenty-three SNP-ethnicity pairs passed bivariate and logistic regression screening ($p < 0.10$) and were included in the metaanalysis. The p values for heterogeneity were non-

Table 3. Metaanalysis[†] results for genetic variants associated with thrombosis in multivariable[††] analyses in either the discovery or replication cohorts.

SNP	Gene	MAF++	Ethnic Group	Discovery Cohort, OR (95% CI), p	Replication Cohort, OR (95% CI), p	OR MH (p)	FDR, p
All thrombosis							
rs6025	*FVL*	0.030	White	2.02 (1.16–3.51), 0.013	1.10 (0.29–4.18), 0.893	1.85 (0.019)	0.11
rs2234628	*XYLB*	0.147	Hispanic	0.48 (0.23–0.99), 0.047	2.22 (0.64–7.69), 0.206	0.95 (0.94)	1
rs9923231	*VKORC1*	0.119	African American	2.44 (1.19–5.01), 0.015	1.29 (0.77–2.87), 0.236	1.74 (0.080)	0.26
rs6049	*F9*	0.003	White	Not enough observations	17.14 (2.10–140.02), 0.008	—	—
rs2066865	*FGG*	0.229	Hispanic	1.69 (0.96–2.98), 0.071	2.79 (1.03–7.56), 0.043	1.91 (0.010)	0.083
rs2001436	*NAT8B*	0.358	African American	1.02 (0.59–1.77), 0.080	0.60 (0.37–0.96), 0.034	0.77 (0.31)	0.84
rs1801131	*MTHFR*	0.316	White	1.17 (0.92–1.48), 0.190	1.65 (1.03–2.65), 0.038	1.30 (0.096)	0.29
rs1801133	*MTHFR*	0.338	White	0.81 (0.64–1.02), 0.076	0.58 (0.34–0.97), 0.04	0.75 (0.042)	0.15
Venous thrombosis							
rs1801131	*MTHFR*	0.316	White	1.53 (1.10–2.13), 0.013	1.42 (0.62–3.28), 0.406	1.52 (0.008)	0.083
rs1801133	*MTHFR*	0.338	White	0.68 (0.47–0.97), 0.035	0.86 (0.35–2.14), 0.75	0.70 (0.040)	0.15
rs6025	*FVL*	0.030	White	2.71 (1.37–5.34), 0.004	2.52 (0.38–16.68), 0.337	2.69 (0.002)	0.050
rs6049	*F9*	0.001	Hispanic	12.78 (1.93–84.41), 0.008	Not enough observations	—	—
rs2066865	*FGG*	0.234	White	1.55 (1.08–2.24), 0.018	1.10 (0.40–3.02), 0.85	1.49 (0.023)	0.11
rs4524	*F5*	0.365	Hispanic	0.38 (0.16–0.86), 0.020	Not enough observations	—	—
rs6048	*F9 Malmo*	0.282	White	0.86 (0.60–1.25), 0.43	0.07 (0.01–0.57), 0.013	0.30 (0.33)	0.84
rs2289252	*F11*	0.404	White	0.85 (0.61–1.19), 0.335	3.10 (1.33–7.24), 0.009	1.53 (0.51)	1
rs2036914	*F11*	0.470	White	1.03 (0.75–1.43), 0.838	0.30 (0.12–0.73), 0.008	0.60 (0.40)	0.95
rs3756008	*F11*	0.400	White	0.87 (0.62–1.22), 0.417	3.24 (1.39–7.55), 0.006	1.58 (0.48)	1
Arterial thrombosis							
rs9934438	*VKORC1*	0.183	Asian American	0.36 (0.18–0.75), 0.006	Not enough observations	—	—
rs9923231	*VKORC1*	0.181	Asian American	0.36 (0.18–0.75), 0.006	Not enough observations	—	—
rs2266911	*ODZ1*	0.046	White	0.67 (0.47–0.96), 0.029	1.30 (0.70–2.43), 0.406	0.89 (0.71)	1
rs20455	*KIF6*	0.210	African American	1.49 (0.65–3.46), 0.345	0.56 (0.31–0.99), 0.047	0.87 (0.78)	1
rs2066865	*FGG*	0.229	Hispanic	1.93 (1.04–3.57), 0.037	3.08 (1.13–8.39), 0.028	2.19 (0.003)	0.050
rs2585008	*CASP8AP2*	0.388	African American	0.69 (0.36–1.32), 0.258	0.58 (0.34–0.99), 0.044	0.62 (0.024)	0.11

[†] Random effects. [††] Adjusted for age, sex, disease duration, aPL (antiphospholipid antibody) status, nephritis, smoking history, immunomodulating medication history. MAF++: minor allele frequency in all patients with SLE. White patients in the discovery cohort were also included in an individual patient data metaanalysis of the *FVL* polymorphism[28]. MH: Mantel-Haenszel test; FDR: false discovery rate; SLE: systemic lupus erythematosus; SNP: single-nucleotide polymorphism.

significant, supporting combined analysis using metaanalytic techniques. In a sensitivity analysis, we repeated the analyses excluding the 218 subjects who had miscarriages as their only thrombosis. Results were similar except for 1 additional association found for arterial thrombosis in Hispanics: MBL (OR 2.70, 95% CI 1.23–5.94, p = 0.013).

Metaanalysis results for the main outcome of thrombosis revealed significant association ($p < 0.05$) with the following SNP across cohorts: *FVL* rs6025 (OR 1.85, p = 0.02), methylenetetrahydrofolate reductase (*MTHFR*) rs1801133 (OR 0.75, p = 0.04) for whites, and fibrinogen gamma (*FGG*) rs2066865 (OR 1.91, p = 0.01) for Hispanic Americans. For venous thrombosis, results of metaanalysis revealed the following statistically significant associations across cohorts: *MTHFR* rs1801131 (OR 1.52, p = 0.01), *MTHFR* rs1801133 (OR 0.70, p = 0.04), *FVL* rs6025 (OR 2.69, p = 0.002), and *FGG* rs2066865 (OR 1.49, p = 0.02) in whites. For arterial thrombosis, results of metaanalysis revealed this statistically significant association across cohorts: *FGG* rs2066865 (OR 2.19, p = 0.003) in Hispanics.

To evaluate whether confounding by ethnicity influenced our results, we performed this same analysis among a large subgroup of the discovery cohort (n = 1595) for whom AIM data were available. In a bivariate analysis, we first evaluated whether ancestry was associated with our thrombosis outcomes. When associated, we also adjusted for percentage ancestry in our logistic analyses. Metaanalysis results across cohorts were similar to our main results.

DISCUSSION

We found that genetic variants of *FGG* rs2066865, *MTHFR* (rs1801133 and rs1801131), and *FVL* rs6025 are associated with risk of thrombosis among white patients with SLE, and that *FGG* also appears to be a risk factor for thrombosis in Hispanic patients with SLE. Interestingly, the effect sizes for these associations are similar to those found in a recent genome-wide association study of venous thrombosis in the general white population. Further, when miscarriages are removed from analysis, association with 1 additional SNP (MBL; OR 2.70, 95% CI 1.23–5.93, p = 0.013) was identified for arterial thrombosis among Hispanic Americans. The fact that most initial results remained significant suggests that the genetic predisposition for obstetric and nonobstetric thrombosis may be similar.

In the general population, several inherited genetic polymorphisms have been shown to independently confer risk of DVT. For example, the relative risk for DVT among individuals heterozygous for *FVL* was 6.6 compared to 80 for homozygotes[27]. We recently performed an individual patient data metaanalysis and found that the presence of the *FVL* polymorphism is associated with an almost 3-fold increased thrombosis risk compared to SLE subjects without this polymorphism, even with adjustment for other known risk factors[28]. This lower effect size compared to the *FVL* studies in the general population suggests that for some polymorphisms, other risk factors for thrombosis in SLE may be equally or more important than in the general population. We confirmed this association with *FVL* rs6025 and venous thrombosis in SLE (OR 2.58, p = 0.002) in white patients with SLE in our study.

Certain SNP appear to be more important in SLE in certain ethnic subgroups, e.g., *FGG* rs2066865 in patients of Hispanic ethnicity. Although established genetic risk factors for thrombosis are described mainly for whites to date, other ethnic groups tend to have worse outcomes (African Americans, Hispanic Americans)[29], and genetic risk factors for thrombosis may contribute to this difference beyond traditional explanations (such as socioeconomic status).

Other polymorphisms implicated as risk factors for SLE development, severity, and infections such as MBL[19] have also been implicated in thrombosis risk in SLE. One study found an increased risk of arterial thrombosis among patients with SLE who are homozygous for the MBL variant[30]. Another found that MBL variant alleles were associated only with cerebrovascular events in whites[17]. Yet another found no association between MBL variant alleles and arterial thromboses[31]. Interestingly, Seelen, *et al* found that aCL autoantibodies occurred more frequently in patients with MBL variant alleles[32]. When we removed miscarriages from consideration in a subgroup analysis, we found that MBL variant alleles were associated with arterial thrombosis among Hispanic Americans (OR 2.70, 95% CI 1.23–5.93, p = 0.013). Thus the relationship between MBL alleles and thrombosis in SLE is incompletely understood but may vary by ethnicity. Further study of these variants in non-European ethnic groups is warranted given the relatively smaller numbers of these individuals in the current study.

Polymorphisms in the *MTHFR* gene result in decreased ability to eliminate plasma homocysteine[33,34]. Resulting elevated homocysteine levels may predispose to thrombosis, although this relationship is not well established[35]. Research in SLE has been inconclusive[36,37]. The results of our study suggest that these polymorphisms may be important in thrombosis risk in SLE, at least among white patients.

For stabilization of thrombi to occur, thrombin-induced conversion of fibrinogen to fibrin must take place. Further, elevated plasma fibrinogen levels are associated with both increased platelet aggregation and plasma viscosity. A SNP (rs2066865) in the *FGG* gene has been associated with DVT risk in an Austrian study[38]. We found that this SNP may be important in thrombosis risk in Hispanics.

The *F9 Malmö* rs6048 polymorphism has been associated with venous thrombosis in non-SLE whites[39]. We found that *F9* rs6049 was an important risk factor for thrombosis in Hispanic Americans in our discovery cohort but we did not have enough observations to confirm these results in our replication cohort. Replication of these findings in larger, independent Hispanic patient populations is therefore warranted.

Although we had limited power to investigate less common polymorphisms in non-white ethnic groups, our study is the largest and most ethnically diverse investigation of genetic risk factors for thrombosis to date in SLE and thus provides valuable data for future investigations of this important outcome. This is also the only such study to date to incorporate so many pertinent covariates into the analysis of genetic risk factors. Because these cohorts were not specifically designed to study the outcome of thrombosis, the replication cohort may have underestimated thrombotic events (therefore misclassifying patients with SLE who have actually had a thrombotic event) because only MI, DVT, and CVA were identified in this cohort. However, this would presumably decrease our ability to identify significant genetic associations and thus represents a conservative bias. Finally, we were unable to adjust for important risk factors for thrombosis such as lipid levels, obesity, body mass index, hypertension, and diabetes. Given the average age of our patients (33 years), some of these risk factors (e.g., diabetes) may not have been prevalent, but others (e.g., hypertension from renal disease, early hyperlipidemia) may be important.

Because we did not have AIM data on all patients, population stratification may have influenced our results; however, our subgroup analyses in which AIM data were incorporated suggest that population stratification was not a major confounding factor. Although we limited this study to polymorphisms that have been previously associated with thrombosis or coagulation pathways, the number of comparisons performed raises the risk of false-positive association results. For this reason, we used a discovery/replication cohort study design and used the false discovery rate (FDR) in a sensitivity analysis. Although only 2 results remained significant after multiple testing correction, the consistency of OR and FDR-adjusted p values < 0.1 in Table 3 suggests that there are additional true associations between these loci and thrombosis outcomes that may be confirmed in other populations.

In the future, genetic information may help predict which patients with SLE are at greatest risk for thrombotic events. Neville, *et al* have demonstrated that among aPL-positive subjects who have not experienced thrombosis, there is a significantly increased risk of a thrombotic event[40]. Therefore, identifying risk factors for this subset of patients may be useful in risk-stratifying patients for treatment with anticoagulation in the future, especially if new anticoagulants can be developed

that have safer risk profiles and are easier to monitor than warfarin. Examining genetic risk factors for thrombosis in SLE may not only help to understand pathogenesis but also inform prediction of this SLE complication.

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