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ORIGINAL ARTICLE

Evaluation of the TREX1 gene in a large multi-ancestral lupus cohort

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Systemic lupus erythematosus (SLE) is a prototypic autoimmune disorder with a complex pathogenesis in which genetic, hormonal and environmental factors have a role. Rare mutations in the TREX1 gene, the major mammalian 3′–5′ exonuclease, have been reported in sporadic SLE cases. Some of these mutations have also been identified in a rare pediatric neurological condition featuring an inflammatory encephalopathy known as Aicardi–Goutières syndrome (AGS). We sought to investigate the frequency of these mutations in a large multi-ancestral cohort of SLE cases and controls. A total of 40 single-nucleotide polymorphisms (SNPs), including both common and rare variants, across the TREX1 gene, were evaluated in ~8370 patients with SLE and ~7490 control subjects. Stringent quality control procedures were applied, and principal components and admixture proportions were calculated to identify outliers for removal from analysis. Population-based case–control association analyses were performed. P-values, false-discovery rate q values, and odds ratios (OR) with 95% confidence intervals (CI) were calculated. The estimated frequency of TREX1 mutations in our lupus cohort was 0.5%. Five heterozygous mutations were detected at the Y305C polymorphism in European lupus cases but none were observed in European controls. Five African cases incurred heterozygous mutations at the E266G polymorphism and, again, none were observed in the African controls. A rare homozygous R114H mutation was identified in one Asian SLE patient, whereas all genotypes at this mutation in previous reports for SLE were heterozygous. Analysis of common TREX1 SNPs (minor allele frequency (MAF)> 10%) revealed a relatively common risk haplotype in European SLE patients with neurological manifestations, especially seizures, with a frequency of 58% in lupus cases compared with 45% in normal controls (P= 0.0008, OR= 1.73, 95% CI= 1.25–2.39). Finally, the presence or absence of specific autoantibodies in certain populations produced significant genetic associations. For example, a strong association with anti-nRNP was observed in the European cohort at a coding synonymous variant rs56203834 (P= 2.99E−13, OR= 5.2, 95% CI= 3.18–8.56). Our data confirm and expand previous reports and provide additional support for the involvement of TREX1 in lupus pathogenesis.

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Introduction

Increased expression of interferon (IFN)-regulated genes and disturbance of IFN alpha (IFN-α) homeostasis has a major role in the pathogenesis of the prototypic autoimmune disorder, systemic lupus erythematosus (SLE).[1–3] A perturbation of IFN-α metabolism is also a major pathogenic feature of the inflammatory encephalopathy, Aicardi-Goutières syndrome (AGS).[4] After the discovery of AGS-causing mutations in the *TREX1* gene, distinct heterozygous mutations were described in autosomal dominant diseases such as retinal vasculopathy with cerebral leukodystrophy,[5] and familial chilblain lupus.[6] Subsequent studies demonstrate that up to 2% of patients with SLE harbor pathogenic mutations in TREX1.[7] These rare but highly penetrant causative mutations are not detected in genome-wide studies and, thereby, may explain part of the missing heritability of lupus as well as provide insights into disease pathogenesis.

The shared TREX1 genetics in clinically distinct human disorders points to a common molecular etiology. Indeed, some AGS individuals also fulfill diagnostic criteria for SLE and have antinuclear antibodies including those with antigenic specificity for single-stranded DNA and double-stranded DNA (dsDNA).[8] Similarly, some retinal vasculopathy with cerebral leukodystrophy patients have autoantibodies to nuclear antigens (unpublished, JPA, PHK).

TREX1 (DNase III) is the major 3′–5′ DNA exonuclease of mammalian cells.[9] It has been proposed to have a major role in cell death processes and genomic DNA degradation in which it might minimize immune activation by self-DNA. It is also a key component of the SET complex, a multitasking protein involved in apoptosis, transcription, nucleosome assembly and histone binding. This complex is normally associated with the endoplasmic reticulum. It is mobilized during the cellular response to oxidative damage and is postulated to participate in the oxidative stress response. A connection between TREX1 and immune activation was initially suggested in the TREX1-null mice that develop an inflammatory myocarditis similar to autoimmune cardiomyopathy, and produce type 1 IFN.[10,11] Furthermore, TREX1-deficient cells accumulate single-stranded DNA species that may trigger autoimmunity.[12]

The *TREX1* gene is located on chromosome 3p21.31 and consists of a single exon encoding a 314-amino acid polypeptide. It has three conserved sequence motifs, Exo I, Exo II and Exo III, which form the catalytic site (Figure 1). TREX1 has a hydrophobic carboxyl-terminal region, predicted to form a transmembrane helix, likely important in defining its intracellular localization.[5] In addition, the TREX1 protein contains a proline-rich sequence that is postulated to participate in protein–protein interactions[13] (Figure 1).

Mutations throughout the *TREX1* gene have been identified in patients with several different human diseases.[13] These mutations include null alleles, frameshift mutations and non-synonymous changes in the catalytic domains and the C-terminal region. In AGS, most TREX1 mutations are autosomal recessive and diminish the exonuclease activity of the enzyme, in particular a transition of arginine to histidine at position 114 (R114H). A homozygous change at this locus (R114H) has been found to have a major effect on exonuclease enzyme activity.[14,15] The heterozygous R114H mutation has been reported in one individual with SLE.[7] Other mutations include D200N and D18N, which have been reported in AGS and familial chilblain lupus, respectively, and are inherited in an autosomal-dominant manner (Figure 1). In lupus, most of the mutations reported thus far are heterozygotes and are usually located outside the catalytic domain in the C-terminal region.[7] The functional significance of these mutations is unknown. C-terminal frameshift mutations that retain exonuclease activity are observed in SLE and account for all of the mutations in retinal vasculopathy with cerebral leukodystrophy.[7,13]

In this study, we evaluated these aforementioned mutations as well as common tagged single-nucleotide polymorphisms (SNPs) in the *TREX1* gene in a large, multi-ancestral cohort of SLE cases and controls. This study is the first to investigate TREX1 in populations with a higher prevalence of lupus, including African Americans and Hispanics. Our results confirm and extend the findings in Caucasians and provide additional support for association of SLE with TREX1 in multiple ancestries.

Results

To determine whether TREX1 is associated with SLE, we genotyped 40 SNPs in the TREX1 genomic region, including both previously reported rare SNPs and more common tag SNPs that capture most of the variation in this region (Table 1). After removing the outliers and correcting for population stratification, 15864 samples (8372 cases and 7492 controls) were included in the analysis (Table 2). All SNPs were in Hardy–Weinberg equilibrium and passed stringent quality control thresholds (see Materials and methods).

Observance of rare polymorphisms (MAF < 0.01)

Table 3 shows the rare non-synonymous-coding SNPs (minor allele frequency (MAF) <0.01) in the *TREX1* gene, including some detected only in cases. We observed at least three different types of mutations in patients that were not detected in the corresponding controls (Table 3). In Europeans, five heterozygous mutations were detected at Y305C in lupus cases but none were observed in the European controls. In African-American and Gullah patients (together), five E266G heterozygous mutations were observed, but again, none were observed in the corresponding controls. In Asians, one homozygous and two heterozygous mutations were observed in lupus cases for the R114H polymorphism, but none in controls. Table 4 describes the American College of Rheumatology (ACR) classification criteria fulfilled by these patients and their serological profiles. Interestingly, two of these 13 SLE cases were men, of which 1 carried the homozygous R114H mutation and developed SLE at 14 years of age (Table 4).

Analyses of polymorphisms with MAF > 0.01 and haplotype structure

In addition to these rare SNPs mentioned above, we also evaluated common and tagged SNPs that capture most of the variation in this region, as well as SNPs with

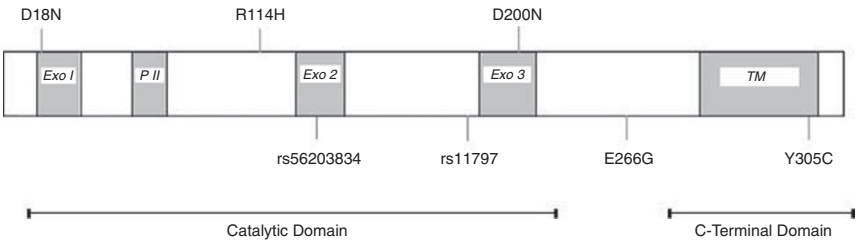

Figure 1 Schematic representation of some important disease-associated TREX1 mutations. Coding synonymous SNP (green); coding non-synonymous SNP (red). Exo I, II and III denote exonuclease domains, PII denotes polyproline II domain and TM denotes transmembrane domain. A full colour version of this figure is available at the *Genes and Immunity* journal online.

Table 1 Selected SNPs in the *TREX-1* gene

SNP	*Position*	*Minor allele*	*Major allele*	*Average MAF in Europeans*	*Average MAF in Africans*	*Average MAF in Asians*	*Average MAF in Hispanics*
rs922075	48 464 402	A	G	0.474	0.2975[a]	0.3558	0.3395
rs6776700	48 471 762	A	G	0.4616	0.2729	0.3566	0.2828
rs6442123	48 475 290	A	G	0.469	0.4512	0.3589	0.3109
rs34426134	48 480 236	A	G	0.0005568	0.04316	0	0.003878
rs62264269	48 480 342	A	G	0.00008173	0.0003569	0	0
rs7626978	48 480 835	C	A	0.003968	0.1098	0	0.01517
rs3135935	48 480 928	A	G	0	0.01331	0	0.0008929
rs2242150	48 480 968	A	C	0.4676	0.4552	0.3558	0.311
rs3135936	48 481 222	0	C	0	0	0	0
rs3135938	48 481 308	A	G	0.0002393	0.0003521	0	0.0005984
rs36041404	48 481 452	A	G	0.000555	0.04846	0	0.007139
rs12486046	48 482 186	G	A	0.03295	0.004374	0.0001983	0.02826
rs3135940	48 482 579	A	G	0.001928	0.0003521	0.0004002	0.0009063
rs3135941	48 482 671	G	A	0.1787	0.03012	0.04005	0.1778
rs3135942	48 482 826	0	G	0	0	0	0
D18N	48 483 110	0	G	0	0	0	0
rs55938060	48 483 247	A	G	0.001311	0.0001779	0.0002006	0.0003081
rs3135943	48 483 256	0	0	NA	NA	NA	NA
R114H	48 483 399	A	G	0.001053	0.0003602	0.0008094	0.0003021
V122A	48 483 423	0	A	0	0	0	0
rs56203834	48 483 454	A	G	0.01349	0.001092	0.001856	0.01587
rs3135944	48 483 520	G	A	0.0001586	0.02172	0	0.00119
A158V	48 483 531	0	G	0	0	0	0
rs55786737	48 483 544	A	G	0.008773	0.003002	0.003843	0.007869
R164X	48 483 548	0	G	0	0	0	0
rs11797	48 483 589	A	G	0.4499	0.2701	0.3499	0.279
D200N	48 483 656	0	G	0	0	0	0
V201D	48 483 660	0	T	0	0	0	0
G227S	48 483 737	0	0	NA	NA	NA	NA
R240S	48 483 778	G	C	0.0000794	0.002451	0	0.0008929
A247P	48 483 797	C	G	0.00007926	0.0014	0	0
E266G	48 483 855	G	A	0.002778	0.0005248	0	0.0002976
P290L	48 483 927	0	0	NA	NA	NA	NA
T303P	48 483 965	0	A	0	0	0	0
rs3135945	48 483 970	A	G	0.000794	0.1282	0	0.01013
Y305C	48 483 972	G	A	0.0001587	0	0	0
G306A	48 483 975	0	C	0	0	0	0
rs3135946	48 484 040	G	A	0.003578	0.1126	0	0.01492
rs56112131	48 484 061	A	G	0	0.0001766	0	0
rs3135947	48 484 132	0	A	0	0	0	0

Abbreviations: MAF, minor allele frequency; NA, not available; SNP, single-nucleotide polymorphism.
[a]For this SNP, minor allele for the African was allele G.

MAF>1%. *TREX1* is a small gene with less than 1000 base pairs and one coding exon (Figure 1). As it is closely linked to the ATR-interacting protein (*ATRIP*) gene, we selected common SNPs to cover both (shown in Table 1). Figures 2a and b demonstrate the haplotype structure of this genomic region in European and African cases and controls using SNPs with MAF>1%. There is a high correlation among all of these common SNPs, especially in the European population (r-squared >0.9). Also, more SNPs are polymorphic (MAF>0.01) in the African-American population than in other racial groups (Table 1).

Case–control association analysis

In a case–control association study, we did not observe significant associations with any of the selected SNPs

Table 2 Demographic distribution of individuals in study

	European-American case/control	*Asian case/control*	*African-American case/control*	*Gullah case/control*[a]	*Hispanic (others) case/control*
Total	3936/3491	1265/1260	1527/1811	152/123	1492/807
Male	344/1151	99/154	121/574	15/18	127/80
Female	3592/2340	1166/1106	1406/1237	137/105	1365/727

[a]The Gullah are African Americans who live in the Low Country of South Carolina and genetically show a much lower admixture rate with non-African populations than other African Americans.

Table 3 Frequency of non-synonymous-coding mutations in lupus cases and controls

SNP[a]	*Position*	*Minor allele*	*Major allele*	*EA case (AA/AB/BB)*	*EA control (AA/AB/BB)*	*AA case*	*AA control*	*Gullah case*	*Gullah control*	*Asian case*	*Asian control*
D18N	48 483 110	0	G	0/0/3921	0/0/3479	0/0/1524	0/0/1801	0/0/152	0/0/123	0/0/1262	0/0/1257
R114H	48 483 399	A	G	0/9/3856	0/5/3389	0/1/1511	0/1/1734	0/0/146	0/0/120	**1/2/1232**	**0/0/1236**
V122A	48 483 423	0	A	0/0/3906	0/0/3456	0/0/1509	0/0/1800	0/0/152	0/0/123	0/0/1262	0/0/1259
A158V	48 483 531	0	G	0/0/3932	0/0/3480	0/0/1526	0/0/1800	0/0/152	0/0/123	0/0/1263	0/0/1257
R164X	48 483 548	0	G	0/0/3928	0/0/3487	0/0/1527	0/0/1807	0/0/151	0/0/123	0/0/1262	0/0/1257
D200N	48 483 656	0	G	0/0/3920	0/0/3489	0/0/1527	0/0/1807	0/0/151	0/0/123	0/0/1263	0/0/1258
V201D	48 483 660	0	T	0/0/3935	0/0/3490	0/0/1525	0/0/1809	0/0/152	0/0/123	0/0/1264	0/0/1260
R240S	48 483 778	G	C	0/1/3919	0/0/3491	0/7/1519	0/11/1798	0/3/149	0/1/122	0/0/1265	0/0/1260
A247P	48 483 797	C	G	0/0/3936	0/1/3489	0/3/1524	0/7/1803	0/0/152	0/2/121	0/0/1264	0/0/1260
E266G	48 483 855	G	A	0/22/3909	0/19/3466	**0/4/1523**	**0/0/1811**	**0/1/151**	**0/0/123**	0/0/1265	0/0/1260
T303P	48 483 965	0	A	0/0/3918	0/0/3458	0/0/1511	0/0/1789	0/0/151	0/0/123	0/0/1263	0/0/1260
Y305C	48 483 972	G	A	**0/5/3924**	**0/0/3489**	0/0/1527	0/0/1811	0/0/152	0/0/123	0/0/1265	0/0/1260
G306A	48 483 975	0	C	0/0/3936	0/0/3491	0/0/1527	0/0/1811	0/0/152	0/0/123	0/0/1265	0/0/1260

Abbreviations: AA, African American; EA, European American; SNP, single-nucleotide polymorphism.
Bold fonts indicate the observed mutations only in cases.
[a]Non-synonymous SNPs with MAF <1%.

using the presence of SLE as a phenotype in any of the racial groups. Because of similarities between lupus and neurological conditions such as AGS, we hypothesized that lupus patients with neurological manifestations might be enriched for risk alleles in the *TREX1* gene. Indeed, in the European population the presence of neurological manifestations (ACR criteria), especially the presence of seizures (79 European cases), produced significant results at multiple common SNPs when compared with healthy controls (Table 5). The haplotype-risk alleles (AAAAAA) (Figure 2a, Table 5) were relatively common in the European population with a frequency of 58% in lupus cases compared with 45% in normal controls ($P = 0.0008$, false discovery rate (FDR) $q = 0.007$, odds ratio (OR) = 1.73, 95% confidence interval (CI) = 1.25–2.39). In addition, in a case-only study in which these 79 SLE cases with seizure manifestations were compared with 2405 lupus patients, with no previous neurological findings, similar haplotype association results were obtained ($P = 0.0008$, FDR $q = 0.003$, OR = 1.75 95% CI = 1.25–2.37). As neurological manifestations in SLE also correlate with previous cerebrovascular accidents and the presence of antiphospholipid syndrome, we evaluated these subgroups separately, but the results were not significant (data not shown). There was also no evidence of associations with other ACR criteria, gender or age of onset in any population. Of note, all five European patients with a mutation at Y305C also had at least one copy of this risk haplotype (two homozygous and three heterozygous patients for the risk allele).

We also evaluated SLE samples for an association with autoantibodies (anti-Ro, anti-La, anti-RNP, anti-Sm and anti-dsDNA). In Asian population, 567 SLE patients lacking anti-Ro antibodies were less likely to carry the same common haplotype mentioned above (AAAAAA) compared with 1260 healthy controls (32% in cases versus 36% in controls ($P = 0.01$, FDR q = 0.03, OR = 0.82 95% CI 0.71–0.96)). Interestingly, the same haplotype was observed less frequently among 260 Asian patients with positive anti-nRNP compared with 1260 healthy controls (30% in cases with positive anti-nRNP versus 36% in controls ($P = 0.003$, FDR $q = 0.008$, OR = 0.75, 95% CI = 0.61–0.92)). In a case-only study, these comparisons were not statistically significant, although the frequency of this haplotype was more frequent in Asian cases with positive anti-Ro (335 cases) in comparison with those lupus cases with negative anti-Ro (567 cases) (36 versus 32% ($P = 0.22$)) and for anti-nRNP autoantibody, less frequent in cases with positive anti-nRNP compared with cases with negative anti-RNP (30% for positives (260 cases) versus 35% for negatives (789 cases) ($P = 0.05$, FDR $q = 0.12$)).

In addition, the presence of anti-nRNP in the European population was strongly associated with another coding synonymous SNP rs56203834 that was extremely rare in the Asian population. In this subgroup, 269 European cases, positive for anti-nRNP, were compared with European controls. The MAF for this SNP was only 1% in controls (Table 1) but 5% in European cases with positive anti-nRNP ($P = 2.99$E-13, FDR $q = 5.97$E-12, OR = 5.2, 95% CI = 3.18–8.56). Furthermore, in case-only

Table 4 Phenotypic characterization of 13 SLE patients with non-synonymous mutations

	SLE cases												
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Detected mutations	R114H (HZ)	R114H (HTZ)	R114H (HTZ)	E266G (HTZ)	E266G (HTZ)	E266G (HTZ)	E266G (HTZ)	E266G (HTZ)	Y305C (HTZ)	Y305C (HTZ)	Y305C (HTZ)	Y305C (HTZ)	Y305C (HTZ)
Gender	M	F	F	F	F	F	F	M	F	F	F	F	F
Race	Asian	Asian	Asian	African American	African American	African American	African American	African Gullah	European	European	European	European	European
Age of SLE onset	14	18	34	16	36	22	50	43	37	27	NA	31	37
Malar rash	+	–	–	–	–	–	–	–	–	+	–	+	+
Discoid rash	+	–	–	–	–	–	+	–	–	+	–	–	–
Photosensitivity	+	–	–	+	–	–	–	+	+	+	+	+	–
Oral ulcers	–	+	–	+	–	–	–	–	–	+	+	–	–
Arthritis	–	–	+	–	+	–	+	+	+	+	+	+	–
Serositis	–	+	–	–	+	–	+	+	–	+	+	+	+
Nephritis	–	+	+	+	–	+	+	–	–	–	–	–	+
Neurological	–	–	–	–	+	–	–	–	–	+	–	+	–
Hematological	–	–	–	+	–	+	–	–	–	+	+	+	+
Immunological	+	+	+	+	+	+	+	+	+	+	+	+	+
Anti-dsDNA	+	–	+	+	–	+	–	+	+	–	NA	NA	NA
Anti-Sm	–	–	–	–	+	+	–	–	–	–	NA	NA	NA

Abbreviations: F, female; HTZ, heterozygous; HZ, homozygous; M, male; NA, not available; SLE, systemic lupus erythematosus.

study, if these 269 cases were compared with 1413 SLE patients with negative anti-nRNP, again similar results were obtained ($P = 2.74E{-}06$, FDR $q = 2.73E{-}05$, OR = 3.33 95% CI 1.95–5.65). In the Hispanic population, suggestive results were also obtained but the number of samples for subset analysis was small.

Discussion

To explore the frequency of mutations in the *TREX1* gene and their relationship with SLE, we evaluated 40 SNPs, including rare variants, previously reported to be associated with several human diseases in large multi-ethnic sample populations. The large sample size of cases and controls (>15800) provide enough power to evaluate rare variations in TREX1. One important mutation described in AGS (R114H) is on the dimer interface of the protein.[14,15] Homozygous mutations at this position (that is, $TREX1^{R114H/R114H}$) degrade dsDNA 300-fold less efficiently than TREX1 wild type.[16] Although AGS cases are associated with homozygous R114H mutations, their parents, who are heterozygous carriers of the mutation, have no abnormal phenotypes reported.[17] A heterozygous mutation at this location has been reported in one European SLE case.[7] In our European population, we identified nine SLE cases with heterozygous mutations at this locus and five heterozygotes in the European healthy controls (Table 3). In the Asian population, on the other hand, we identified one homozygous SLE case. In addition, two heterozygous SLE cases were detected in the same population, but none in Asian controls. The homozygous case was a male lupus patient with early-onset lupus, positive anti-dsDNA antibody and predominant skin manifestations, but no neurological manifestations. The latter is intriguing, as all previously reported homozygous R114H mutations have been in AGS patients, who invariably have had central nervous system disease in early childhood (Table 4).

We identified five European lupus cases with heterozygous mutations at another variant (Y305C), but none in controls. This is a missense (coding) mutation located outside of the catalytic domain of TREX1. This polymorphism was previously reported in one European lupus patient.[7] None of these five patients were carriers for R114H mutant alleles, although all of them were carriers for the common risk haplotype (AAAAAA) (two homozygotes and three heterozygotes). This suggests a correlation of this common risk haplotype with coding mutation at Y305C that could be functionally important.

In the African population, we also identified five lupus patients with the E266G mutation, which was absent in African controls (Figure 1). This mutation was detected in Europeans, but as previously reported in European cohorts, there was no significant difference in cases and controls (Table 3).[7] This association has not been previously reported in the African population. Overall, the coding mutation frequency in our SLE cases was ~0.5%.

Aicardi-Goutières syndrome, an autosomal disease (usually recessive but rare dominant case) characterized by progressive encephalopathy of early onset, basal ganglia calcifications and chronic cerebrospinal fluid lymphocytosis, is also associated with increased levels of IFN-α in the cerebrospinal fluid. SLE patients likewise

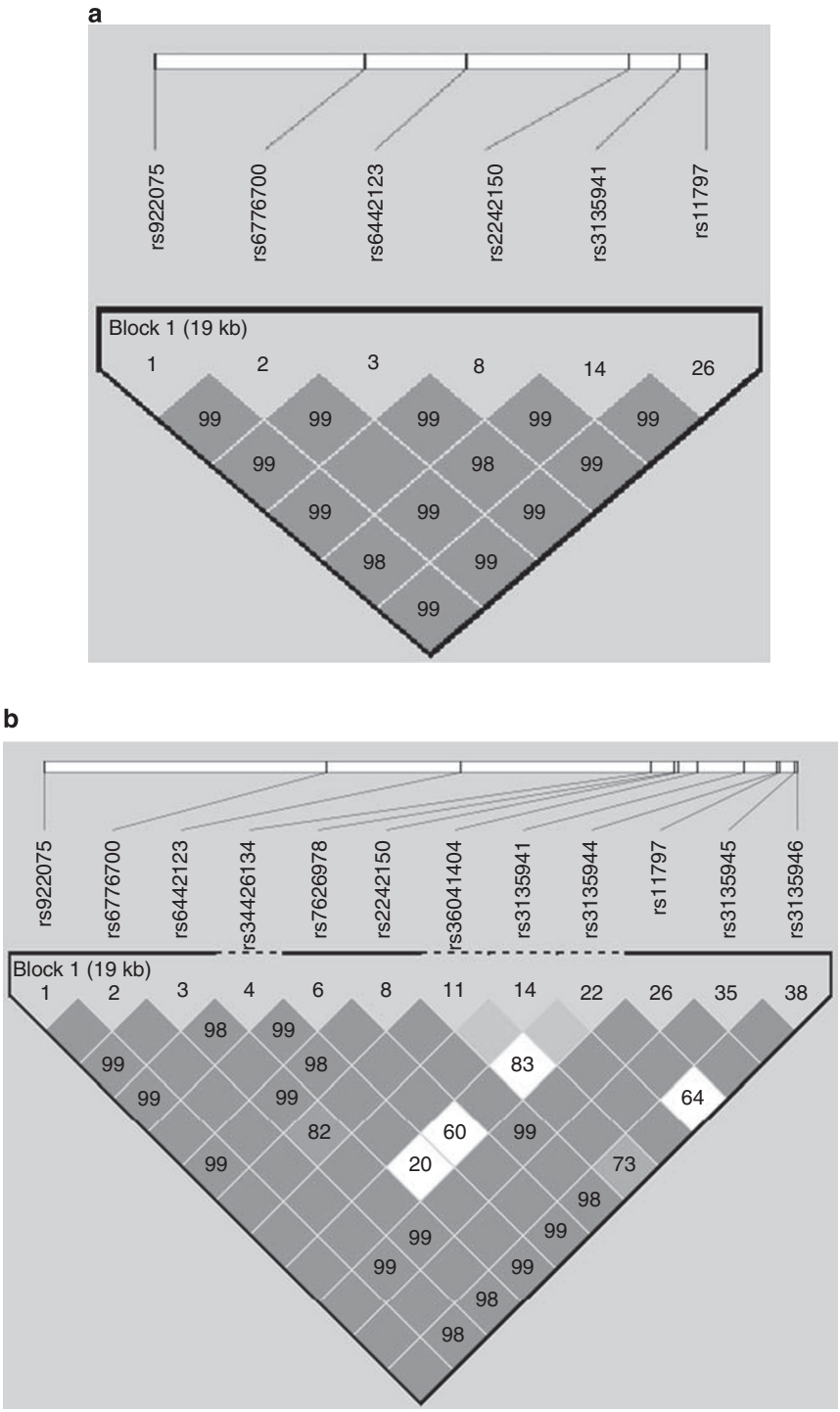

Figure 2 European-American (**a**) and African-American (**b**) haplotype block structure. Blocks connecting SNP pairs are shaded according to the strength of the linkage disequilibrium among the SNPs, from 0.0 (white) to 1.0 (bright red), as measured by the disequilibrium coefficient D′. A full colour version of this figure is available at the *Genes and Immunity* journal online.

may have high level of IFN-α and neurological manifestations similar to AGS, including seizures. Indeed, neuroimaging in patients with SLE may show calcifications, white matter changes and atrophy, as typically observed in patients with AGS.[18–20] Given these overlapping phenotypes, one may speculate that a common pathogenic mechanism underlies the neurological phenotype of AGS and cerebral lupus.

In a recent study, up to 60% of patients with AGS presented with clinical findings such as skin rash, arthritis, oral ulcer as well as laboratory findings commonly seen in patients with lupus, including ANAs, reduced complement levels, thrombocytopenia and leukocytopenia. Furthermore, if seizures are taken into account, up to 75% of those patients with AGS showed manifestations of lupus.[21] Because of these similarities,

Table 5 Case–control association results in European SLE cases, with presence of seizure (79 cases), compared with controls

SNP	*BP*	*Minor allele*	*Cases*	*Controls*	*Major allele*	χ^2	P-*value*	*FDR* q	*OR*	*L95*	*U95*
rs922075	48464402	A	0.5949	0.4718	G	9.387	0.002185	0.007	1.644	1.192	2.267
rs6776700	48471762	A	0.5921	0.4622	G	10.09	0.001491	0.007	1.689	1.218	2.343
rs6442123	48475290	A	0.6053	0.4674	G	11.34	0.000757	0.004	1.747	1.258	2.427
rs2242150	48480968	A	0.5886	0.4662	C	9.301	0.002291	0.007	1.638	1.189	2.257
rs3135941	48482671	G	0.1429	0.1856	A	1.83	0.1761	0.23	0.7312	0.4637	1.153
rs11797	48483589	A	0.5823	0.4485	G	11.16	0.000835	0.005	1.714	1.245	2.36
rs3135945	48483970	A	0.01266	0.000287	G	42.15	8.46E-11	1.69E−09	44.65	6.25	319

Abbreviations: BP, base pair; OR, odds ratio; SLE, systemic lupus erythematosus; SNP, single-nucleotide polymorphism.
The bold SNPs represent the common risk haplotype (AAAAAA).

Vries *et al*.[22] sequenced genomic DNA of 60 European lupus patients with neurological manifestations for exonic TREX1 mutations and identified a novel R128H mutation in one of these SLE patients. Brain magnetic resonance imaging of this patient showed generalized atrophy, extensive symmetric cerebral white matter hyperintensities and cerebellar infarcts, without evidence for ischemia. This rare mutation is located within the highly conserved second exonuclease domain (ExoII) of the *TREX1* gene.

In our study, we also tested this hypothesis and identified a relatively common risk haplotype in the *TREX1* gene among European lupus patients with seizure (58% in SLE cases with seizure compared with 45% in normal controls). The frequency of this haplotype in normal European controls was consistent with the HapMap data for the CEU (CEPH Utah residents with ancestry from northern and western Europe) study population (45%). This haplotype spans 19 kb, and covers both *TREX1* and *ATRIP* genes. These two genes are closely linked and some mRNAs encode ATRIP and TREX1 in different reading frames. In fact, these two genes previously were considered a single gene (*NCBI-35*). ATRIP is an essential component of the DNA-damage checkpoint, and binds to single-stranded DNA and interacts with proteins such as ataxia telangiectasia, Rad3-related protein and breast-ovarian cancer susceptibility 1.[23] It has central role in checkpoint activation in response to DNA damage and is important for chromosomal stability. Because of the high-linkage disequilibrium among SNPs in this risk haplotype in Europeans (Figure 2a), conditional analyses were not conclusive; however, analyses on this haplotype suggest that rs11797, a common synonymous SNP located in the exonic region of TREX1, can better explain the whole association in this haplotype and, therefore, the effect likely originates from the *TREX1* gene.

There was also evidence for association of certain SNPs with the presence of autoantibodies in SLE. In particular, there was a positive association with the presence of anti-nRNP in Europeans for SNP rs56203834 ($P = 2.99E{-}13$, FDR $q = 5.97E{-}012$, OR = 5.2, 95% CI = 3.18–8.56). This is another synonymous SNP located in the TREX1 exon and is less than 60 base pairs away from R114H (Figure 1). Because of low MAF in this SNP, no clear correlation between this SNP and the common risk haplotype for neurological manifestation in European can be detected, and in fact, all available European cases with seizure manifestations were homozygous for the major wild-type allele for this SNP, suggesting that these two effects might be independent. This SNP was extremely rare in the Asian and African populations, whereas in the Hispanic population the result was suggestive.

With regard to the Asian population, the common haplotype that was associated with neurological manifestations in Europeans was also more frequently seen in Asian patients with this phenotype (42% in cases compared with 38% in controls, $P = \text{NS}$); although it was not significant. As described in the Results, in Asian population, this haplotype was less frequently observed with the presence of anti-nRNP or in patients with absence of anti-Ro antibodies. In fact, this negative correlation with anti-Ro in TREX1 has been previously reported in the same population.[24] The reason for this opposite association between anti-Ro and anti-nRNP in Asian with the same haplotype is not clear, and requires additional confirmation. As SLE is an extremely heterogenous disease, this could be partly related to different disease manifestations. In complex diseases such as SLE, many subtle inherited elements could directly or indirectly affect these autoantibodies with subsequent clinical sequel. Some autoantibodies associated with lupus tend to cluster together and usually result in specific clinical manifestations. For example, the association of anti-Ro with secondary Sjogren's syndrome or leukopenia, anti-RNP with Raynaud's phenomenon and anti-dsDNA with nephritis have been noted and replicated in many studies. In addition, anti-Ro antibodies have been reported as one of the independent predictors of neurological damage in lupus.[25] This correlation could explain our results with regard to the risk haplotype and anti-Ro antibodies.

Overall, our results with TREX1 indicate a complex relationship between genetic loci, SLE sub-phenotypes and different population ancestry that demands further studies of this gene. Our data, combined with the findings in Trex1-deficient mice, which develop lethal autoimmunity, accompanied with a high production of type I IFN, suggest that TREX1 is involved in lupus pathogenesis and probably essential for the prevention of autoimmunity.

Materials and methods

Recruitment and biological sample collection

The participants were enrolled in the Lupus Family Registry and the Repository and Lupus Genetics Studies at the Oklahoma Medical Research Foundation as

described,[26] and by collaborators.[27–30] A total of 15864 study participants were used in the current study (Table 2). Protocols were approved by the institutional review boards at each respective institution. Patients met 4 of the 11 revised 1997 ACR criteria for the classification of SLE.[31] Ethnicity was self-reported and verified by principal component and admixture proportion calculations.

Genotyping
This genotyping project was part of the Lupus Large Association Study that involved different investigators and more than 32 000 SNPs. Data were generated using the Illumina iSelect technology at the Oklahoma Medical Research Foundation. Genotype calls were made using all samples to maximize the accuracy of the cluster plots. Following genotype scoring, SNP clusters were evaluated electronically using the Illumina BEADSTUDIO(r) software package (http://www.illumina.com). Ambiguous SNP clusters were evaluated manually and SNPs with poor cluster characteristics were flagged.

Genotypic data were only used from samples with a call rate > 90% (average sample call rate = 99.1%) and from SNPs with a call frequency > 90% (average SNP call rate = 99.0%). Initial quality control analyses were performed by plate, by lot of reagents and by date genotyped to be certain that systematic error did not find its way into our data. A sample report was generated for every sample attempted in the project, including sample barcode, ethnicity, gender, pedigree information, no calls, calls, call rate, genotype frequency and Gencall score. Any sample with previous genotype data was analyzed for concordance. A summary SNP report was also generated containing chromosome and location, call rate, genotype frequency and Gencall score.

Statistical analyses
Testing for association was completed using PLINK.[32] Haploview version 4.0 (see ref.33) was used to estimate the linkage disequilibrium between markers and haplotypes in the different racial groups. Conditional haplotype analyses were conducted using the WHAP program version 2.09.[34] To correct for multiple testing, FDR methods were used and q values were calculated using PLINK.[32] Q values correspond to the proportion of false positives among the results. Thus, q values <0.05 signify less than a 5% false-positive rate and are taken as a measure of significance. For each SNP, missing data proportions for cases and controls, minor allele frequencies, ORs, 95% CI intervals, P-values and exact tests for departures from Hardy–Weinberg expectations were calculated. SNPs needed to pass stringent quality control criteria that included: Hardy–Weinberg proportions with a $P > 0.01$ in the controls and >0.0001 in cases, total proportion missing $<5\%$, and $P > 0.05$ for differential missingness between cases and controls. Samples with a $<90\%$ call rate or increased heterozygosity (>5 s.d. around the mean) were excluded from the analysis. The remaining samples were then evaluated for duplicates or related individuals. Genetic outliers were removed from further analysis as determined by principal components analysis,[35] and admixture proportions calculated using ADMIXMAP (http://admixmap.sourceforge.net). Principal components were calculated using all SNPs and admixture proportions were calculated using 347 ancestry informative markers.

Conflict of interest

The authors declare no conflict of interest.

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