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A functional haplotype of *UBE2L3* confers risk for Systemic Lupus Erythematosus

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P.M.G., C.G.M., K.L.M., C.J.L., J.A.K., K.M.K., C.D.L., and J.B.H. selected SNPs and were responsible for the study design. J.M.G., M.E.A.R., G.S.A., J.M.A., S.C.B., S.A.B., E.E.B., M.A.P., R.R.G., J.D.R., L.M.V., L.A.C., J.C.E., B.I.F., G.S.G., C.O.J., J.A.J., D.L.K., R.P.K., J.M., J.T.M., T.B.N., B.A.P.E., A.M.S., B.P.T., L.M.V., T.J.V., J.B.H., K.L.M., and P.M.G. assisted in the collection and characterization of the SLE cases and controls. A.A., K.M.K. and P.M.G. performed the genotyping. S.B.G., A.W., J.Z., M.E.C., M.M., J.A.K., K.M.K. and C.D.L. performed genotyping quality control. I.A., S.W., G.W., C.J.L., C.G.M. and P.M.G. performed association analyses and imputation. G.W., B.E.W., C.L., E.K.W., S.W. and P.M.G. performed sequencing. G.W., I.A., S.W., C.J.L., S.B.G., C.G.M. and P.M.G. performed sequencing data analysis. S.W. and P.M.G. performed functional studies. S.W., I.A., G.W., C.G.M., and P.M.G. prepared the manuscript and all authors approved the final draft.

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The authors declare no competing financial interests.

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Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease with diverse clinical manifestations characterized by the development of pathogenic autoantibodies manifesting in inflammation of target organs such as the kidneys, skin and joints. Genome-wide association studies have identified genetic variants in the *UBE2L3* region that are associated with SLE in subjects of European and Asian ancestry. *UBE2L3* encodes an ubiquitin-conjugating enzyme, UBCH7, involved in cell proliferation and immune function. In this study, we sought to further characterize the genetic association in the region of *UBE2L3* and use molecular methods to determine the functional effect of the risk haplotype. We identified significant associations between variants in the region of *UBE2L3* and SLE in individuals of European and Asian ancestry that exceeded a Bonferroni corrected threshold ($P < 1 \times 10^{-4}$). A single risk haplotype was observed in all associated populations. Individuals harboring the risk haplotype display a significant increase in both *UBE2L3* mRNA expression ($P = 0.0004$) and UBCH7 protein expression ($P = 0.0068$). The results suggest that variants carried on the SLE associated *UBE2L3* risk haplotype influence autoimmunity by modulating UBCH7 expression.

Keywords

Systemic Lupus Erythematosus; UBE2L3; Multi Ethnic Association Study; UBCH7 Expression

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by self-reactive antibodies that form immune complexes leading to systemic inflammation and organ failure. SLE susceptibility is strongly influenced by both genetic and environmental factors. Recent candidate gene and genome-wide association studies (GWAS) have identified more than 30 susceptibility loci for SLE [1–8]. Single nucleotide polymorphisms (SNPs) in the region of *UBE2L3*, which encodes the ubiquitin conjugating enzyme, UBCH7, demonstrate association with SLE in multiple independent SLE cohorts of European and African American ancestry [9,10] and correlate most significantly with patients developing anti-dsDNA antibodies [11]. Variants in the region of *UBE2L3* have also been reported to be associated with several other autoimmune disorders such as Crohn's disease [12, 13], celiac disease [14] and rheumatoid arthritis [9, 14]. Gene expression studies suggest that variants in the vicinity of *UBE2L3* regulate *UBE2L3* expression, thus providing a potential mechanism by which *UBE2L3* influences susceptibility to autoimmune diseases [13].

Post-translational ubiquitination of proteins is an important process in eukaryotes that is responsible for the degradation of short-lived and abnormal cytosolic proteins and the regulation of cellular signaling pathways [15]. Three classes of enzymes, ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s) and ubiquitin-protein ligases (E3s) constitute the system by which ubiquitin is transferred to target proteins. *UBE2L3*, located at chromosome 22q11.2, is a member of the E2 ubiquitin conjugating enzyme family and has been demonstrated to participate in the ubiquitination of p53 [16], c-Fos, and the NF-κB precursor p105 in vitro [17, 18]. Recent studies have further revealed that *UBE2L3* is involved in cell proliferation [19].

In order to more thoroughly evaluate the *UBE2L3* locus in SLE, we fine mapped and imputed SNPs in five diverse ethnic populations using a custom genotyping array, publicly available datasets of human variation and a targeted resequencing dataset enriched for subjects with SLE risk haplotypes. We identified a single 67kb risk haplotype associated with SLE and characterized the effect of the risk haplotype on gene expression by using quantitative-PCR and Western blotting. Our data demonstrate that both *UBE2L3* mRNA transcripts and UBCH7 protein expression is increased by variants carried on the SLE risk haplotype, suggesting a mechanism by which variants in the region of *UBE2L3* influence the pathogenesis of SLE.

RESULTS

Genome-wide association studies have identified genetic association with variants in the vicinity of *UBE2L3* and multiple autoimmune diseases. In an effort to identify the causal variants responsible for association with SLE, we genotyped 57 SNPs in and around *UBE2L3* along with 347 ancestry-informative markers (AIMs) in 8922 independent SLE cases and 8077 independent controls across five ethnic populations (Table 1, Supplementary Figure 1, Supplementary Tables 1, 2 and 3). After applying a series of quality control filters, 55 genotyped SNPs and 262 AIMs were available for further analyses. To enrich our dataset for additional untyped SNPs, we imputed a minimum of 285 SNPs from the 1000 Genomes Project. Single-marker logistic regression analyses, adjusting for gender and global ancestry estimates, revealed significant associations between multiple SNPs and SLE surpassing a Bonferroni corrected $P < 1 \times 10^{-4}$. In individuals of European-ancestry the strongest signal was observed at rs131658 ($P = 6.50 \times 10^{-7}$, odds ratio [OR] = 1.24, 95% confidence interval [CI] = 1.14–1.35, Figure 1A). In the Asian population the strongest signal occurred at rs5754177 ($P = 1.98 \times 10^{-6}$, OR = 1.33, 95% CI = 1.18–1.50, Figure 1B). We also observed weaker evidence of association not exceeding the Bonferroni corrected threshold in other populations with the optimal signals at rs11089629 for African Americans ($P = 1.23 \times 10^{-3}$, OR = 1.18, 95% CI = 1.07–1.30, Figure 1C), rs390408 for Hispanics ($P = 2.89 \times 10^{-3}$, OR = 1.23, 95% CI = 1.07–1.42, Figure 1D) and rs11705317 for Gullah ($P = 1.74 \times 10^{-2}$, OR = 0.27, 95%CI = 0.09–0.79, Figure 1E). When all populations were combined in meta-analysis, rs7444 produced the most significant association ($P_{combined} = 2.21 \times 10^{-14}$, Supplementary Table 4) with no evidence of heterogeneity (the Cochran's Q test $P = 0.672$ and the inconsistency index $I^2 = 0\%$, see Methods).

To capture novel variants enriched on the *UBE2L3* risk haplotype that were not genotyped or imputed with the 1000 Genomes Project reference panel, we resequenced 174 subjects of European-ancestry enriched for SLE risk haplotypes including *UBE2L3*. The phased haplotypes of these sequenced individuals were then imputed into the European-ancestry dataset. This procedure added 5 novel variants (3 SNPs and 2 deletion/insertion polymorphisms [DIPs]) that were not present in dbSNP 132 (Supplementary Table 5). Among these five variants, a single base insertion located in the 3' UTR of *UBE2L3* demonstrated significant association with SLE ($P = 2.56 \times 10^{-6}$, OR = 1.23, 95% CI = 1.13–1.33) and is in strong linkage disequilibrium with the most significant SNP in European-ancestry (rs131658, $r^2 = 0.99$).

To determine if differences in the linkage disequilibrium patterns across populations (trans-population mapping) could help define a minimal risk segment, we performed haplotype analysis using the thirty-four variants with $P < 1 \times 10^{-4}$ defined in subjects of European-ancestry (Table 2). In the European-ancestry population we observed a single 67 kb risk haplotype ($P = 1.17 \times 10^{-7}$) spanning the *UBE2L3* region (haplotype H2, Figure 2). Similarly, a single risk haplotype harboring the majority of alleles in the EA risk haplotype was also present in Asian (haplotype H2, Supplementary Figure 2A), African American (haplotypes H2, Supplementary Figure 2B), and Hispanic populations (haplotype H2, Supplementary Figure 2C). Strong linkage disequilibrium was observed on the risk haplotype in all four populations and limited the utility of trans-population mapping or conditional analysis to further isolate a minimal risk segment. These results suggest that a single risk effect common to these populations may be responsible for the association with SLE.

Previous studies have demonstrated that variants in the region of *UBE2L3* influence *UBE2L3* transcript expression [13], therefore, we evaluated whether the SLE associated risk haplotype produced a similar molecular phenotype. To evaluate *UBE2L3* mRNA and UBCH7 protein expression, quantitative real-time PCR and western blotting was performed in an independent set of EBV-transformed B cell lines under resting conditions. Cell lines were selected based on whether they contained 0, 1, or 2 copies of the *UBE2L3* risk haplotype as defined by the rs7444-C risk allele. Concordant with other published studies, we observed increased *UBE2L3* mRNA expression and increased expression of UBCH7 protein as a function of the number of copies of the risk haplotype ($P = 0.0004$ and $P = 0.0068$, respectively (one-way ANOVA), Figures 3A, 3B and Supplementary Figure 3).

DISCUSSION

In this study, we observed significant associations between variants in *UBE2L3* and SLE in individuals of European, Asian, and African-American ancestry. Weaker association evidence was also observed in the Hispanic and Gullah populations due in part to the smaller samples sizes of these two groups (Table 1). Risk variants were carried on a 67 kb risk haplotype tagged by the proxy SNP rs7444, in all populations demonstrating association with SLE. Since the variants in this haplotype block were highly correlated across the different populations, we were unable to further narrow this SLE associated DNA segment using conditional analyses or trans-population mapping.

In line with data published in Crohn's Disease, we observed higher levels of *UBE2L3* mRNA and UBCH7 protein expression in EBV cell lines carrying the risk haplotype. This suggests that similar molecular mechanisms in *UBE2L3* that influence susceptibility to autoimmunity are shared between SLE and CD. The precise mechanism by which causal variants on the *UBE2L3* risk haplotype influence expression of *UBE2L3* is not yet defined but we hypothesize that this could be due to the modification of mRNA stability and/or modification of the binding affinity of transcription factors to the *UBE2L3* promoter. Further studies geared toward identification of the causal variant(s), which underlies the effect on gene and protein expression are required.

Ubiquitination is a critical post-translational protein modification for regulation of NF-κB signaling [20], however, little is known about how UBCH7 mediated ubiquitination might impact NF-κB signaling. In a cell free system, Orian et al. demonstrated that the NF-κB precursor protein, p105, was a substrate for UBCH7 mediated ubiquitination. At rest, p105, encoded by the gene, NF-κB1, undergoes constitutive proteosomal processing to yield the NF-κB subunit, p50 [18]. Unprocessed p105 functions as an inhibitor of NF-κB by retaining p50 homodimers in the cytoplasm using ankyrin repeats located in the C-terminal portion of the protein [21]. Following cellular activation, p105 is phosphorylated and undergoes complete proteosomal degradation, allowing bound p50 homodimers to translocate to the nucleus. It is possible that UBCH7 mediated ubiquitination of p105 may result in increased proteosomal processing and/or degradation of p105, resulting in increased levels of free p50 homodimers.

UBCH7 has been demonstrated to function with the HECT (homologous to the E6-associated protein carboxy terminus) family E3 ubiquitin ligase, ITCH, in *in vitro* ubiquitination assays [22, 23]. ITCH participates in regulation of NF-κB along with RNF11, TAX1BP1 and A20 as part of a protein complex known at the ubiquitin-editing complex [24]. Recent data demonstrates that UBCH7 is restricted to HECT and RBR (RING-in-between-RING) type E3 ligases which underscores the possibility that UBCH7 and ITCH could function together to ubiquitinate substrate proteins, however, to our knowledge, a physical interaction between ITCH and UBCH7 has not yet been demonstrated in vivo.

In summary, our data support a role for variants in the *UBE2L3* locus in the predisposition to SLE in multiple ethnic populations. The *UBE2L3* locus demonstrates low haplotype diversity with a single risk haplotype associated with SLE. This risk haplotype carries causal variants that result in increased expression of *UBE2L3* transcripts and UBCH7 protein. Future work will now focus on the isolation and characterization of the variants that result in this expression phenotype and on the role of UBCH7 function in immune cell signaling.

MATERIALS AND METHODS

Subjects

In this study, the following independent case and control subjects were collected, respectively: African-American (1,569/1,893), Asian (1,328/1,348), European-ancestry (4,248/3,818), African-American Gullah (155/131) and Hispanic enriched for the Amerindian-European admixture (1,622/887) populations (Supplementary Table 1). SLE cases were determined by meeting at least four of the eleven 1997 ACR revised criteria for SLE. Case and control samples were obtained from multiple sites with the Institutional Review Board (IRB) approval from each institution and processed at the Oklahoma Medical Research Foundation (OMRF) under the OMRF IRB.

Genotyping and Quality Control

The Illumina iSelect platform at OMRF was employed to genotype 57 SNPs and 347 genome-wide ancestry-informative markers (AIMs) [25, 26]. SNP quality control (QC) measures included well-defined cluster scatter plots, a call rate >90%, a minor allele frequency >0.001 and Hardy-Weinberg proportion test p-value in controls >0.001 for inclusion. For the AIMs, we removed AIMs with low call rates (<90%), low minor allele frequencies (<0.001), and that are in LD with each other (r^2>0.2). We did not perform the Hardy-Weinberg proportion test for the AIM QC to avoid AIMs being inadvertently dropped due to monomophic states in one of the ethnic groups. Principal components [27] calculated using R and global ancestry estimated using ADMIXMAP [28, 29] (with ancestral allele frequencies from African, European, American, Indian, and East Asian population) were utilized to pinpoint population outliers (Supplementary Figure 1) and to adjust the logistic regression models for controlling population structure in our association analyses. A total of 1,135 samples were removed because they were duplicates (the proportion of alleles shared identity by descent (IBD) >0.4), sample heterozygosity outliers (>5 standard deviation from the mean), population outliers, low call rate (<90%), or gender discrepancies between reported gender and genetic data (Supplementary Table 3). The final dataset, following quality control exclusions comprised 55 SNPs and 262 AIMs and 15,864 samples (Table 1).

Association Analyses

Single marker association analyses were calculated using the logistic regression function in PLINK v1.07 [30] under the additive model adjusting for gender and global ancestry estimates (African, European, and East Asian). Meta-analyses to combine p-values from different populations were performed using a weighted *Z*-score METAL [31]. We used both the Cochran's *Q* test statistic *and* I^2 index to test for heterogeneity in the meta-analysis. The Cochran's *Q* test calculates the weighted sum of the squared deviations between individual study effects and the overall effect across studies [32] whereas the I^2 index measures the degree or percentage of inconsistency across studies due to heterogeneity rather than by chance [33]. LD between variants was estimated and probable haplotypes were calculated using Haploview 4.2 [34] followed by haplotypic association for all haplotypes formed by the associated markers across the various populations. Constructing haplotypes using all variants yielded the same haplotypes as the analysis using only the associated SNPs (results not shown).

Imputation

IMPUTE2 software [35] was used to impute SNPs from 20.21 Mb to 20.34 Mb on chromosome 22 with genotype data as the source of observed genotypes and the 1000 Genomes Project from Phase I interim release (June 2011) for 1,094 individuals from

Africa, Asia, Europe, and the Americas (Supplementary Table 6) as reference genotypes. Imputation using the sequence data from our European-ancestry samples along with the 1000 Genome Project haplotypes was also performed. IMPUTE2 calculates posterior probabilities for the three possible genotypes (i.e. AA, AB, and BB). These probabilities were converted to the most possible genotypes with a threshold of 0.8. Imputed SNPs with the information measure less than 0.4 were excluded.

Resequencing, Variant Detection and Quality Control

We resequenced 74 SLE cases and 100 controls of European-ancestry then included the sequenced haplotypes into the genotype imputation. For each sample 3–5 ug of whole genomic DNA were sheared and prepared using an Illumina Paired-End Genomic DNA Sample Prep Kit. The SureSelect Target Enrichment System was used to enrich targeted regions of interest from each sample by utilizing a custom designed bait pool. Resequencing was performed on an Illumina GAIIx platform using standard procedures with minimum average fold coverage of 25X. Illumina Pipeline software v.1.7 was the used to process post sequence data.

Duplicate reads were excluded using a custom script followed by alignment to the human reference genome build hg18 using BWA alignment software version 0.5.9 [36]. Realignment of reads around insertion/deletion sites and problematic areas, base quality score recalibration, and variation detection were processed using the Genome Analysis Tool Kit (GATK) software suite version 1.0 [37, 38]. Variants clustered within 10 base pairs were filtered out, as well as any variant with a quality score less than 30, a quality by depth score less than 5, inclusion within a homopolymer run of 5 or more bases, or a strand bias score of greater than −0.1. The program Beagle version 3.3 [39] was utilized to determine variant phase. PLINK and IMPUTE2 format files were created using the vcftools software suite version 0.1.3 [40].

In order to assess the quality of the sequence data, the sequence-based variant calls were compared with common SNPs previously genotyped with the Illumina iSelect platform. More than 99% concordance was observed suggesting high quality of our sequence data. Samples with more than 5% of variants inconsistent with genotype calls required a manual inspection of the assembled contig sequence to determine the sequence quality using the Integrative Genomics Viewer (IGV) program [41]. The assembled contig sequence of each novel variant identified by our sequencing was also inspected using IGV.

Cell Culture

EBV-transformed B cell lines were requested from the Lupus Family Registry and Repository (LFRR) at OMRF with IRB approval. All cell lines in this study were EA samples and were stratified by rs7444 genotype, which is a proxy of the *UBE2L3* risk haplotype. Cell lines are either homozygous (carry two copies) of non-risk haplotype, heterozygous (one copy of the risk haplotype and one copy of non risk haplotype), or homozygous (carry two copies) of risk haplotype. Cell lines were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin, streptomycin, L-glutamine, and 55μM beta-mercaptoethanol. Equal numbers of cells were harvested under basal culture condition in log-phase growth.

RNA Isolation and Quantitative RT-PCR

Total RNA was isolated using the Trizol total RNA isolation reagent (Invitrogen Inc., Carlsbad, CA). The concentrations of total RNA were determined by using nanodrop, and were diluted with 20ng/μL of MS2-RNA (Hoffmann-La Roche, Inc., Nutley NJ) to a final concentration of 0.5μg/μL. Total RNA was treated with DNase and cDNA was synthesized using the iScript cDNA Synthesis Kits purchased from Bio-Rad Laboratories, Inc., Hercules, CA. Quantitative PCR was carried out using the SYBR Green method to determine the mRNA expression of *UBE2L3*. A pair of primers was designed and synthesized: sense, 5'-TTAGTGCCGAAAACTGGAAGC-3'; anti-sense, 5'-ATTCACCAGTGCTATGAGGGAC-3'. The PCR product corresponds to 346bp-416bp of *UBE2L3* mRNA. Human HMBS gene was used in quantitative RT-PCR as a reference. The RT^2 qPCR Primer Assay-SYBR Green Human HMBS Kit was purchased from SABiosciences Inc., Frederick, MD. mRNA expression of UBE2L3 was normalized to HMBS.

UBE2L3 Protein Expression

EBV-transformed B cells were harvested and lysed in Whole Cell Extraction Buffer (25mM Tris, 1% Triton X-100, 150mM NaCl, 1mM EDTA and protease inhibitors). Concentrations of protein in each cell line were determined using Quick Start Bradford Protein Assay Kits and were adjusted to a final protein concentration of 2mg/mL. Anti-*UBE2L3* and Anti-GAPDH antibodies were purchased from Cell Signaling Technology, Inc., Danvers, MA, and were used to detect protein expression of UBCH7 and GAPDH, respectively. ECL Plus Western Blotting Detection System was purchased from GE Heathcare, Inc., Amersham, UK. The intensity of each band was analyzed using Image J (NIH) software. Protein expression of UBCH7 was normalized to GAPDH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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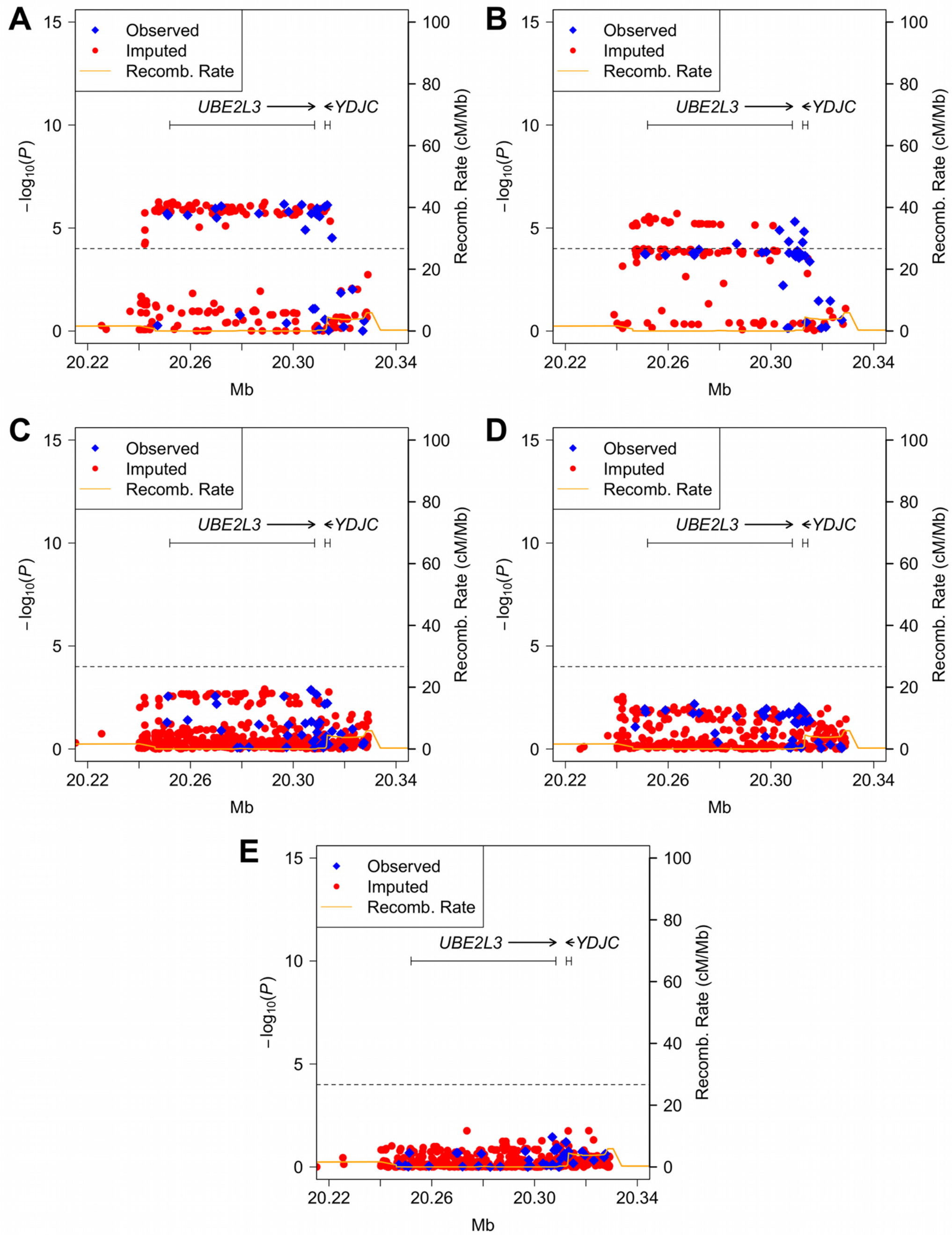

Figure 1.

SNPs in and around the *UBE2L3* region associated with SLE. (A) European-ancestry, (B) Asian, (C) African American, (D) Hispanic and (E) African-American Gullah populations. The dashed line in each panel signifies the Bonferroni corrected level of significance ($P = 1 \times 10^{-4}$). The orange solid line denotes the recombination rate calculated from the combined HapMap CEU, YRI and CHB+JPT data.

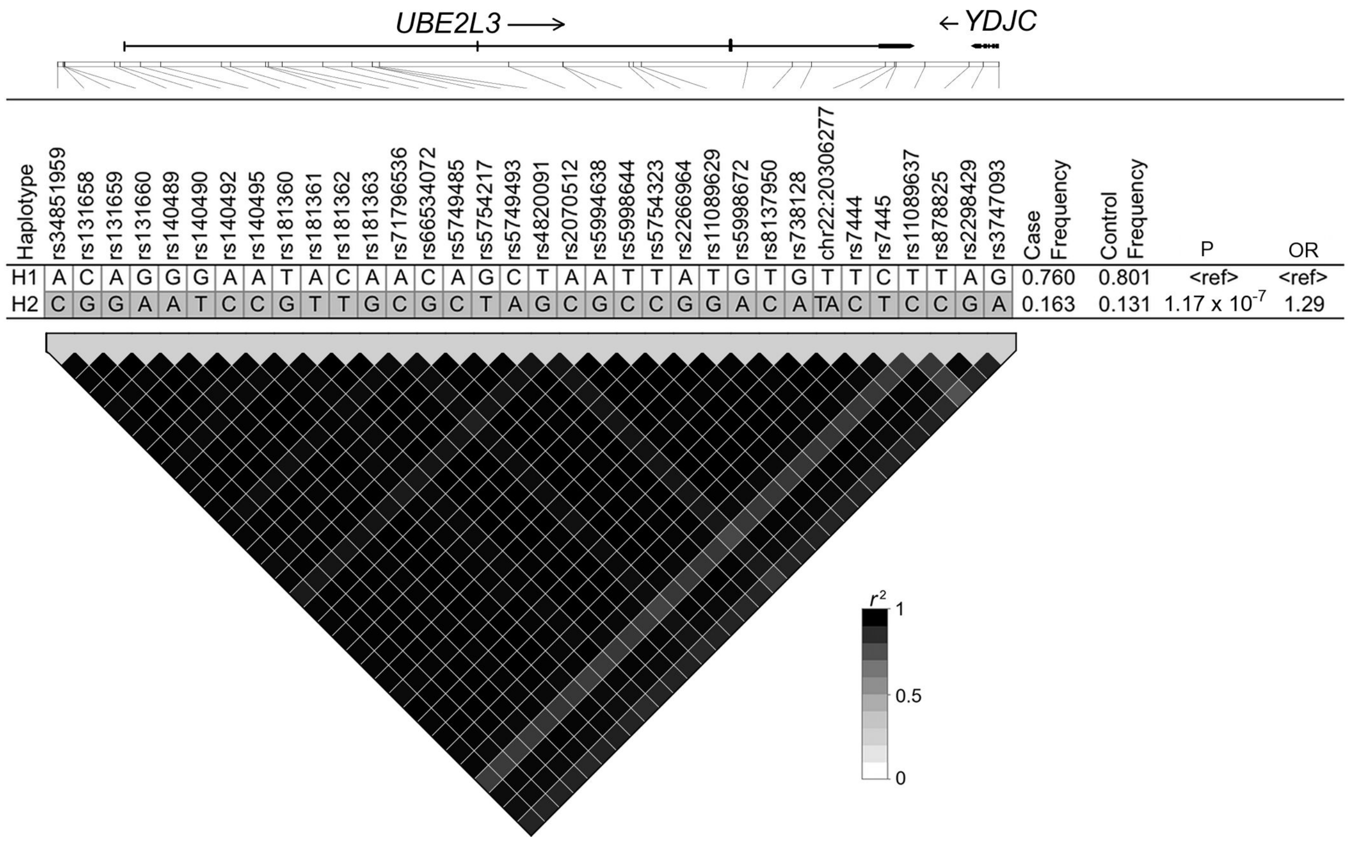

Figure 2.

Analyses of 34 associated SNPs present on *UBE2L3* region in European-ancestry population. Top: *UBE2L3* haplotype association analysis with haplotype frequencies > 5%. Alleles in white boxes represent the major alleles and those in gray boxes represent the minor allele for each haplotype. Bottom: the plot of the pairwise linkage disequilibrium (LD) of 34 associated SNPs with the intensity color for r^2 superimposed.

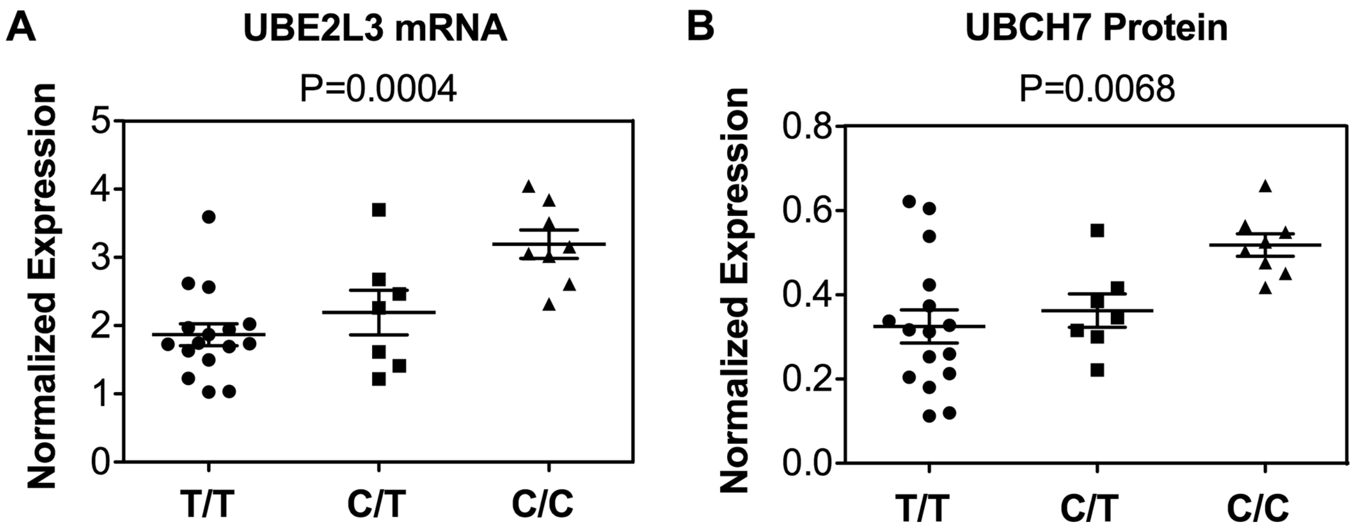

Figure 3.

Effect of the risk haplotype on *UBE2L3* (A) mRNA and UBCH7 (B) protein expression. On the X-axis, the three different genotypes for SNP rs7444 are displayed corresponding to homozygote of risk haplotype (C/C), heterozygote (C/T), and homozygote of non-risk haplotype (T/T). On the Y-axis is the level of normalized expression for *UBE2L3* for each assay. Each data point represents the expression level of *UBE2L3* mRNA or UBCH7 protein for one individual.

Table 1

Samples available for analysis following quality control adjustments.

Population	Number of Samples	Case	Control	Male	Female
African-American	3338	1527	1811	695	2643
Asian	2525	1265	1260	253	2272
European-ancestry	7427	3936	3491	1495	5932
AA-Gullah	275	152	123	33	242
Hispanic[1]	2299	1492	807	207	2092
Total	15864	8372	7492	2683	13181

[1] enriched for Amerindian-European admixture

Table 2

SNPs in the region of *UBE2L3* associated with SLE.

SNP	BP (hg18)	SNP Status[a]	Alleles[b]	European-ancestry			Asian			African-American			Hispanic		
				MAF[c]	OR[d]	P[e]	MAF[c]	OR[d]	P[e]	MAF[c]	OR[d]	P[e]	MAF[c]	OR[d]	P[e]
rs34851959	20247239	i-seq	A/C	0.198	1.22(1.12–1.33)	4.72E-06	NA	NA	NA	NA	NA	NA	NA	NA	NA
rs131658	20247626	i-1kGP	C/G	0.204	1.24(1.14–1.35)	6.50E-07	0.464	1.23(1.1–1.37)	0.0002279	0.418	1.11(1–1.23)	0.04387	0.422	1.18(1.04–1.35)	0.01071
rs131659	20247708	i-1kGP	A/G	0.205	1.23(1.13–1.34)	1.41E-06	0.456	1.22(1.09–1.37)	0.0004743	0.418	1.17(1.05–1.3)	0.003587	0.420	1.17(1.03–1.33)	0.01988
rs131660	20247757	i-1kGP	G/A	0.204	1.24(1.14–1.35)	9.46E-07	0.448	1.25(1.12–1.4)	0.0001051	0.420	1.17(1.05–1.29)	0.004152	0.422	1.17(1.03–1.33)	0.01787
rs140489	20251294	g	G/A	0.212	1.23(1.13–1.33)	2.41E-06	0.470	1.24(1.11–1.38)	0.0001899	0.451	1.17(1.05–1.29)	0.002789	0.425	1.17(1.03–1.33)	0.01682
rs140490	20251686	i-1kGP	G/T	0.204	1.22(1.12–1.33)	2.92E-06	0.468	1.24(1.11–1.39)	0.0001357	0.451	1.17(1.06–1.29)	0.002583	0.425	1.18(1.04–1.34)	0.01292
rs140492	20253144	i-1kGP	A/C	0.203	1.23(1.13–1.33)	2.66E-06	0.468	1.24(1.11–1.39)	0.0001321	0.451	1.17(1.05–1.29)	0.002767	0.425	1.18(1.04–1.34)	0.01307
rs140495	20254589	i-seq	A/C	0.198	1.23(1.13–1.34)	2.43E-06	NA	NA	NA	NA	NA	NA	NA	NA	NA
rs181360	20258916	g	T/G	0.210	1.23(1.13–1.33)	2.36E-06	0.470	1.23(1.1–1.38)	0.0002145	0.136	1.17(1.01–1.36)	0.03956	0.401	1.18(1.04–1.35)	0.01308
rs181361	20259566	i-1kGP	A/T	0.206	1.22(1.12–1.33)	4.28E-06	0.453	1.24(1.11–1.39)	0.0001672	0.451	1.17(1.06–1.29)	0.002585	0.425	1.18(1.04–1.34)	0.01324
rs181362	20262068	i-1kGP	C/T	0.206	1.23(1.13–1.34)	1.88E-06	0.467	1.24(1.11–1.39)	0.0001435	0.451	1.17(1.05–1.29)	0.002853	0.425	1.18(1.04–1.34)	0.01269
rs181363	20262264	i-1kGP	A/G	0.205	1.23(1.13–1.34)	1.82E-06	0.467	1.24(1.11–1.39)	0.0001453	0.471	1.16(1.04–1.28)	0.005006	0.427	1.19(1.04–1.35)	0.00928
rs71796536	20263251	i-seq	A/C	0.195	1.23(1.12–1.33)	3.80E-06	NA	NA	NA	NA	NA	NA	NA	NA	NA
rs66534072	20266152	i-1kGP	C/G	0.207	1.23(1.13–1.34)		0.467	1.24(1.11–1.39)	0.0001438	0.451	1.17(1.05–1.29)	0.002834	0.425	1.18(1.04–1.34)	0.01249
rs5749485	20268224	i-1kGP	A/C	0.205	1.23(1.13–1.33)	2.18E-06	0.467	1.24(1.11–1.38)	0.0001458	0.439	1.18(1.06–1.3)	0.001982	0.424	1.19(1.04–1.35)	0.00949
rs5754217	20269675	g	G/T	0.213	1.23(1.13–1.34)	1.13E-06	0.468	1.24(1.11–1.38)	0.0001626	0.452	1.17(1.06–1.29)	0.002735	0.425	1.17(1.03–1.33)	0.01885
rs5749493	20269687	i-1kGP	C/A	0.207	1.23(1.13–1.34)	1.70E-06	0.467	1.24(1.11–1.38)	0.0001477	0.448	1.17(1.06–1.29)	0.002355	0.425	1.17(1.03–1.34)	0.01508
rs4820091	20270189	g	T/G	0.198	1.23(1.13–1.34)	3.17E-06	0.467	1.23(1.1–1.38)	0.0002087	0.446	1.15(1.04–1.27)	0.006538	0.411	1.2(1.05–1.37)	0.00657
rs2070512	20279411	i-1kGP	A/C	0.205	1.23(1.13–1.33)	2.30E-06	0.467	1.24(1.11–1.38)	0.0001648	0.451	1.17(1.05–1.29)	0.002846	0.426	1.18(1.04–1.34)	0.01191
rs5994638	20283276	i-1kGP	A/G	0.206	1.23(1.13–1.34)	1.80E-06	0.464	1.24(1.11–1.39)	0.0001257	0.472	1.15(1.04–1.27)	0.005997	0.428	1.18(1.03–1.34)	0.01349
rs5998644	20283288	i-1kGP	T/C	0.205	1.23(1.13–1.34)	2.13E-06	0.464	1.24(1.11–1.39)	0.0001257	0.472	1.15(1.04–1.27)	0.005997	0.428	1.18(1.03–1.34)	0.01349
rs5754323	20287992	i-1kGP	T/C	0.207	1.23(1.13–1.34)	1.68E-06	0.467	1.24(1.11–1.38)	0.0001449	0.464	1.15(1.04–1.28)	0.005267	0.427	1.19(1.04–1.35)	0.00980
rs2266964	20288304	i-1kGP	A/G	0.206	1.23(1.13–1.34)	1.83E-06	0.467	1.24(1.11–1.38)	0.0001449	0.451	1.17(1.06–1.29)	0.002659	0.426	1.18(1.04–1.34)	0.01161
rs11089629	20288872	i-1kGP	T/G	0.206	1.23(1.13–1.34)	1.16E-06	0.467	1.24(1.11–1.38)	0.0001809	0.473	1.18(1.07–1.3)	0.001234	0.428	1.18(1.04–1.34)	0.01319
rs5998672	20296442	g	G/A	0.214	1.24(1.14–1.34)	7.01E-07	0.463	1.24(1.11–1.39)	0.0001527	0.452	1.17(1.05–1.29)	0.002772	0.426	1.17(1.03–1.33)	0.01667
rs8137950	20299640	i-1kGP	T/C	0.204	1.22(1.12–1.33)	4.35E-06	0.460	1.22(1.09–1.37)	0.0003816	NA	NA	NA	0.417	1.15(1.01–1.32)	0.03141
rs738128	20301010	i-1kGP	G/A	0.206	1.23(1.13–1.33)	2.32E-06	0.464	1.24(1.11–1.39)	0.0001217	0.451	1.16(1.05–1.29)	0.002988	0.426	1.18(1.04–1.34)	0.01195

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SNP	BP (hg18)	SNP Status[a]	Alleles[b]	European-ancestry			Asian			African-American			Hispanic		
				MAF[c]	OR[d]	P^e	MAF[c]	OR[d]	P^e	MAF[c]	OR[d]	P^e	MAF[c]	OR[d]	P^e
chr22:20306277	20306277	i-seq	T/TA	0.203	1.23(1.13–1.33)	2.56E-06	NA	NA	NA	NA	NA	NA	NA	NA	NA
rs7444	20306934	i-1kGP	T/C	0.206	1.23(1.13–1.34)	1.37E-06	0.462	1.26(1.13–1.41)	4.56E-05	0.496	1.18(1.07–1.3)	0.001353	0.433	1.17(1.03–1.33)	0.01698
rs7445	20307047	g	C/T	0.211	1.23(1.13–1.33)	2.00E-06	0.465	1.24(1.11–1.39)	0.0001635	0.161	1.15(1–1.32)	0.04677	0.403	1.17(1.03–1.33)	0.01962
rs11089637	20309096	g	T/C	0.178	1.24(1.14–1.36)	2.16E-06	0.464	1.24(1.11–1.38)	0.0001858	0.445	1.17(1.06–1.3)	0.002278	0.411	1.17(1.03–1.34)	0.01802
rs878825	20312249	g	T/C	0.215	1.23(1.13–1.34)	9.30E-07	0.470	1.23(1.1–1.38)	0.0002118	0.488	1.15(1.04–1.27)	0.006683	0.432	1.18(1.04–1.34)	0.01276
rs2298429	20313260	g	A/G	0.209	1.24(1.14–1.35)	7.64E-07	0.471	1.23(1.1–1.38)	0.0002233	0.469	1.15(1.04–1.27)	0.006024	0.426	1.17(1.03–1.33)	0.01845
rs3747093	20314379	i-1kGP	G/A	0.212	1.19(1.09–1.29)	4.68E-05	0.466	1.23(1.1–1.38)	0.0003042	NA	NA	NA	0.433	1.16(1.02–1.32)	0.02673

[a]SNP Status: genotyped (g), imputed SNP from the 1000 Genomes Project (i-1kGP), or imputed SNP from sequencing data (i-seq).

[b]Major/minor.

[c]Minor allele frequency.

[d]The odds ratio (OR) was calculated with respect to the minor allele.

[e]Adjusted for sex and global ancestry estimates.