Variation in the ATP-binding cassette transporter 2 gene is a separate risk factor for Systemic Lupus Erythematosus within the MHC

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Abstract

The ATP-binding cassette transporter (TAP) proteins are functionally relevant candidates for predisposition to Systemic Lupus Erythematosus (SLE) by virtue of their role in autoantigen presentation and location in the MHC. We tested if variation in the TAP genes (TAP1 and TAP2) is associated with SLE. We genotyped tag single nucleotide polymorphisms (SNPs) and performed family-based association analysis on 390 Caucasian pedigrees. We found significant evidence of association between TAP2 and SLE (rs241453, \( P = 1.33 \times 10^{-6} \)). Conditional logistic regression analysis suggests that this TAP2 effect is separate from the HLA-DRB1 alleles. Our analyses show that both rs241453 (\( P = 1.6 \times 10^{-4} \)) and HLA-DRB1*03xx (\( P = 2.3 \times 10^{-4} \)) have significant autonomous effects not due to linkage disequilibrium. Moreover, these loci exhibit a significant statistical interaction (\( P < 1.0 \times 10^{-6} \)), demonstrated by an increase in the odds ratio for the TAP2 association from OR = 2.00 (CI=1.17-3.42) in HLA-DRB1*03xx-negative subjects to OR = 4.29 (CI=1.88-9.76) in the subjects with at least one HLA-DRB1*03xx allele group. We report the largest association study of the TAP genes with SLE to date, and the first to test for its separate effect and interaction with the HLA alleles consistently associated with SLE.
Keywords
Systemic Lupus Erythematosus; TAP2; HLA-DRB1; family-based association analysis; conditional logistic regression analysis; interaction analysis

Introduction
Systemic lupus erythematosus (SLE [MIM 152700]) is a chronic and severe systemic autoimmune disease characterized by the production of high titers of autoantibodies directed against native DNA and other cellular constituents. Based on the most current estimates, the prevalence of SLE in the U.S. is estimated between 0.05% and 0.1% of the population, disproportionately affecting women and African Americans (0.009% of white men, 0.066% of white women, 0.038% of African-American men, and 0.282% of African-American women). Although the exact pathogenesis of SLE is unknown, multiple lines of evidence demonstrate that SLE susceptibility in humans is strongly influenced by genetic factors.

The Major Histocompatibility Complex (MHC) has been the most consistent genetic risk factor associated with SLE and autoantibody production, specifically the associations with the class II alleles HLA-DR2 (DRB1*1501) and HLA-DR3 (DRB1*0301) in Caucasian populations. One study has also reported an association with the HLA-DR8 allele.

The transporter associated with antigen processing (TAP1 and TAP2) genes are located within the MHC class II region between the HLA-DOB and HLA-DMB loci. Peptides of self or of foreign (e.g. viral) origin are transported by a heterodimer formed by the TAP proteins from the cytoplasm into the lumen of the endoplasmic reticulum for assembly with HLA class I or class II molecules. These peptide-loaded HLA molecules are ultimately presented on the cell surface of antigen-presenting cells. The TAP proteins have some specificity for the peptides they will transport.

Given their role in autoantigen presentation and location in the MHC class II region, the TAP proteins are potential candidates for SLE predisposition. Several early association analyses have provided conflicting results. Thus, we genotyped single nucleotide polymorphisms (SNPs) in the TAP genes and tested them for association with SLE. In addition, we formally tested if the association was due to LD, and for interaction with the HLA-DRB1 alleles. Herein, we describe the largest association study of the TAP genes and SLE reported to date, and the first to assess its autonomy from the HLA alleles consistently associated with SLE. In addition, our study is one of the first to formally test for a gene-gene interaction in this disease.

Results
Our sample of 390 genotyped Caucasian SLE trio pedigrees was predominantly female (96%) with a mean±SD age-of-onset of 31±12 years-old. All SNPs had over 90% genotyping success except for rs241453 (86.0%) and rs241425 (88.9%). In the trios analyzed, the genotyped SNPs that met our quality criteria thresholds achieved a mean $r^2 = 0.87$ across 8.76 kb of TAP1 (6 tag SNPs), and a mean $r^2 = 0.68$ across 16.94 kb of TAP2 (9
tag SNPs). From the total of 390 genotyped pedigrees, we had an average of 256 fully genotyped trios for the TAP genes. This sample yields 0.80 power to detect odds ratios (OR) greater than 1.5 under an additive model for alleles whose frequency is greater than 0.20.

We found significant evidence of association between TAP and SLE status (Table 1), as shown by the increased risk of SLE for individuals with the G allele at rs241453 ($P = 1.3 \times 10^{-6}$, O.R. = 2.39). This effect is considered significant even if we use a stringent Bonferroni multiple testing correction on the total number of tested SNPs (n=15) to set a significance threshold of $P = 0.0033$. We questioned the genotyping accuracy for this SNP (86.0%), but as explained below, we believe that this association is corroborated by that of its neighbor rs241447, whose genotyping success was 97.5%.

More modest effects within TAP2 were also observed, with an increased risk for individuals with the T allele at rs241447 ($P = 0.0021$, O.R. = 1.46), with the G allele at rs183585 ($P = 0.02$, O.R. = 1.32), and with the G allele at rs3819714 ($P = 0.027$, O.R = 1.31). Figure 1 shows the LD structure of TAP2 with the localization of the nine genotyped SNPs. The SNPs rs241453 and rs241447 are 525 bp from each other and are in strong linkage disequilibrium (LD) ($r^2 = 0.93$) in this sample. Haplotype analysis of the TAP2 gene showed that the haplotype defined by rs241453 and rs241447 was significant ($P = 0.0011$ for the GT haplotype) (data not shown), but it was not as strong an association as rs241453 in isolation. Thus, the association at rs241447 might be due to LD with rs241453.

Depending on the isoform, rs241453 ($P = 1.3 \times 10^{-6}$, O.R. = 2.39) localizes to the 3′UTR or the last intron of TAP2; it may thus be associated with altered stability of the TAP2 mRNA. Interestingly, rs241447 ($P = 0.0021$; O.R. = 1.46), which is in a conserved region with regulatory potential, locates to either the last intron or in a predicted last exon, in a human mRNA from GenBank (UCSC browser under URLs).

The rs241453 effect seems to be separate from the HLA haplotypes consistently associated with SLE. Using the HapMap browser (see URLs), we searched for variants in LD ($r^2 > 0.5$) with this SNP in a region of 550 kb around TAP2, from upstream HLA-DRB1 to HLA-DPB1 (Table 2). According to the HapMap B35 assembly, rs241453’s highest LD ($r^2 = 1$) in this region is with two TAP2 SNPs, one adjacent to it in the 3′UTR (rs241452), and the other in the last intron (rs2857104). A third intronic SNP in TAP2 also shows high LD with rs241453 (rs241442, $r^2=0.82$). Outside of TAP2, the highest LD ($r^2 = 0.76$) is found with an intronic SNP in HLA-DQA2 (rs17500482). Nineteen other SNPs, none in proximity to known genes, show modest LD ($r^2 \sim 0.5-0.6$) with rs241453.

Given the established SLE association with HLA-DRB1 alleles, we specifically focused on the LD with this locus and its HLA-DR2 and DR3 alleles. Based on the HapMap B35 data (see URLs) the LD between rs241453 and the tag SNPs that capture the HLA-DRB1*1501 and HLA-DRB1*030112 alleles is extremely low ($r^2 < 0.02$, Table 2). The LD values are similar between these HLA-DRB1 SNPs and the four aforementioned SNPs in LD with rs241453. The highest LD observed between these HLA-DRB1 and any TAP2 and SNPs is low ($r^2 < 0.4$). These data suggest that the association at rs241453 is not due to LD with HLA-DRB1.
We formally tested the hypothesis that the association observed with TAP2 is separate from the HLA-DRB1 locus, namely the DRB1*15xx (HLA-DR2), DRB1*03xx (HLA-DR3) and DRB1*08xx (HLA-DR8) allele groups. In our collection of 191 trios, the conditional logistic regression analysis continued to show differential transmission at rs241453 \((P = 1.8 \times 10^{-5}, \text{OR} = 2.56)\). The HLA-DRB1*03xx allele group was also significant \((P = 1.4 \times 10^{-6}, \text{OR} = 2.63)\), but the HLA-DRB1*15xx and HLA-DRB1*08xx alleles were not associated with SLE.

After separately adjusting for either HLA-DRB1*15xx, HLA-DRB1*03xx or HLA-DRB1*08xx, in the TDT conditional logistic regression model, rs241453 remained associated with SLE \((P = 1.3 \times 10^{-5}\) adjusting for HLA-DRB1*15xx, \(P = 1.6 \times 10^{-4}\) adjusting for HLA-DRB1*03xx, and \(P = 6.0 \times 10^{-6}\) adjusting for HLA-DRB1*08xx, Table 1). This suggests that the TAP2 rs241453 association is a separate effect from HLA-DRB1*15xx, HLA-DRB1*03xx and HLA-DRB1*08xx (Table 1). Both rs241453 \((P = 1.6 \times 10^{-4})\) and HLA-DRB1*03xx \((P = 2.3 \times 10^{-4})\) provide evidence for association with SLE in this model. When we include an interaction term in the conditional logistic regression analysis, the \(P\)-value for the interaction is highly significant for HLA-DRB1*03xx \((P < 1.0 \times 10^{-6})\), but not for HLA-DRB1*15xx or HLA-DRB1*08xx. These data provide preliminary evidence of a potential interaction between TAP2 and HLA-DRB1*03xx \((P < 1.0 \times 10^{-6})\). Given the modest sample size of 191 trios, this interaction forms an interesting hypothesis in need of a well-powered independent replication study. It is noteworthy that the presence of at least one HLA-DRB1*03xx allele group increases the OR for the TAP2 association. In the 113 trios where the SLE diagnosed offspring were homozygote DRB1*03xx-negative affecteds OR = 2.00 (CI=1.17-3.42), increasing to OR= 3.83 (CI=1.56-9.41) in the 65 DRB1*03xx-heterozygote trios, and to OR= 7.00 (CI=0.86-56.90) in the 13 homozygote DRB1*03xx-positive affected trios. Overall, having at least one HLA-DRB1*03xx allele (78 trios) increased the OR to 4.29 (CI=1.88-9.76), from OR = 2.00 (CI=1.17-3.42) in 113 homozygous DRB1*03xx-negative trios, thus supporting the evidence of an interaction between TAP2 and HLA-DRB1*03xx.

There was no evidence of an association between the SNPs in TAP1 and SLE status (Table 1). This may represent a true lack of association or it may reflect that the fact that the allele frequencies are low for the SNPs genotyped and the resulting magnitude of any association in TAP1 is less than we are powered to detect.

**Discussion**

Here we report the largest and most complete association study of the TAP genes with SLE to date, and one of the first studies of SLE to formally test for a gene-gene interaction. We have captured an average of 87% of the variation in TAP1 and found no evidence of association. In contrast, we captured an average of 68% of the variation within TAP2 and found strong association with a SNP that localizes in either an intron or the 3’UTR and mRNA of the gene, depending on the isoform (rs241453, \(P = 1.3 \times 10^{-6}\), O.R. = 2.39). Past studies of TAP2 association with SLE have focused on three specific amino-acids (V379I (rs1800454), A565T (rs2228396) and T665A (rs241447)), had modest sample sizes (~100-200 cases) in ethnically diverse populations and were collectively inconclusive.7,11 Of these three SNPs, only rs241447 was included in our analyses, and it yielded a modest
association. Interestingly, associations of TAP2 have also been reported with anti-Ro/SSA production.9,10

Our strongest effect was observed with rs241453, with an increased risk of SLE for individuals with the G allele ($P = 1.3 \times 10^{-6}$, O.R. = 2.39). In some of the predicted isoforms, this variant localizes to the 3’UTR and mRNA, and may thus be potentially important for the stability of the TAP2 transcript. Also, this SNP is in LD with nine other SNPs within TAP2, either intronic or in the 3’UTR. It is difficult to speculate about the function of the intronic SNPs, but having another 3’UTR SNP in strong LD ($r^2 = 1$) suggests that there is at least one potential effect in this part of the gene. The SNPs in high LD with these nine variants are also within TAP2. Interestingly, our next most significant effect, rs241447 ($P = 0.0021$) is not in LD with rs241453, suggesting that there may be multiple genetic effects within TAP2 that predispose to SLE.

Extensive LD patterns across the HLA region raised the possibility that association with rs241453 was driven by alleles in long range LD with rs241453. Previous studies have demonstrated increased risk for SLE in individuals carrying combinations of HLA-DRB1 haplotypes. Using the HapMap browser, we searched an extended region of 550 kb flanking TAP2 for SNPs in LD ($r^2 > 0.5$) with rs241453 and the two TAP2 SNPs in perfect LD with rs241453 (rs2857104 and rs241452). The region screened included HLA-DRB1 and HLA-DQB1, and we specifically looked at the tag SNPs that capture the HLA-DRB1*1501 and HLA-DRB1*0301 alleles.12 We did not find any SNPs in these loci to be in LD with the TAP2 SNPs. We did, however, find an intronic variant in HLA-DQA2 to be in LD with rs241453 ($r^2 = 0.76$). HLA-DQA2 is not a known risk factor for SLE, suggesting that the effect we found on TAP2 is separate from previously identified associations with other HLA genes. This result is supported by lack of evidence for LD found between the TAP genes and HLA-DR, -DP, and -DQ in Caucasians from southern Spain (the Andalusian population)13 and a large study of the MHC in rheumatoid arthritis that found an independent risk effect of TAP2.14 A possible explanation is the existence of a recombination hotspot near DQB1, between TAP2 and DRB1.15,16 We also performed a conditional logistic regression analysis to formally test for the autonomous effect of the significant TAP2 SNP. We found that its effect is indeed separate from the HLA alleles consistently associated with SLE (HLA-DR2, HLA-DR3 and HLA-DR8). Moreover, we found evidence of a potential interaction between rs241453 and HLA-DRB1*03xx, further supported by the fact that the OR for the TAP2 association increases in the subjects with at least one HLA-DRB1*03xx allele group. Few studies of SLE have formally tested for gene-gene interactions, and only one reports modest evidence for such between two cytokine genes.17 Our study is thus among the first studies of SLE that have formally tested and found a gene-gene interaction. Replication in an independent cohort is necessary to delineate the role of this polymorphism from that of the HLA haplotypes.

TAP2 has previously been found to be associated with anti-Ro/SSA production in two studies of 20 and 49 SLE patients, respectively.9,10 In our collection, we have 90 patients positive for this autoantibody, which has enough statistical power ($P > 0.80$) to detect associations at OR > 2 for allele frequencies greater than 25%. We did not detect statistically
The TAP2 gene is critical in the antigen processing pathway. Our study supports the hypothesis that TAP2 may predispose to SLE by influencing antigen presentation and that disease-associated variants are enriched in patients. Additional analyses will be important to replicate and expand this finding, as well as pinpoint the mechanism by which genetic variation might affect TAP2 function. Identification of the causative alleles will advance our understanding of the molecular mechanisms that lead to systemic autoimmune diseases, hopefully leading to the development of improved therapies.

Materials and Methods

Clinical Samples

Three hundred and ninety SLE pedigrees of self-reported Caucasian ethnicity were recruited as part of the family collection at the University of Minnesota. Family ascertainment, demographics, clinical features, genotyping, and Mendelian inheritance testing of the family collection has been described in detail elsewhere. All patients fulfilled the revised ACR criteria for SLE. This study was approved by the University of Minnesota Institutional Review Board for research on human subjects, and informed consent was obtained from all subjects enrolled in the study.

SNP Markers and Genotyping

Tag SNPs were chosen for the TAP genes using Tagger (HapMap Phase II (release 19; NCBI build 35) with the following settings: “aggressive” (multimarker mode), r^2 > 0.8 for allele and haplotype, allele frequency > 0.05, and HapMap CEU population Phase II data. These polymorphisms were genotyped by primer extension of multiplex products with detection by matrix-assisted laser desorption ionization-time of flight (MALDI-ToF) mass spectroscopy using a Sequenom platform. Only SNPs that met the following three criteria were used for association analysis: 1) Hardy-Weinberg P value ≥ 0.01, 2) more than 85% successful genotypes, and 3) no Mendelian errors. Pedigrees that showed Mendelian errors were eliminated, providing the 390 families used for the association analysis. Genotyping was performed for seven SNPs in TAP1 and ten SNPs in TAP2; one SNP in each gene failed to meet the above criteria and was rejected. In addition, 191 of these pedigrees had high-resolution four-digit HLA-DRB1 genotype data on the SLE affected offspring and at least one parent. DRB1 genotyping was performed by PCR-sequence-specific oligonucleotide (SSO) “linear array” methodology as previously described. Briefly, multiplex amplification of exon 2 of the DRB1 locus was performed with a set of 10 upstream PCR primers, corresponding to 10 sequence motifs in the first hypervariable region of the exon, and one common downstream primer. All primers were biotinylated, and resulting biotinylated PCR products were denatured and hybridized to a series of oligonucleotides, corresponding to known DRB1 sequence motifs, immobilized on a backed nylon membrane. Binding was detected by treatment of the membrane with streptavidin conjugated horseradish peroxidase followed by the colorimetric substrate tetramethylbenzidine. Probe binding was assessed using StripScan software (Roche Molecular Systems, Pleasanton, CA),
and genotype calls were made using the Sequence Compilation and Rearrangement Evaluation (SCORE) software.\textsuperscript{25} Allele calls were reported at four-digit resolution.

**Statistical Analysis**

**Family-based Association Analysis**—Standard Transmission Disequilibrium Test (TDT) and haplotype estimation of the TAP1 and TAP2 genes were performed using Haploview v4.0\textsuperscript{26} under default settings. The Pedigree Disequilibrium Test (PDT) was performed with UNPHASED using the EM option.\textsuperscript{27} The Odds Ratio (OR) for each SNP was calculated for the over-transmitted allele as the ratio of transmissions to non-transmissions.

**Conditional Logistic Regression Analysis**—We formally tested the hypothesis that the association observed with TAP2 is not due to LD, separate or autonomous from the HLA-\textit{DRB1} locus, namely the HLA-\textit{DRB1}\text{*15xx} (HLA-DR2), HLA-\textit{DRB1}\text{*03xx} (HLA-DR3) and HLA-\textit{DRB1}\text{*08xx} (HLA-DR8) allele groups, using conditional logistic regression.\textsuperscript{28} The models include cases with at least one genotyped parent, but we did not infer the transmissions of the ungenotyped parent. A total of 191 trios were in the conditional logistic regression analysis. We coded the high-resolution HLA-\textit{DRB1} alleles as binary in the following manner: HLA-\textit{DRB1}\text{*15xx} was coded as HLA-DR2 versus all the other HLA-\textit{DRB1} alleles, \textit{DRB1}\text{*03xx} as HLA-DR3 versus all the other HLA-\textit{DRB1} alleles, and \textit{DRB1}\text{*08xx} as HLA-DR8 versus all the other HLA-\textit{DRB1} alleles. We converted the allelic transmissions at each trio to units amenable to logistic regression as described.\textsuperscript{19} Briefly, for each marker, the transmission of the reference allele from each parent was coded as +1, the nontransmission as -1, or no effect as 0. For each of the three binary HLA-\textit{DRB1} alleles coded as described above, the conditional logistic model was computed with HLA-\textit{DRB1} and rs241453 loci in the model and with and without the interaction variable. The regression analysis was thus performed for rs241453 and HLA-\textit{DRB1}\text{*15xx}, rs241453 and HLA-\textit{DRB1}\text{*03xx}, and rs241453 and HLA-\textit{DRB1}\text{*08xx}, with and without the interaction term. We focused only on the three known HLA-\textit{DRB1} risk alleles for SLE and analyzed them separately so that we could evaluate their individual effects.

**Power analysis**—We used the Quanto software for our power analysis (see Quanto URL).

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Figure 1.
LD structure of TAP2. The nine genotyped SNPs are indicated by the red lines, numbered as shown in Table 1.
Table 1
Single Marker Family-Based Association and Conditional Logistic Regression Analysis of *TAP1* and *TAP2*.

| Gene | SNP         | Alleles | Single Marker Association | Conditional Analysis |
|------|-------------|---------|---------------------------|----------------------|
|      |             |         | OverT<sup>a</sup> T<sup>a</sup>:U<sup>a</sup> MAF<sup>a</sup> O.R. 95% C.I. TDT P value<sup>a</sup> PDT P value<sup>b</sup> HLA-DR2 P value<sup>c</sup> HLA-DR3 P value<sup>c</sup> HLA-DR8 P value<sup>c</sup> |
| 1    | TAP2        | rs241453| G:A                       | 98:41 0.201 2.39 1.66,3.44 1.3 × 10<sup>-6</sup> 2.2 × 10<sup>-4</sup> 1.3 × 10<sup>-5</sup> 1.6 × 10<sup>-4</sup> 6.0 × 10<sup>-6</sup> |
| 2    | TAP2        | rs241447| T:C                       | 149:102 0.240 1.46 1.14,1.88 0.0021 0.0035 |
| 3    | TAP2        | rs1015166| C:T                     | 158:131 0.348 1.21 0.96,1.52 0.101 0.053 |
| 4    | TAP2        | rs183585| G:A                       | 143:108 0.246 1.32 1.03,1.70 0.020 0.049 |
| 5    | TAP2        | rs4148873| C:T                     | 77:73 0.126 1.05 0.77,1.45 0.744 0.611 |
| 6    | TAP2        | rs3819714| G:A                       | 149:114 0.332 1.31 1.02,1.67 0.027 0.106 |
| 7    | TAP2        | rs241426| A:T                       | 153:139 0.380 1.10 0.87,1.38 0.413 0.696 |
| 8    | TAP2        | rs17583244| T:C                   | 44:33 0.058 1.33 0.85,2.09 0.251 0.301 |
| 9    | TAP2        | rs241425| G:A                       | 133:121 0.410 1.10 0.86,1.41 0.452 0.582 |
| 10   | TAP1        | rs6457684| T:C                       | 153:142 0.432 1.08 0.86,1.35 0.522 0.936 |
| 11   | TAP1        | rs1800453| T:C                       | 80:74 0.140 1.08 0.79,1.48 0.629 0.620 |
| 12   | TAP1        | rs4148882| A:G                       | 139:134 0.377 1.04 0.82,1.32 0.762 0.807 |
| 13   | TAP1        | rs2127679| G:A                       | 80:74 0.140 1.08 0.79,1.48 0.629 0.620 |
| 14   | TAP1        | rs4148880| T:C                       | 91:80 0.161 1.14 0.84,1.54 0.400 0.103 |
| 15   | TAP1        | rs4148879| G:A                       | 68:51 0.105 1.33 0.93,1.92 0.119 0.121 |

SNPs ordered by position along chromosome 6.

<sup>a</sup>Transmission Disequilibrium Test,

<sup>b</sup>Pedigree Disequilibrium Test,

<sup>c</sup>P-value for rs241453 after conditioning on each HLA-DRB1 allele group: HLA-DRB1*15xx (HLA-DR2), HLA-DRB1*03xx (HLA-DR3) or HLA-DRB1*08xx (HLA-DR8). OverT – over-transmitted allele; T - Transmission of the OverT allele; U - Under(non)-transmission of the OverT allele; O.R. – Odds Ratio of the over-transmitted allele (as given by T/U); C.I. – Confidence Interval.
Table 2

HapMap LD (as measured by the $r^2$) between rs241453 on TAP2 and selected SNPs.

| SNP        | Location      | rs241453 (TAP2) |
|------------|---------------|-----------------|
| rs241452   | TAP2          | 1.00            |
| rs2857104  | TAP2          | 1.00            |
| rs241442   | TAP2          | 0.82            |
| rs17500482 | HLA-DQA2      | 0.76            |
| rs3135388  | DR2tag\(^a\)  | 0.01            |
| rs2040410  | DR3tag\(^a\)  | 0.014           |

\(^a\) tag SNPs that capture the HLA-DRB1*1501 (DR2tag) and HLA-DRB1*0301 (DR3tag) alleles according to de Bakker et al.\(^{12}\)