Published in final edited form as:

Genes Immun. 2009 June ; 10(4): 350–355. doi:10.1038/gene.2009.21.

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

Paula S. Ramos[1], **Carl D. Langefeld**[1], **LeeAnn Bera**[2], **Patrick M. Gaffney**[3], **Janelle A. Noble**[4], and **Kathy L. Moser**[3]

[1]Paula S. Ramos, PhD, Carl D. Langefeld, PhD: Section on Statistical Genetics and Bioinformatics, Department of Biostatistical Sciences and Center for Public Health Genomics, Division of Public Health Sciences, Wake Forest University Health Sciences, Winston-Salem, North Carolina, USA

[2]LeeAnn Bera, MS: Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota, USA

[3]Patrick M. Gaffney, MD, Kathy L. Moser, PhD: Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA

[4]Janelle A. Noble, PhD: Children's Hospital Oakland Research Institute, Oakland, California, USA

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.

Address correspondence and reprint requests to: Kathy Moser, Ph.D, Oklahoma Medical Research Foundation, Arthritis and Immunology Program, 755 Research Parkway, Suite 540, Oklahoma City, OK 73104, USA. Phone: 405-271-2534; Fax: 405-271-3045; moserk@omrf.org.

URLs: International HapMap project: http://www.hapmap.org/, Release 21a/PhaseII Jan2007, dbSNP b125
Quanto: http://hydra.usc.edu/gxe
SAS: http://www.sas.com
UCSC Genome Browser: http://genome.ucsc.edu/, March 2006 freeze

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).[1] Although the exact pathogenesis of SLE is unknown, multiple lines of evidence demonstrate that SLE susceptibility in humans is strongly influenced by genetic factors.[2]

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.[3]

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.[4,5] The TAP proteins have some specificity for the peptides they will transport.[6]

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.[7-11] 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 *TAP2* 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*0301*[12] 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 ($P = 1.8 \times 10^{-5}$, OR = 2.56). The *HLA-DRB1*03xx* allele group was also significant ($P = 1.4 \times 10^{-6}$, 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* alelles.[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

significant evidence for association to anti-Ro/SSA. Future work, including larger sample sizes, is warranted to more fully evaluate this possibility.

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.[18,19] All patients fulfilled the revised ACR criteria for SLE.[20] 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[21] (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.[22] 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.[23,24] 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 tetramethlybenzidine. 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.[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[26] under default settings. The Pedigree Disequilibrium Test (PDT) was performed with UNPHASED using the EM option.[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-DRB1* locus, namely the *HLA-DRB1*15xx* (*HLA-DR2*), *HLA-DRB1*03xx* (*HLA-DR3*) and *HLA-DRB1*08xx* (*HLA-DR8*) allele groups, using conditional logistic regression.[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-DRB1* alleles as binary in the following manner: *HLA-DRB1*15xx* was coded as *HLA-DR2* versus all the other *HLA-DRB1* alleles, *DRB1*03xx* as *HLA-DR3* versus all the other *HLA-DRB1* alleles, and *DRB1*08xx* as *HLA-DR8* versus all the other *HLA-DRB1* alleles. We converted the allelic transmissions at each trio to units amenable to logistic regression as described.[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-DRB1* alleles coded as described above, the conditional logistic model was computed with *HLA-DRB1* and rs241453 loci in the model and with and without the interaction variable. The regression analysis was thus performed for rs241453 and *HLA-DRB1*15xx*, rs241453 and *HLA-DRB1*03xx*, and rs241453 and *HLA-DRB1*08xx*, with and without the interaction term. We focused only on the three known *HLA-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).

Acknowledgments

We thank the families and their referring physicians for their participation in this study. We are grateful for Lindsey A. Criswell and Lisa F. Barcellos' support. We would like to acknowledge Julie A. Lane for generating the *HLA-DRB1* genotyping results, and Lingyi Lu and Miranda Marion for assistance with programming. This research was supported by NIH/NIAMS R01 AR043274 (KLM), R01 AR052300 (LAC, LFB), Lupus Foundation of Minnesota (KLM) and the Center for Public Health Genomics at Wake Forest University Health Sciences (CDL).

References

1. Helmick CG, Felson DT, Lawrence RC, Gabriel S, Hirsch R, Kwoh CK, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. Arthritis Rheum. 2008; 58:15–25. [PubMed: 18163481]
2. Harley JB, Alarcon-Riquelme ME, Criswell LA, Jacob CO, Kimberly RP, Moser KL, et al. Genome-wide association scan in women with systemic lupus erythematosus identifies

susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. Nat Genet. 2008; 40:204–210. [PubMed: 18204446]

3. Graham RR, Ortmann WA, Langefeld CD, Jawaheer D, Selby SA, Rodine PR, et al. Visualizing human leukocyte antigen class II risk haplotypes in human systemic lupus erythematosus. Am J Hum Genet. 2002; 71:543–553. [PubMed: 12145745]
4. Huang AY, Bruce AT, Pardoll DM, Levitsky HI. In vivo cross-priming of MHC class I-restricted antigens requires the TAP transporter. Immunity. 1996; 4:349–355. [PubMed: 8612129]
5. Malnati MS, Marti M, LaVaute T, Jaraquemada D, Biddison W, DeMars R, et al. Processing pathways for presentation of cytosolic antigen to MHC class II-restricted T cells. Nature. 1992; 357:702–704. [PubMed: 1614517]
6. Kageyama G, Kawano S, Kanagawa S, Kondo S, Sugita M, Nakanishi T, et al. Effect of mutated transporters associated with antigen-processing 2 on characteristic major histocompatibility complex binding peptides: analysis using electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom. 2004; 18:995–1000. [PubMed: 15116427]
7. Correa PA, Molina JF, Pinto LF, Arcos-Burgos M, Herrera M, Anaya JM. TAP1 and TAP2 polymorphisms analysis in northwestern Colombian patients with systemic lupus erythematosus. Ann Rheum Dis. 2003; 62:363–365. [PubMed: 12634240]
8. Davies EJ, Donn RP, Hillarby MC, Grennan DM, Ollier WE. Polymorphisms of the TAP2 transporter gene in systemic lupus erythematosus. Ann Rheum Dis. 1994; 53:61–63. [PubMed: 8311559]
9. Kanagawa S, Morinobu A, Koshiba M, Kageyama G, Hayashi N, Yoshino S, et al. Association of the TAP2*Bky2 allele with presence of SS-A/Ro and other autoantibodies in Japanese patients with systemic lupus erythematosus. Lupus. 2003; 12:258–265. [PubMed: 12729048]
10. Martin-Villa JM, Martinez-Laso J, Moreno-Pelayo MA, Castro-Panete MJ, Martinez-Quiles N, Alvarez M, et al. Differential contribution of HLA-DR, DQ, and TAP2 alleles to systemic lupus erythematosus susceptibility in Spanish patients: role of TAP2*01 alleles in Ro autoantibody production. Ann Rheum Dis. 1998; 57:214–219. [PubMed: 9709177]
11. Takeuchi F, Nakano K, Nabeta H, Hong GH, Kuwata S, Ito K. Polymorphisms of the TAP1 and TAP2 transporter genes in Japanese SLE. Ann Rheum Dis. 1996; 55:924–926. [PubMed: 9014588]
12. de Bakker PI, McVean G, Sabeti PC, Miretti MM, Green T, Marchini J, et al. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. Nat Genet. 2006; 38:1166–1172. [PubMed: 16998491]
13. Alvarado-Guerri R, Cabrera CM, Garrido F, Lopez-Nevot MA. TAP1 and TAP2 polymorphisms and their linkage disequilibrium with HLA-DR, -DP, and -DQ in an eastern Andalusian population. Hum Immunol. 2005; 66:921–930. [PubMed: 16216677]
14. Lee HS, Lee AT, Criswell LA, Seldin MF, Amos CI, Carulli JP, et al. Several regions in the major histocompatibility complex confer risk for anti-CCP-antibody positive rheumatoid arthritis, independent of the DRB1 locus. Mol Med. 2008; 14:293–300. [PubMed: 18309376]
15. Cullen M, Perfetto SP, Klitz W, Nelson G, Carrington M. High-resolution patterns of meiotic recombination across the human major histocompatibility complex. Am J Hum Genet. 2002; 71:759–776. [PubMed: 12297984]
16. Miretti MM, Walsh EC, Ke X, Delgado M, Griffiths M, Hunt S, et al. A high-resolution linkage-disequilibrium map of the human major histocompatibility complex and first generation of tag single-nucleotide polymorphisms. Am J Hum Genet. 2005; 76:634–646. [PubMed: 15747258]
17. Parks CG, Pandey JP, Dooley MA, Treadwell EL, St C E, Gilkeson GS, et al. Genetic polymorphisms in tumor necrosis factor (TNF)-alpha and TNF-beta in a population-based study of systemic lupus erythematosus: associations and interaction with the interleukin-1alpha-889 C/T polymorphism. Hum Immunol. 2004; 65:622–631. [PubMed: 15219382]
18. Gaffney PM, Kearns GM, Shark KB, Ortmann WA, Selby SA, Malmgren ML, et al. A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. Proc Natl Acad Sci U S A. 1998; 95:14875–14879. [PubMed: 9843983]

19. Gray-McGuire C, Moser KL, Gaffney PM, Kelly J, Yu H, Olson JM, et al. Genome scan of human systemic lupus erythematosus by regression modeling: evidence of linkage and epistasis at 4p16-15.2. Am J Hum Genet. 2000; 67:1460–1469. [PubMed: 11078476]
20. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997; 40:1725. [PubMed: 9324032]
21. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Genet. 2005; 37:1217–1223. [PubMed: 16244653]
22. O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet. 1998; 63:259–266. [PubMed: 9634505]
23. Bugawan TL, Apple R, Erlich HA. A method for typing polymorphism at the HLA-A locus using PCR amplification and immobilized oligonucleotide probes. Tissue Antigens. 1994; 44:137–147. [PubMed: 7839345]
24. Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, et al. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. Diabetes. 2008; 57:1084–1092. [PubMed: 18252895]
25. Helmberg W, Lanzer G, Zahn R, Weinmayr B, Wagner T, Albert E. Virtual DNA analysis--a new tool for combination and standardised evaluation of SSO, SSP and sequencing-based typing results. Tissue Antigens. 1998; 51:587–592. [PubMed: 9694350]
26. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]
27. Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol. 2003; 25:115–121. [PubMed: 12916020]
28. Harley JB, Moser KL, Neas BR. Logistic transmission modeling of simulated data. Genet Epidemiol. 1995; 12:607–612. [PubMed: 8787981]

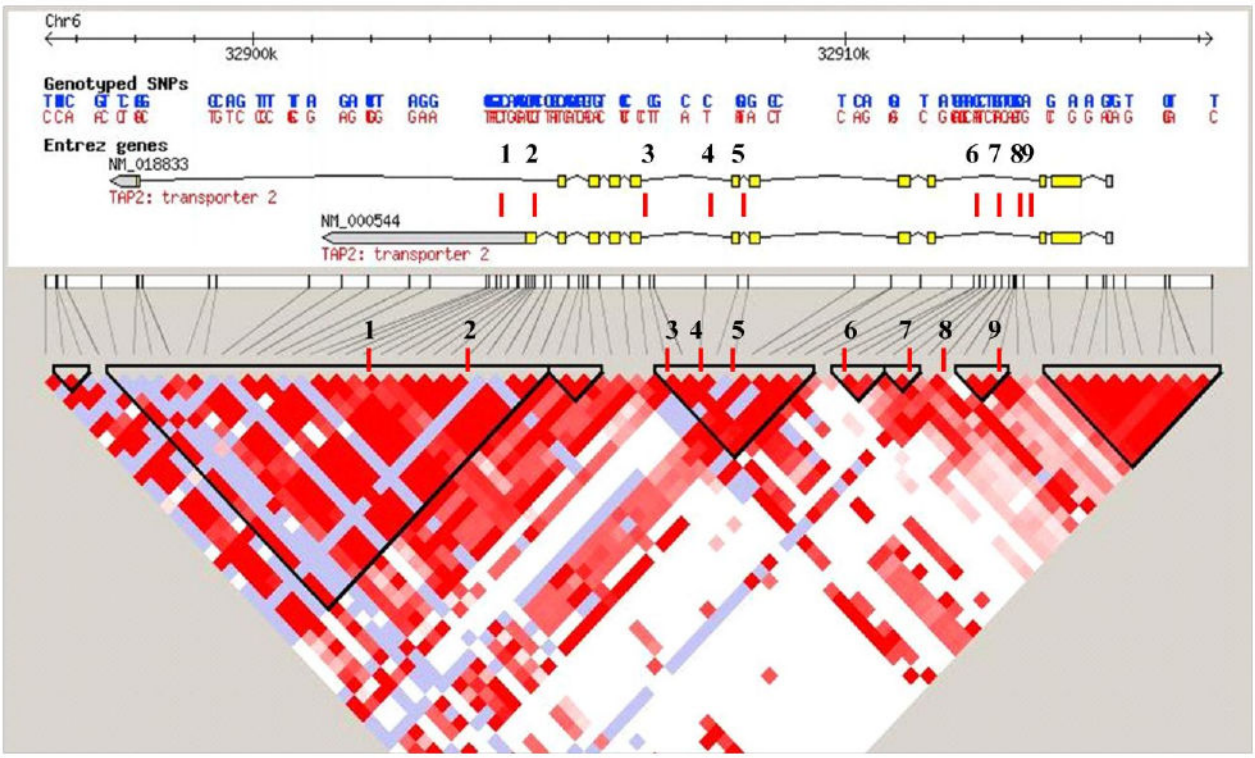

Figure 1.
LD structure of *TAP2*. The nine genotyped SNPs are indicated by the red lines, numbered as shown in Table 1.

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

SNPs ordered by position along chromosome 6.

[a] Transmission Disequilibrium Test,

[b] Pedigree Disequilibrium Test,

[c] *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) alelles according to de Bakker *et al.*[12]