# Evidence for Genetic Association and Interaction Between the *TYK2* and *IRF5* Genes in Systemic Lupus Erythematosus

ANNA HELLQUIST, TIINA M. JÄRVINEN, SARI KOSKENMIES, MARCO ZUCCHELLI, CHRISTINA ORSMARK-PIETRAS, LINDA BERGLIND, JAANA PANELIUS, TAINA HASAN, HEIKKI JULKUNEN, MAURO D'AMATO, ULPU SAARIALHO-KERE, and JUHA KERE

***ABSTRACT.*** ***Objective***. Several candidate genes have been implicated in susceptibility for systemic lupus erythematosus (SLE), a complex autoimmune disease. The proposed genes include members of the type I interferon (IFN) pathway and genes involved in immunological defense functions. Our aim was to systematically replicate 6 such genes, *TYK2*, *IRF5*, *CTLA4*, *PDCD1*, *FCGR2A*, and *NOD2*.

***Methods.*** Single-nucleotide polymorphisms in *TYK2*, *IRF5*, *CTLA4*, *PDCD1*, *FCGR2A*, and *NOD2* were genotyped in 277 SLE patients and 356 healthy controls from Finland, giving a power of 42%–70% for different genes at published allele frequencies.

***Results.*** Significant association was seen for rs2304256 (p = 0.0001) and rs12720270 (p = 0.0031) in *TYK2* and rs10954213 (p = 0.0043) in *IRF5* in our samples, but not for the other genes. We found evidence for genetic interaction (p = 0.014) between rs2304256 in *TYK2* and rs10954213 in *IRF5*, both members of the type I IFN pathway, strengthening the role of the type I IFN pathway in the pathogenesis of SLE.

***Conclusion.*** The IFN pathway genes *IRF5* and *TYK2* may act epistatically in increasing risk for SLE, but our lack of replication does not exclude effects of the other genes studied. (First Release July 1 2009; J Rheumatol 2009;36:1631–8; doi:10.3899/jrheum.081160)

*Key Indexing Terms:*

| | | |
|---|---|---|
| SYSTEMIC LUPUS ERYTHEMATOSUS | GENETIC ASSOCIATION | *IRF5* |
| GENETIC INTERACTION | DISEASE SUSCEPTIBILITY | *TYK2* |

Systemic lupus erythematosus (SLE) is a complex autoimmune disease, characterized by production of pathogenic autoantibodies against nuclear antigens due to a breakdown in self-tolerance. This subsequently leads to the formation of immune complexes, followed by tissue inflammation in multiple organs, such as the skin, joints, heart, and kidneys. As a result, individuals with SLE have a wide range of clinical manifestations and the diagnosis of SLE is therefore based on

*From the Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; Department of Dermatology, Helsinki University Central Hospital and Biomedicum Helsinki, University of Helsinki, Helsinki; Department of Medical Genetics, University of Helsinki, and Folkhälsan Institute of Genetics, Helsinki; Helsinki Biomedical Graduate School LERU PhD Program in Biomedicine, Helsinki, Finland; Clinical Research Centre, Karolinska University Hospital, Huddinge, Sweden; Department of Dermatology, University of Tampere and Tampere University Hospital, Tampere; Department of Rheumatology, Helsinki University Central Hospital, Peijas Hospital, Vantaa, Finland; and Section of Dermatology, and Department of Clinical Science and Education, Karolinska Institutet at Stockholm Söder Hospital, Stockholm, Sweden.*

*Supported by Finska Läkaresällskapet, Academy of Finland, Sigrid Jusélius Foundation and Helsinki University Central Hospital Research Fund (project TYH 2008248), Helsinki University Research Foundation and Biomedicum Helsinki Foundation and the Finnish Union of Experts in Science (TMJ), Medical Research Fund of Tampere University Hospital (TH), and the Swedish Research Council (US-K).*

*A. Hellquist, MSc, Department of Biosciences and Nutrition, Karolinska Institutet; T.M. Järvinen, MSc, Department of Biosciences and Nutrition, Karolinska Institutet, Department of Dermatology, Helsinki University Central Hospital and Biomedicum Helsinki, Department of Medical Genetics, University of Helsinki, Folkhälsan Institute of Genetics, Helsinki Biomedical Graduate School LERU PhD Program in Biomedicine; S. Koskenmies, MD, PhD, Department of Dermatology, Helsinki University Central Hospital and Biomedicum Helsinki, Department of Medical Genetics, University of Helsinki, Folkhälsan Institute of Genetics; M. Zucchelli, PhD; C. Orsmark-Pietras, MSc, Department of Biosciences and Nutrition, Karolinska Institutet; L. Berglind, MSc, Clinical Research Centre, Karolinska University Hospital; J. Panelius, MD, PhD, Department of Dermatology, Helsinki University Central Hospital and Biomedicum Helsinki, University of Helsinki; T. Hasan, MD, PhD, Department of Dermatology, University of Tampere and Tampere University Hospital; H. Julkunen, MD, PhD, Department of Rheumatology, Helsinki University Central Hospital, Peijas Hospital; M. D'Amato, PhD, Department of Biosciences and Nutrition, Karolinska Institutet; U. Saarialho-Kere, MD, PhD, Professor, Department of Dermatology, Helsinki University Central Hospital and Biomedicum Helsinki, University of Helsinki; J. Kere, MD, PhD, Professor, Department of Biosciences and Nutrition, Karolinska Institutet, Department of Medical Genetics, University of Helsinki, Folkhälsan Institute of Genetics.*

*A. Hellquist and T.M. Järvinen contributed equally to this report.*

*Address correspondence to Prof. J. Kere, Department of Biosciences and Nutrition, Karolinska Institutet, Hälsovägen 7-9, S-141 57 Huddinge, Sweden. E-mail: juha.kere@ki.se*

*Accepted for publication March 5, 2009.*

criteria set by the American College of Rheumatology (ACR)[1,2]. SLE has a low prevalence (12–64 cases per 100,000 in European-derived populations)[3] and occurs primarily in women of childbearing age[4]. Although the underlying pathogenic mechanisms of SLE remain unclear, the disease is complex and involves multiple genes and aggravating environmental components, such as ultraviolet radiation, certain drugs, and estrogen[5-7]. The genetic component in SLE is strong, with familial aggregation studies showing a sibling risk ratio (λs) of 20–29[6,8]. Twin studies further support a strong genetic component, with a 10-fold concordance ratio of affected monozygotic twins (24%–58%) over dizygotic twins (2%–5%)[5].

Recently, our understanding of SLE genetics has improved with the identification of several novel susceptibility genes. Programmed cell death 1 (*PDCD1*) and cytotoxic T-lymphocyte-associated protein 4 (*CTLA4*), among others, have been associated with susceptibility to SLE in several studies, but require more conclusive evidence to establish their role in SLE pathogenesis[9,10]. The nucleotide-binding oligomerization domain 2/caspase activation recruitment domain 15 (*NOD2/CARD15*) gene, implicated in inflammatory bowel disease, has been suggested to have a role in SLE[11].

The importance of the type I interferon (IFN) pathway in SLE pathogenesis has become evident in several studies, including 3 recent high-density genome-wide association (GWA) studies that showed strong association with 2 genes in this pathway: interferon regulatory factor 5 (*IRF5*)[12-17] and signal transducer and activator of transcription 4 (*STAT4*)[14-16,18,19]. Further, tyrosine kinase 2 (*TYK2*) has shown association with SLE in 2 reports[12,20], but not in any of the 3 high-density GWA studies[14-16]. In addition, the Fc receptor 2A (*FCGR2A*) and protein tyrosine phosphatase nonreceptor-type 22 (*PTPN22*) could also be regarded as relatively certain risk factors in SLE susceptibility given their confirmation in one GWA study[15] and several other studies as reviewed[9,10]. There are many reasons why true susceptibility genes may remain without replication in GWA studies, and therefore replication studies of suggested genes continue to be relevant.

Our aim was to investigate systematically whether certain susceptibility genes implicated in the recent SLE literature can be replicated in the Finnish population, and also to examine genetic interactions between the possibly replicated genes.

## MATERIALS AND METHODS

*Patients and controls*. Finnish patients with SLE from 2 separate collections were included in this study. The majority were recruited together with their families as described[21]. All SLE patients included in the family group were interviewed by the same physician, and case records from hospitals where the patients were treated were reviewed by permission of the study participants. All patients met the ACR criteria for the diagnosis of SLE[2]. Blood samples from patients and their relatives were obtained from a total of 192 families. Clinical characteristics of the SLE patients included in this collection are described in Table 1.

An additional collection of SLE patients has been described[22]. Briefly, all patients with clinical diagnosis of SLE attending the Departments of Dermatology at Helsinki or Tampere University Central Hospitals during 1995-2005, and visiting these hospitals for routine lupus controls during 2005-2006, were recruited. The presence of LE-specific skin manifestations diagnosed by a dermatologist at some point during the disease course was a prerequisite for inclusion in the study. Case hospital records were scrutinized to confirm correct clinical diagnosis; the diagnosis of SLE was based on the ACR criteria[2], always verified by a rheumatologist, and included the presence of systemic manifestations together with positive serology and skin manifestations. Participating patients were clinically examined by the investigating doctors (SK, TH, JP) and interviewed using a structured questionnaire. Altogether 85 patients with SLE participated in the study; clinical characteristics are given in Table 1. Unaffected unrelated family members (spouses and common-law spouses) were asked to participate as control individuals and an existing collection of unrelated Finnish individuals was also used, giving a total of 356 controls included in the study.

All subjects gave written informed consent for participation in genetic studies on SLE and the study protocols were reviewed and approved by local ethical committees (Karolinska Institutet, University of Helsinki, and The Ethical Review Boards of Helsinki and Tampere University Central Hospitals).

To obtain the maximal statistical power of association analysis, the probands (n = 192) from each family with SLE were added to the SLE case-control collection, thus giving a total of 277 SLE patients and 356 controls for analysis. Part of the family group, consisting of 109 SLE patients and 121 unaffected pedigree members, had been included earlier in a study of *IRF5* (with a different single-nucleotide polymorphism, however), *TYK2*, *FCGR2A*, and *PDCD1*[12], and thus

*Table 1*. Clinical characteristics of the 2 SLE sample collections. Data are percentages unless otherwise indicated.

| | Family Data, n = 236 | Case-Control Data, n = 85 |
|---|---|---|
| Female, yrs | 94 | 93 |
| Mean age at onset (range) | 29 (1–66) | 36 (8–85) |
| Mean age at diagnosis, yrs (range) | 33 (6–72) | 40 (13–89) |
| Butterfly rash | 51 | 72 |
| Discoid rash | 10 | 44 |
| Photosensitivity | 69 | 80 |
| Mouth ulcers | 18 | 18 |
| Arthritis | 83 | 64 |
| Nephritis | 30 | 20 |
| Leukopenia | 68 | 37 |
| Thrombocytopenia | 16 | 17 |

for these 4 genes, the current study is only in part independent. As an association to the *IRF5* and *TYK2* genes was observed previously in a smaller dataset, the expanded dataset serves as a comparison for associations for *FCGR2A* and *PDCD1*.

*Single-nucleotide polymorphism (SNP) selection*. A subset of our Finnish family group, consisting of 109 SLE patients and 121 unaffected pedigree members, was included in the original report of *TYK2* and *IRF5* association with SLE, showing significant association to several SNP[12]. Motivated by this collaborative work on *TYK2* and *IRF5*[12] and after careful review of the recent SLE literature preceding GWA studies[14-16], the following SNP were selected for genotyping: rs12720270, rs2304256 and rs12720356 in *TYK2*, rs2004640 and rs10954213 in *IRF5*, rs1801274 in *FCGR2A*, rs231775, rs3087243, rs231726, rs231727 and rs1991416 in *CTLA4*, rs11568821 in *PDCD1*, rs2076756, rs2066843 and rs2066845 in *NOD2*, and rs2476601 in *PTPN22*. All these SNP have shown either significant linkage or association with SLE[11-13,20,23-29].

Out of the genotyped markers, rs2004640 in *IRF5*, rs2066843 in *NOD2*, and rs2476601 in *PTPN22* failed to meet our genotyping quality criteria (described below) and were thus excluded from further analyses. The *NOD2* SNP rs2066845 was found to be monomorphic in our sample set (minor allele frequency < 0.5%) and was excluded as well.

*DNA extraction and genotyping*. Genomic DNA was extracted from whole-blood samples by standard nonenzymatic methods (Flexigene DNA kit, Qiagen GmbH, Hilden, Germany, or Puregene blood kit, Gentra Systems Inc., Minneapolis, MN, USA). All SNP genotyping was performed at the Mutation Analysis Facility at Karolinska Institutet, Huddinge, Sweden (www.maf.ki.se) using the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry iPLEX method[30] (Sequenom Inc., San Diego, CA, USA; www.sequenom.com) according to the manufacturer's instructions. Assays for all SNP were designed using SpectroDesigner software (Sequenom). The resulting mass spectra were analyzed for peak identification using the SpectroTyper RT 3.3.0 software (Sequenom).

The genotyping of each assay was initially validated by comparing genotype concordance from the genotyping on our platform in a set of 14 CEU trios (CEPH Utah residents with ancestry from northern and western Europe), with genotypes available through the HapMap consortium (www.hapmap.org). Perfect concordance was shown for all markers with data available in the HapMap database, with the exception of rs2066843 in *NOD2* and rs200464 in *IRF5*, which also had low success rates (< 85%) and were thus excluded from further genotyping. For additional markers, internal concordance was analyzed on 14 unrelated individuals of Caucasian descent, showing perfect concordance. All markers were in Hardy-Weinberg equilibrium when analyzed in a total of 55 unrelated individuals from the described validation sample sets.

In the genotyping of the study sample set, the success rate of genotyped SNP was over 90% for all markers, with the exception of rs2476601 in *PTPN22*. Hardy-Weinberg calculations were performed in controls to ensure that each marker was in equilibrium also in the study sample set. In the family material, PedCheck[31] was used to detect Mendelian inconsistencies, and markers deviating > 10% from expected were excluded from the analysis. Corresponding SNP in *FCGR2A*, *PDCD1*, and *TYK2* had previously been genotyped in a subset of the family material[12] and the concordance between the 2 different methods was over 97% for all markers.

*Statistical analyses*. Haploview program v. 4.0[32] was used to investigate the descriptive genotype data: to assess linkage disequilibrium through pairwise D′ and $r^2$ values and allele and haplotype frequencies as well as construct haplotypes. Allelic and haplotypic associations were investigated using a chi-square test, and 2-tailed odds ratios (OR) with their corresponding 95% confidence intervals were calculated with GraphPad Prism v. 4.03 (GraphPad Software, La Jolla, CA, USA). As this was a replication study, nominal p values < 0.05 were considered significant. The observed associations were confirmed by the permutation approach (10,000 iterations) to obtain empirical p value.

A multiple logistic regression model was used to estimate the interactive effects of the SNP in *TYK2* and *IRF5* by adding an interaction term between the genotypes of interest (Stata software, v. 8.0; Stata, College Station, TX, USA), as described[33-36]. A model free coding with variables for common homozygote, heterozygote, and rare homozygote was used as a first approach. For SNP with too few rare homozygotes, dominant coding was used. P values estimate a departure from a multiplicative interaction model on the OR scale indicating whether the effect (OR) of one genotype is altered by the effects of another genotype. P values were obtained by likelihood-ratio tests between the models with and without interaction term. A logistic regression model was used to estimate the additive joint effects of the 4 SNP in *TYK2* and *IRF5*. Due to the strong linkage disequilibrium between the *TYK2* SNP, the 3 markers were included in the analysis as a haplotype. Patients and controls were divided into subgroups according to risk allele counts, and individuals carrying zero or one risk allele were used as reference group. Analyses were performed using SPSS v. 15.0 (SPSS Inc., Chicago, IL, USA). Nominal p values < 0.05 were considered significant.

To study any association between associated *TYK2* and *IRF5* SNP and clinical features, the SLE patients were stratified according to clinical characteristics: elevated antinuclear antibodies, elevated extractable nuclear antibodies (probands were not included), Ro/SSA antibody positivity (because of the differences in laboratory methods and reference values, only patients from Helsinki were included), double-stranded DNA, low complement 4 values (probands were not included), leukopenia, lymphopenia, thrombocytopenia, anemia (probands were not included), renal involvement, arthritis,

serositis (probands were not included), and self-reported photosensitivity. Test variables were selected based on reported associations[37,38] and our clustering results[22]; the most important differentiating variables between clusters were used. The association between risk-allele carrier status and each clinical variable was examined using a chi-square or Fisher exact test, when appropriate. Analyses were done with SPSS v. 15.0; 2-tailed p values < 0.05 after Bonferroni multiple testing correction were considered significant.

The statistical power of the study sample (n = 277 + 356) using reported minor allele frequencies in the Finnish patients[12] at significance level 0.05 varied from 42% in *FCGR2A* up to 70% in *TYK2*. The statistical power was estimated with the Power for Association With Error program v. 1.2 (http://linkage.rockefeller.edu/pawe/).

## RESULTS

*TYK2 and IRF5 showed strong association to SLE*. SNP in *TYK2*, *IRF5*, *FCGR2A*, *CTLA4*, *PDCD1*, and *NOD2* were successfully genotyped in accord with our quality criteria and subsequently analyzed. Significant single-marker association with SLE in the Finnish population was found in *TYK2* and *IRF5*, while none of the other genes tested reached the level of significant association (Table 2). In *TYK2*, the C allele of rs2304256 (p = 0.0001, OR 1.68, 95% CI 1.29–2.18) and the G allele of rs12720270 (p = 0.0031, OR 1.57, 95% CI 1.16–2.21) showed significant association (Table 2). In *IRF5* the A allele of rs10954213 (p = 0.0043, OR 1.42, 95% CI 1.12–1.81) showed significant association (Table 2). Haplotype association analysis was done for *TYK2* and *CTLA4*, of which the first showed association to 2 separate haplotypes: GCA (p = 0.0002, OR 1.63, 95% CI 1.26–2.12) and AAA (p = 0.0030, OR 0.64, 95% CI 0.47–0.86) (Table 3).

*TYK2 and IRF5 showed signs of gene-gene interaction*. A possible gene-gene interaction between SNP in *TYK2* and *IRF5* was studied based on the biological basis that they both are members of the type I IFN pathway and thus might increase the risk for SLE disproportionately by multiple risk alleles. Significant overall interaction could be observed between rs10954213 in *IRF5* and rs2304256 in *TYK2* (p = 0.014), where the risk alleles for rs10954213 in *IRF5* (AA) and rs2304256 in *TYK2* [CC (valine)] contributed the most to the overall interaction (p < 0.0001, OR 2.73, 95% CI 1.73–4.30). The remaining combinations were nonsignificant (rs10954213 AA vs rs2304256 AA/AC, p = 0.62, OR 1.14, 95% CI 0.69–1.89; and rs10954213 GG/AG vs rs2304256 CC, p = 0.10, OR 1.43, 95% CI 0.93–2.20; Figure 1). A tendency for interaction could be observed between rs12720270 and rs10954213 (p = 0.096) as well as rs12720356 and rs10954213 (p = 0.083).

The disease risk was further analyzed by studying the effect of carrying multiple risk factors. The risk of SLE increases as a function of the number of risk factors (Table 4). The OR for SLE is 2.44 (95% CI 1.22–4.85) for carriers of 2 risk alleles and 4.55 (95% CI 2.25–9.22) for carriers of 4 risk alleles compared to those with zero or one risk allele.

*No evidence of association between TYK2, IRF5, and clinical variables*. To investigate whether there was an association between associated SNP and clinical characteristic for SLE, the material was stratified by various parameters and the association between risk allele carrier status and each parameter was examined. No variable tested showed significant association to risk allele carrier status.

## DISCUSSION

We analyzed a panel of SNP previously implicated in SLE susceptibility for association in Finnish population samples, and found a significant association to *TYK2* and *IRF5*. This replication is not completely independent of previous studies, as a subset of our sample was studied earlier for the same genes, but with partly different markers[12]. As these genes thus could be considered positive controls for association in our

*Table 2*. Single-marker association results.

| Gene | SNP | Chromosome | SNP Alleles | Associated Allele | Case Allele Ratio Count | Control Allele Ratio Count | Case Frequency | Control Frequency | Chi-square | p | OR (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FCGR2A | rs1801274 | 1 | A/G | G | 288:242 | 345:343 | 0.54 | 0.50 | 2.11 | 0.15 | |
| CTLA4 | rs231775 | 2 | A/G | A | 263:249 | 333:343 | 0.51 | 0.49 | 0.52 | 0.47 | |
| CTLA4 | rs3087243 | 2 | A/G | G | 344:178 | 449:237 | 0.66 | 0.66 | 0.03 | 0.87 | |
| CTLA4 | rs231726 | 2 | C/T | C | 296:234 | 374:318 | 0.56 | 0.54 | 0.39 | 0.53 | |
| CTLA4 | rs231727 | 2 | A/G | G | 296:236 | 374:318 | 0.56 | 0.54 | 0.31 | 0.58 | |
| CTLA4 | rs1991416 | 2 | A/G | A | 291:235 | 368:322 | 0.55 | 0.53 | 0.48 | 0.49 | |
| PDCD1 | rs1168821 | 2 | C/T | C | 499:27 | 632:54 | 0.95 | 0.92 | 3.58 | 0.06 | |
| IRF5 | rs10954213 | 7 | A/G | A | 367:161 | 424:264 | 0.70 | 0.62 | 8.16 | 0.0043* | 1.42 (1.12–1.81) |
| NOD2 | rs2076756 | 16 | A/G | A | 449:87 | 589:101 | 0.84 | 0.85 | 0.59 | 0.44 | |
| TYK2 | rs12720270 | 19 | A/G | G | 437:79 | 528:150 | 0.85 | 0.78 | 8.78 | 0.0031* | 1.57 (1.16–2.21) |
| TYK2 | rs2304256 | 19 | A/C | C | 417:113 | 473:215 | 0.79 | 0.69 | 15.00 | 0.0001* | 1.68 (1.29–2.18) |
| TYK2 | rs12720356 | 19 | A/C | A | 497:35 | 623:65 | 0.93 | 0.91 | 3.28 | 0.07 | |

* Significant associations. SNP: single-nucleotide polymorphism.

*Table 3*. Haplotype association results. SNP included in the haplotypes are in the following order: rs231775, rs3087243, rs231726, rs231727, rs1991416 *(CTLA4)* and rs12720270, rs2304256, rs12720356 (*TYK2*). * Significant associations.

| Gene | Associated Haplotype | Haplotype Frequency | Case Allele Ratio Count | Control Allele Ratio Count | Case Frequency | Control Frequency | Chi-square | p | OR (95% CI) |
|---|---|---|---|---|---|---|---|---|---|
| CTLA4 | GGTAG | 0.43 | 230:304 | 302:390 | 0.43 | 0.44 | 0.05 | 0.83 | |
| CTLA4 | AACGA | 0.34 | 183:351 | 236:456 | 0.34 | 0.34 | 0.00 | 0.97 | |
| CTLA4 | AGCGA | 0.16 | 92:442 | 102:590 | 0.17 | 0.15 | 1.39 | 0.24 | |
| CTLA4 | GGCGG | 0.02 | 10:524 | 18:674 | 0.02 | 0.03 | 0.70 | 0.40 | |
| CTLA4 | GGCGA | 0.02 | 13:521 | 15:677 | 0.03 | 0.02 | 0.16 | 0.69 | |
| CTLA4 | GGTAA | 0.02 | 6:528 | 16:676 | 0.01 | 0.02 | 2.20 | 0.14 | |
| TYK2 | GCA | 0.73 | 417:117 | 472:216 | 0.78 | 0.69 | 13.63 | 0.0002* | 1.63 (1.26–2.12) |
| TYK2 | AAA | 0.19 | 80:454 | 149:539 | 0.15 | 0.22 | 8.81 | 0.0030* | 0.64 (0.47–0.86) |
| TYK2 | GAC | 0.08 | 36:498 | 66:622 | 0.07 | 0.10 | 3.20 | 0.07 | |

* Significant associations.

extended sample set, it is noteworthy that none of the other genes showed significant associations, in spite of a reasonable power.

TYK2 is a member of the Janus kinase (JAK) family of nonreceptor tyrosine kinases that play a critical role in initiating signaling cascades of a large number of cytokine receptors[39]. In the type I IFN pathway, which plays a crucial role in SLE pathogenesis (as reviewed[40]), TYK2 is stably associated to the type I IFN receptor complex with JAK1. After binding of type I IFN to the receptor, TYK2 is phosphorylated and thereby activated and initiates a JAK/STAT signaling cascade leading to the transcription of IFN signature genes[41-43]. In addition to the type I IFN pathway, recent studies have implicated a role of TYK2 in the response to interleukin 12 (IL-12)

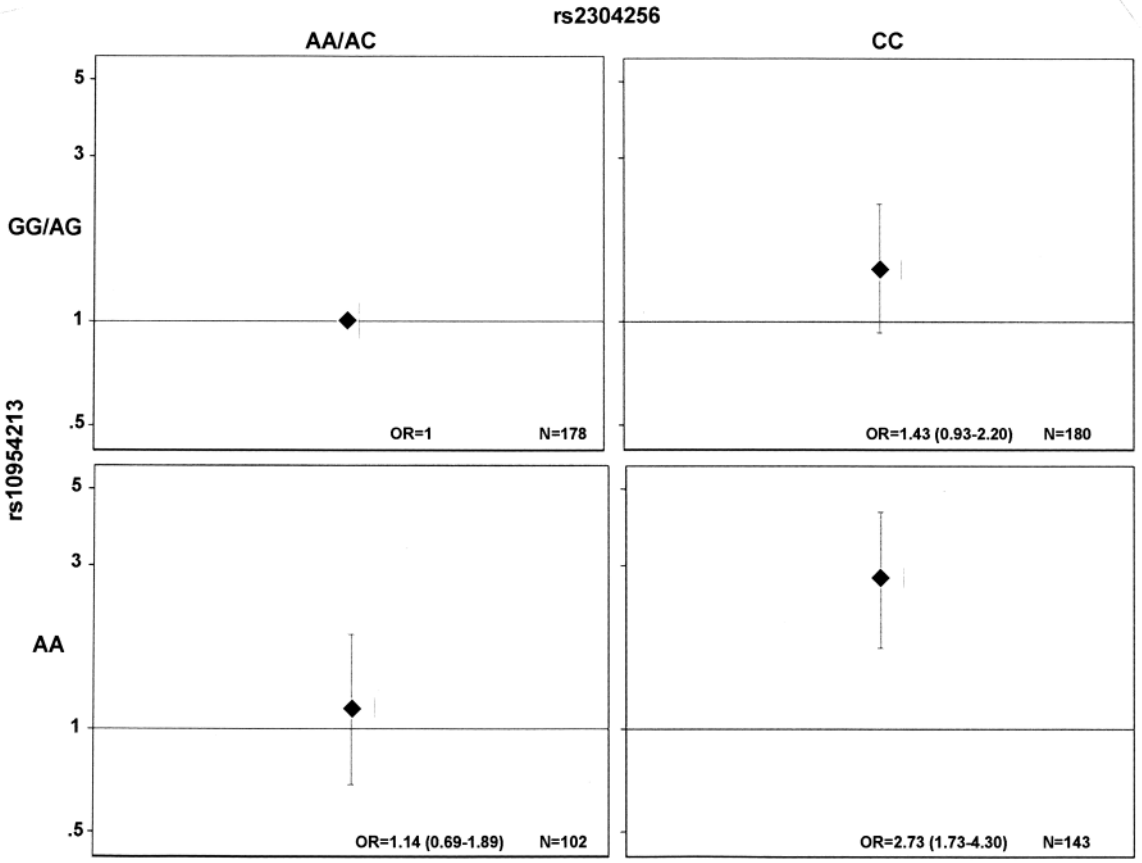


*Figure 1*. Genetic interaction between *TYK2* and *IRF5*: 2 × 2 table illustrates the interactive effect of *TYK2* rs2304256 and *IRF5* rs10954213 in SLE. Individuals homozygote for the rs2304256 risk allele C and the rs10954213 risk allele A show a significantly increased risk effect compared to individuals homozygote for no-risk alleles or heterozygote. OR (95% CI) for each combination is shown. N: number of subjects in each category. p = 0.014 for overall interaction.

*Table 4.* Joint effect of the risk alleles in *TYK2* and in *IRF5*.

| No. of Risk Factors | Joint Effect* p | OR (95% CI) |
|---|---|---|
| 0 or 1 | | Reference |
| 2 | 0.011 | 2.44 (1.22–4.85) |
| 3 | 0.020 | 2.22 (1.13–4.36) |
| 4 | 0.00003 | 4.55 (2.23–9.22) |

* Due to strong linkage disequilibrium *TYK2* was included in the analyses as a haplotype.

and IL-23, as well as several members of the IL-6 and IL-10 receptor families (as reviewed[44]).

All SNP analyzed in *TYK2* have been associated with SLE in 2 different studies, one including a subset of our Finnish SLE family group together with Swedish and Icelandic patients with SLE[12], and the other including UK SLE families[20]. In the Scandinavian study, a strong association signal was observed for rs2304256 in all patients and for rs12720356 in the Swedish patients only[12]. The rs2304256 marker was reported to cause a phenylalanine (A allele) to valine (C allele) switch, the C allele being prominent in SLE. This variation is located in a part of a larger JH4 domain of TYK2, crucial for the interaction of TYK2 with IFNAR1 and its function, and for maintaining expression of IFNAR1 on cell membranes[12]. The SNP rs12720356 causes a substitution of serine (C allele) to isoleucine (A allele) in the pseudokinase region JH2 of TYK2[12]. Neither of these SNP showed an association in isolation in the UK study; however, rs2304256 was reported to lie within an identified core associated region of 2.8 kb[20]. The strongest association seen in the UK data was to rs12720270, with the G allele being overtransmitted in SLE families[20]. This variant is situated close to an intron/exon boundary, thus a role for mis-splicing events in molecular pathogenesis was implicated[20].

We reproduced the association between the C allele of rs2304256 and risk for SLE (Table 2). We found also that the G allele of rs12720270 was present significantly more often in SLE cases than in controls (Table 2). The rs12720356 marker did not show association to SLE in the Finnish population individually; however, 2 haplotypes including all 3 *TYK2* markers showed either significantly increased risk (GCA) or decreased susceptibility (AAA). Over- and undertransmission of haplotypes containing GCA and AAA, respectively, was also reported in the UK study[20]. Further, in the UK study the core associated region was located between markers rs12720270 and rs280519, given that outside this region the alleles were identical on the major associated haplotypes. Our results strengthen this observation further: rs2304256 and rs12720270 showed significant association, while rs12720356 was not associated.

The *IRF5* gene encodes a transcription factor involved in the control of inflammatory and immune responses (as reviewed[40]) and has been shown to be important in SLE susceptibility in several studies (as reviewed[10]). It belongs to a family of transcription factors activated by IFN-α/ß and inducing expression and also positively regulates the expression of IFN-α/ß itself[40].

In our study, significant association was found to the rs10954213 marker (Table 2), located in a conserved polyA+ signal sequence of *IRF5*. This variation alters the length of the 3′ untranslated region and stability of *IRF5* mRNA, and has been shown to contribute to risk for SLE[29]. Three additional functional markers important in SLE risk have been described: rs2004640, a 30-bp in-frame insertion/deletion (indel) variant of exon 6, and a CGGGG indel polymorphism located 64-bp upstream of the first untranslated exon[17,29]. Strong association was seen to rs2004640 in the subset of Finnish families included in the previous study[12], but unfortunately this SNP failed the genotyping in our study.

None of the additional genes included in the study showed association to SLE in the Finnish data. In consideration of the recent GWA studies[12,14-16], some of these genes may not be strong candidates for SLE, even though well replicated genes may also fail to show up in GWA studies. However, lack of replication may have been a result of the modest power of our study. This may be the case for *FCGR2A*, which has a mild effect on SLE susceptibility[9]. Although many individual studies on *FCGR2A* are inconclusive, GG homozygotes had an increased susceptibility to SLE in one metaanalysis[23] and this association was supported in one of the recent GWA studies[15]. A similar distribution of alleles, with G being more common in SLE cases, was also observed in our study (Table 2).

We were not able to replicate the rs11568821 marker (PD1.3) in *PDCD1*. Interestingly, the A allele previously associated with SLE risk[24] was more frequent among controls. A protective effect of the A allele was also shown in a Spanish case-control study[45]. A role for the *NOD2/CARD15* has also been implicated in SLE, with the rs2066845 marker showing the strongest effect[11]. However, this marker was monomorphic in the Finnish patients with SLE and was thus uninformative for analysis. The only remaining SNP rs2076756, a putative susceptibility SNP in Crohn's disease[26], showed no association in our study. Also, several markers in *CTLA4* have been associated to SLE risk, although the results are inconsistent between studies[9]. Two metaanalyses have been conducted, both showing a moderately increased susceptibility to SLE in individuals carrying the GG genotype of rs231775; however, this was not observed in our study.

To our knowledge, not many studies have looked at the interaction between known SLE susceptibility genes. However, one recent study showed a strong increased risk of SLE in individuals carrying multiple risk alleles of *IRF5* and *STAT4*, both members of the type I IFN signaling pathway[19]. Given that the 2 genes showing association with SLE in this study, *TYK2* and *IRF5*, are both members of the type I IFN signaling pathway, we set out to investigate their possible genetic interaction.

Of note, interaction could be observed between

rs10954213 in *IRF5* and rs2304256 in *TYK2* (Figure 1). Odds ratio interpretation of the data clearly shows that by combining risk genotypes of each respective SNP the risk of disease increases significantly. Thus one can speculate that the phenylalanine (A allele) to valine (C allele) change in *TYK2* (encoded by rs2304256) in combination with altered *IRF5* mRNA (encoded by rs10954213) has functional relevance in the pathogenesis of SLE. This observation should encourage further studies of the interaction of SLE susceptibility genes to get a better understanding of the genes involved in this disease. Further, when the joint effects of GCA risk haplotype in *TYK2* and risk allele in *IRF5* were determined, an increase in OR for each additional risk allele was observed. The risk of SLE was considerably increased in double-homozygote individuals carrying 4 risk alleles (Table 4).

None of the clinical variables tested showed significant association to risk allele carrier status for either *TYK2* or *IRF5*. SLE patients who have manifestations of renal or central nervous system disease or hematological aberrations have been found to show upregulation of IFN-inducible genes[37]. However, we did not observe this, in agreement with a previous study[12].

We identified significant association with SLE and variations in *TYK2* and *IRF5* in a Finnish population. Our study did not, however, replicate previously reported associations with SLE in *CTLA4*, *PDCD1*, *FCGR2A*, or *NOD2*. We also found evidence for interaction between markers in *TYK2* and *IRF5*. These results strengthen the role of the type I interferon pathway in the pathogenesis of SLE.


## ACKNOWLEDGMENT

We thank Prof. Anna-Elina Lehesjoki and Prof. Albert de la Chapelle for providing the control samples, Lotta Koskinen, MSc, and Elisabet Einarsdottir, PhD, for assisting with the association analyses, and Sara Bruce, PhD, for assisting with the interaction analyses.


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