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► To cite this version:

Ann-Christine Syvänen, Johanna K Sandling, Sophie Garnier, Snaevar Sigurdsson, Chuan Wang, et al.. A candidate gene study of the type I interferon pathway implicates and as risk loci for SLE. European Journal of Human Genetics, 2010, 10.1038/ejhg.2010.197 . hal-00602289

HAL Id: hal-00602289

<https://hal.science/hal-00602289>

Submitted on 22 Jun 2011

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1 **A candidate gene study of the type I interferon pathway**
2 **implicates *IKBKE* and *IL8* as risk loci for SLE**

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39 **Running title:** type I interferon genes in SLE

40

41 **ABSTRACT**

42 Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease in which the type
43 I interferon pathway plays a crucial role. We have previously shown that three genes in this
44 pathway, *IRF5*, *TYK2* and *STAT4*, are strongly associated with risk for SLE. Here we
45 investigated 78 genes involved in the type I interferon pathway to identify additional SLE
46 susceptibility loci. First, we genotyped 896 single nucleotide polymorphisms in these 78
47 genes and 14 other candidate genes in 482 Swedish SLE patients and 536 controls. Genes
48 with $P < 0.01$ in the initial screen were then followed up in 344 additional Swedish patients
49 and 1 299 controls. SNPs in the *IKBKE*, *TANK*, *STAT1*, *IL8* and *TRAF6* genes gave
50 nominal signals of association with SLE in this extended Swedish cohort. To replicate
51 these findings we extracted data from a genome-wide association study on SLE performed
52 in a US cohort. Combined analysis of the Swedish and US data, comprising a total of 2 136
53 cases and 9 694 controls, implicates *IKBKE* and *IL8* as SLE susceptibility loci ($P_{\text{meta}} =$
54 0.00010 and $P_{\text{meta}} = 0.00040$, respectively). *STAT1* was also associated with SLE in this
55 cohort ($P_{\text{meta}} = 3.3 \times 10^{-5}$), but this association signal appears to be dependent of that
56 previously reported for the neighbouring *STAT4* gene. Our study suggests additional genes
57 from the type I interferon system in SLE, and highlights genes in this pathway for further
58 functional analysis.

59

60 **Keywords:** systemic lupus erythematosus, type I interferon system, candidate gene study,
61 single nucleotide polymorphism, *IKBKE*, *IL8*

62 INTRODUCTION

63 Systemic Lupus Erythematosus (SLE, OMIM 152700) is an inflammatory autoimmune
64 disease that primarily affects women during their childbearing years. Production of
65 autoantibodies, tissue deposits of immune complexes and inflammation in kidneys, skin,
66 joints and central nervous system are hallmarks of SLE. Despite a strong heritability of the
67 disease, linkage studies have failed to identify genes outside the major histocompatibility
68 complex (MHC) region as risk factors for SLE. Association studies have since proven a
69 more fruitful approach. The confirmed findings from genome-wide association studies
70 (GWAS) include genes that were originally discovered in candidate gene studies, such as
71 the interferon regulatory factor 5 (IRF5)¹ and the signal transducer and activator of
72 transcription 4 (STAT4)² genes from the type I IFN system. IFNs are cytokines with anti-
73 viral activity that are produced in response to viral infections, of which the type I IFNs
74 bind the IFN alpha receptor (IFNAR). Today there are more than 20 confirmed SLE
75 susceptibility loci³⁻⁴, of which several are in the type I IFN system.

76 The type I IFN system is activated in SLE patients⁵⁻⁶, and an important role
77 of the type I IFN system in the disease process was confirmed by studies showing an
78 increased expression of type I IFN-inducible genes in SLE patients (an “IFN-signature”)⁷.
79 A direct causative role of the type I IFN system in the etiopathogenesis of SLE was
80 suggested by the observation that individuals treated with IFN- α can develop an SLE
81 syndrome indistinguishable from the naturally occurring disease⁸. Moreover, a phase I
82 clinical trial with a monoclonal antibody against IFN- α , reports reduction of disease
83 activity as well as neutralization of the IFN-signature in SLE patients⁹⁻¹⁰. Encouraged by

84 the compelling evidence for the involvement of the type I IFN system in SLE, we
85 performed an association study to identify additional genes from the type I IFN pathway
86 that confer risk for SLE.

87

88 **MATERIALS AND METHODS**

89 **Subjects and genotyping**

90 Our study included 826 Swedish and 1 310 US SLE patients, fulfilling at least four of the
91 classification criteria for SLE as defined by the American College of Rheumatology
92 (ACR)¹¹, and 9 694 healthy control individuals from the same geographic areas as the
93 patients (Supplementary Table S1). DNA was extracted from blood samples of the patients
94 and controls using standard procedures. The study was approved by the regional ethical
95 boards and all subjects gave their informed consent to participate. The study was
96 performed in three stages:

97 *Discovery phase.* First, a panel of SNPs in 82 genes with key functions related to
98 the type I IFN signalling system and 14 additional genes, with suggested association with
99 SLE, were selected for genotyping in 490 Swedish SLE patients and 543 controls. Patients
100 were from the rheumatology clinics at the Lund, Karolinska (Solna) and Uppsala
101 University Hospitals in Sweden. SNPs were selected based on their average spacing of
102 around 1kb and LD information from the HapMap project ($r^2 < 0.8$ HapMap CEU release
103 16c), excluding SNPs with an Illumina quality score < 0.6 . Genotyping of 1 258 SNPs in
104 the 96 genes was performed using the Golden Gate Assay (Illumina Inc, San Diego, CA,
105 USA). Samples and SNPs with $> 10\%$ missing data, SNPs with Hardy-Weinberg

106 equilibrium test P values < 0.001 and SNPs with MAF < 0.01 were excluded from further
107 analysis. Four parent-offspring trios were included in the genotyping for inheritance
108 checks, and no Mendelian inheritance errors were observed. After exclusion of genetic
109 outliers, duplicate or related samples 482 cases, 536 controls and 896 SNPs in 92 genes
110 were available for analysis.

111 *Confirmatory set.* In the second phase, SNPs in the eight most promising genes
112 were followed up in 393 patients and 1 645 controls from Sweden. Patients were from the
113 rheumatology clinics at the Umeå, Uppsala and Karolinska (Solna) University Hospitals.
114 For the patients and 972 of the controls genotyping of 25 SNPs was performed using the
115 12-plex and 48-plex SNPStream systems¹² (Beckman Coulter Inc., Brea, CA, USA).
116 Primer sequences are provided in Supplementary Table S2. The same quality control filters
117 as for the discovery cohort were applied, and samples overlapping with the discovery
118 phase were excluded. Additional Swedish population-based controls from the Stockholm
119 area (n = 673), previously genotyped using the Infinium II assay on human 1M v1 bead
120 arrays¹³ (Illumina Inc.), were also included after applying the following quality filters:
121 MAF $> 1\%$, HWE $P > 1 \times 10^{-6}$, SNP and sample call rates $> 95\%$. After exclusion of
122 genetic outliers, duplicate or related samples, a total of 344 cases, 1 299 controls and 21
123 SNPs were available for analysis.

124 *Genome-wide association study.* We sought replication of our results by using data
125 from a genome-wide association study on SLE in US Caucasians¹⁴. In brief 1 435 North
126 American SLE cases of European descent and 3 583 controls had been genotyped on
127 HumanHap550 bead arrays (Illumina Inc.). An additional 4 564 controls were also

128 included as previously described⁴. After strict quality control¹⁴ 1 310 cases and 7 859
129 controls remained.

130

131 **Additional quality control and imputation**

132 For the 673 Swedish controls genotyped on the 1M bead arrays, genotypes for 13
133 confirmatory phase SNPs that were not directly genotyped were imputed using the
134 software IMPUTE and phasing data from the HapMap project¹⁵. In the US GWAS dataset
135 genotypes were imputed for 15 SNPs using the IMPUTE software. SNPs had imputation
136 confidence scores ≥ 0.90 with one exception (rs4694178, confidence 0.85 in the US data).

137 For all the Swedish SLE patients, and for more than half of the Swedish controls
138 genotyped using the GoldenGate or SNPstream methods, data from 6 060 uncorrelated
139 ancestry informative markers (AIMs) became available during the course of our study⁴.

140 Using this data, genetic outliers were identified with principal component analysis by the
141 EIGENSTRAT software¹⁶ and excluded from the study (the ten first principal components
142 were inferred and a cut off of $\sigma > 6$ was used to identify outliers). These samples were also
143 checked for cryptic relatedness by investigation of identity-by-state (IBS) status in
144 PLINK¹⁷ (<http://pngu.mgh.harvard.edu/purcell/plink/>) using a set of 12k previously
145 genotyped SNPs. For the Swedish controls genotyped on the 1M arrays, all markers were
146 used to identify cryptically related samples and 6 035 successfully genotyped AIMs were
147 used to identify genetic outliers.

148

149 **Association analysis and power calculation**

150 The association analysis of the directly genotyped SNPs was performed by comparing
151 allele frequencies in cases and controls with Fisher's exact or Chi2 tests using PLINK. A
152 null distribution for the quantile-quantile (Q-Q) plot was generated with PLINK and
153 plotted using R (<http://www.r-project.org/>). Analyses including imputed genotypes were
154 performed using SNPTEST¹⁵, which takes imputation uncertainty into account. SNPs
155 which were not captured by imputation were only analyzed in the directly genotyped
156 samples. Conditional logistic regression analysis assuming an additive model was
157 performed using PLINK to test for independence of association signals observed in
158 neighbouring genes, or between associated SNPs within the same gene. Tests for pair-wise
159 SNP interactions were performed using the epistasis command in PLINK. The combined
160 analysis of the Swedish and US case-control data was performed using the software Metal
161 (<http://www.sph.umich.edu/csg/abecasis/Metal/index.html>). Pooled odds ratios were
162 calculated using the Mantel-Haenszel method under a fixed effects model, and tests for
163 heterogeneity of odds ratios between studies were calculated using the MedCalc software
164 (<http://www.medcalc.be/>). Power calculations were performed using the software
165 QUANTO (<http://hydra.usc.edu/GxE/>) assuming a log-additive model, and a prevalence
166 for SLE in Sweden of 0.05%.

167

168 **RESULTS**

169 We selected a panel of SNPs in 78 genes with key functions related to the type I IFN
170 signalling system to study their association with SLE in a Swedish case-control cohort.
171 The selected genes encode Toll-like receptors (TLRs) and intracellular sensor molecules

172 for nucleic acids (i.e. RIG-I-like receptors: RLR) and members of their signalling
173 pathways, including several transcription factors that are active in the IFN producing pDCs
174 and membrane proteins of the pDCs. Genes encoding the members of the type I IFN
175 family and other genes regulated by the TLRs that are involved in the response to the type
176 I and type III IFNs, and genes for which the expression is directly regulated by type I IFNs,
177 were also included in the panel. Although the type I and type III IFNs bind two different
178 receptors, the IFNAR and IFN- λ receptor, they share downstream signalling and IFN- λ
179 induced genes are also induced by type I IFNs. A total of 14 additional genes that are not
180 directly involved in the type I IFN system, but have been suggested to be associated with
181 SLE were also included (Supplementary Table S3). This analysis identified 21 SNPs in
182 seven genes that yielded unadjusted P-values < 0.01: *IKBKE*, *TANK*, *STAT1*, *IL8*, *NRP1*,
183 *TRAF6*, and *PIAS4* (Figure 1 and Supplementary Table S3). At this significance level only
184 nine associated SNPs would be expected to yield association signals by chance, which
185 indicates the presence of true association signals in our data (Supplementary Figure S1).
186 The power for the discovery phase, which included 482 SLE cases and 536 controls, was
187 65% to detect an OR of 1.5 at 0.01 significance for a 10% frequency allele. However, our
188 power to detect genes with an OR of 1.2 was considerably lower (10%), and thus we
189 cannot exclude that genes which remained undetected in our study may contribute to the
190 risk for SLE.

191 To increase power we designed a panel of SNPs in the *IKBKE*, *TANK*, *STAT1*,
192 *IL8*, *NRP1*, *TRAF6*, and *PIAS4* genes for genotyping in an independent collection of
193 Swedish SLE patients and controls. The *STAT5B* gene was also included in the follow-up

194 study, since initially one SNP in the gene showed a P-value < 0.01. This was however prior
195 to an additional quality control step, by which related samples and population outliers were
196 excluded. For genes where multiple SNPs showed P-values < 0.05 in the discovery phase,
197 candidate SNPs for follow-up were tested for independence of their association signal in
198 relation to the most strongly associated SNP in each gene (Supplementary Table S4). A
199 partly redundant set of 21 SNPs that accounted for the association signals from the
200 discovery phase was then analysed in the second Swedish case-control cohort. Also in this
201 confirmatory cohort genetic outliers were excluded. By combining the data from the
202 Swedish discovery and confirmatory cohorts, totalling 826 cases and 1 835 controls, we
203 observed strong association signals with $P < 5 \times 10^{-4}$ for the SNPs rs2030171 in *STAT1*
204 and rs4694178 in *IL8* (Table 1 and Supplementary Table S5). Multiple linked SNPs in the
205 TANK and TRAF6 genes also gave suggestive signals of association with SLE in the
206 combined Swedish cohort. In addition, two weakly correlated SNPs in the IKBKE gene
207 (rs1539243 and rs17433930, LD $r^2 = 0.34$) displayed P-values < 0.05. Conditional
208 regression analysis did not provide evidence for more than one allele contributing to risk
209 for SLE in genes with multiple associated SNPs (Supplementary Table S5), nor was there
210 any evidence for epistatic interactions between SNPs at different loci.

211 We sought independent replication of our results by using data from a genome-
212 wide association study on SLE in European Americans¹⁴. The SNPs rs1539243 and
213 rs17433930 in the IKBKE gene showed signals of association with SLE also in the US
214 data (P = 0.0028 and P = 0.0021, respectively) (Table 2 and Supplementary Table S6). The
215 SNP rs1539243 had been directly genotyped in the US cohort, and for rs17433930 imputed

216 genotypes were analysed. Combined analysis of the data from the Swedish and US cohorts
217 revealed convincing association signals with SLE for these SNPs ($P_{\text{meta}} = 0.00026$, OR =
218 1.19, and $P_{\text{meta}} = 0.00010$, OR = 1.33, for rs1539243 and rs17433930, respectively), a
219 result which was significant also after Bonferroni correction ($P_{\text{meta_corr}} < 0.01$). The two
220 *IKBKE* SNPs rs17433930 and rs1539243 which we found to be associated with SLE are
221 located in the tenth intron and fourth exon of the gene respectively, where rs1539243 is a
222 synonymous SNP in amino acid residue 67 (Ile) of the IKK ϵ kinase.

223 Although the SNP rs4694178, located 3.3 kb downstream of the *IL8* gene, had only
224 a trend-wise significant P-value in the US cohort compared to the more convincing
225 association with SLE in the Swedish cohort ($P_{\text{US}} = 0.064$, $P_{\text{SWE}} = 5 \times 10^{-5}$), its association
226 signal remained significant in the combined analysis after Bonferroni correction ($P_{\text{meta}} =$
227 0.00040, OR = 1.17, $P_{\text{meta_corr}} < 0.01$) (Table 2). This SNP was imputed in the US data
228 (confidence = 0.85), however the directly genotyped SNP rs9999446 which is strongly
229 correlated with the SNP rs4694178 ($r^2 = 0.86$) yielded a similar result ($P = 0.085$) for
230 association with SLE. For the SNP rs10199181 in the *STAT1* gene we observed combined
231 P values $< 1 \times 10^{-3}$. The *STAT1* gene is located close to the *STAT4* gene in a region of high
232 LD on chromosome 2q32.2. *STAT4* contains two linked SNPs, rs10181656 and rs7582694,
233 which are strongly associated with SLE¹⁸, and conditional regression analysis of the data
234 from the combined Swedish cohort indicates that the association signals from the *STAT1*
235 and *STAT4* SNPs are not independent of each other (data not shown, remaining $P_{\text{conditional}} >$
236 0.3).

237

238 **DISCUSSION**

239 Our association study of genes from the type I IFN pathway and additional candidate genes
240 for SLE highlighted two genes, *IKBKE* and *IL8*, as potential risk factors for SLE. In
241 addition, the genes *TRAF6* and *TANK* showed significant association with SLE in the
242 Swedish cohorts. Furthermore, *IRF5*, *TYK2*, *STAT4*, *IFIH1*, *IRAK1*, *IRF8* and the
243 *PHRF1/IRF7* region have been reported by us elsewhere to be associated with SLE^{1,4,18}.
244 Thus, polymorphisms in multiple genes connected to the type I IFN signalling system are
245 important for SLE disease susceptibility.

246 *IKBKE*, the inhibitor of nuclear factor kappa-B kinase subunit epsilon gene,
247 encodes IKK ϵ , a kinase that together with the TANK-binding kinase (TBK1) plays a role
248 in the innate antiviral response. IKK ϵ and TBK1 are activated when two intracellular RLR
249 helicases, encoded by the *IFIH1* and *DDX58* genes, recognize viral RNA in virus-infected
250 cells (Figure 2). These kinases are also activated upon stimulation of endosomal TLR3 by
251 double stranded DNA, or cell membrane TLR4 by bacterial lipopolysaccharide (LPS).
252 Together with TBK1, IKK ϵ mediates phosphorylation of the transcription factors IRF3 and
253 IRF7, which leads to their activation and subsequent transcription of type I IFN and other
254 inflammatory cytokines, but also activation of NF κ B has been reported¹⁹⁻²⁰. The activation
255 of IRF3 and IRF7 can be inhibited by an ubiquitin editing enzyme (A20), encoded by the
256 *TNFAIP3* gene. Since also variation in *TNFAIP3* and *IFIH1* are associated with SLE^{4,21-22},
257 this further supports an important role for the RLR pathway in the disease process. There is
258 also evidence that IKK ϵ can phosphorylate STAT1, and thus contribute to the type I IFN
259 signalling via the IFNAR²³.

260 The *IKBKE* gene has recently been implicated in risk for rheumatoid arthritis
261 (RA)²⁴. The two polymorphisms with the most significant association with RA were tested
262 in the discovery phase of our study (rs2151222 and rs3748022 with P = 0.084 and P = 0.49
263 for association to SLE, respectively), however these variants appear to be independent
264 from the *IKBKE* variants rs1539243 and rs17433930 that we identified as risk alleles for
265 SLE ($r^2 < 0.1$ discovery phase). The conjecture that *IKBKE* plays a role in arthritis is
266 supported by data from an animal model. *IKKε* knock-out mice have been shown to be less
267 sensitive to induction of arthritis and exhibit less joint destruction than control mice²⁵. In a
268 published GWAS on women with SLE²⁶ the *IKBKE* SNP rs1539243 was tested, but no
269 association was observed (dbGaP, www.ncbi.nlm.nih.gov/gap). The power of that study
270 was however only 23% at the P = 0.05 level to detect the association we observe.

271 The chemokine IL-8 has a wide range of pro-inflammatory effects, and its
272 production can be triggered by immune complexes that also have the capacity to induce
273 type I IFN production²⁷. Recent data also suggests that IL-8 production in virus-infected
274 cells is IFN dependent²⁸. SLE patients with renal²⁹ or CNS involvement³⁰ have elevated
275 IL-8 levels in their serum and cerebrospinal fluid, respectively, and serum IL-8 levels and
276 disease activity correlate in SLE patients³¹. Because SLE disease flares are associated with
277 increased IFN- α production⁶, these observations provide a link between the SLE disease
278 process, IL-8 and the type I IFN system.

279 While we observed a significant association of *IL8* with SLE in the present study,
280 there are prior conflicting reports on the association between *IL8* and SLE^{26,32-34}, only two
281 of which, a Spanish case-control study and a GWAS in women with SLE, having similar

282 power to our discovery phase. The power of these studies was around 50% at the 0.05
283 significance level to detect the effect for *IL8* that we observe. The LD between the SNP
284 rs2227306 tested in the Spanish study and the SNP rs4694178 in our study is very high (r^2
285 = 0.97 in the HapMap CEU population), but they do not observe any association with SLE
286 for this SNP³⁴. In our study the association signal is mainly contributed by the Swedish
287 cohort, which has a higher frequency of the risk allele than both the US and Spanish
288 cohorts (control frequency of rs4694178 C: Sweden = 0.46, USA = 0.41 and the linked
289 allele rs2227306 T: Spain = 0.41). In the women GWAS²⁶, a SNP perfectly correlated with
290 *IL8* rs4694178 (rs4694636, $r^2 = 1$ HapMap CEU) was tested, and showed an odds ratio
291 suggestive of association with SLE (OR CI: 1.01-1.49, dbGaP). The modest power to
292 detect an effect of this size, or genetic heterogeneity between populations could explain
293 these conflicting results.

294 Although the TRAF family member-associated NF κ B activator (*TANK*) and TNF
295 receptor-associated factor 6 (*TRAF6*) genes, which gave clear association signals ($p <$
296 0.001) in the Swedish cohort, did not replicate in the US GWAS data, these genes remain
297 interesting candidates. TANK signals immediately upstream of IKK ϵ in the TLR4 and
298 IFIH1/DDX58 mediated activation of type I IFNs and inflammatory cytokines in response
299 to bacterial and viral stimuli, respectively (Figure 2). Association of *TANK* with SLE
300 would thus further support an important role for the RLR pathway in SLE. TRAF6 is a
301 ubiquitin ligase that mediates signal transduction from for example members of the TLR
302 family leading to activation of NF κ B and IRFs. Polyubiquitination of IRF5 after its
303 interaction with IRAK1 is mediated by TRAF6, which enables IRF5 to translocate to the

304 nucleus and exert its effect on gene expression. On the other hand TNFAIP3 can inhibit
305 TLR induced activity of NFκB by de-ubiquitination of TRAF6³⁵. Interestingly, also *IRAK1*
306 has been associated with risk for SLE³⁶, and because SNPs in the *TRAF6* region and in
307 *TNFAIP3* have also been associated with risk for rheumatoid arthritis (RA)³⁷⁻³⁹, it seems as
308 variants of all these genes have the potential to contribute to loss of tolerance and
309 autoimmune reactions. Further studies will be needed to determine whether the TANK and
310 TRAF6 genes have an effect on SLE, and whether this effect is specific for Scandinavian
311 populations.

312 Our study confirms the important role of the type I IFN system in SLE, and
313 suggests multiple genes from this pathway as candidates for functional studies and as
314 interesting therapeutic targets (Figure 2). These results also point more specifically to the
315 importance of genes in the RLR pathway, which is activated in response to viral infections
316 because of the ability of IFIH1/DDX58 to recognize cytoplasmic viral RNA. This pathway
317 is active in cells other than the pDCs, including monocyte derived dendritic cells. In
318 addition to IFIH1, factors such as TANK, IKBKE, and TNFAIP3 contribute to signalling
319 in the RLR pathway (reviewed in 40). However, there is also evidence for involvement in
320 SLE of the MYD88-dependent pathway activated by endosomal TLR7/9 by RNA/DNA
321 from dying cells, immune complexes (IC) or by viral RNA/DNA, because *IRF8*, *IRAK1*,
322 *FCGR2A*, and potentially *TRAF6*, in this pathway are associated with SLE. Thus at least
323 two pathways seem important in SLE, both leading to production of type I IFN and
324 inflammatory cytokines via activation of IRF3, IRF5 and IRF7 and additional
325 transcriptions factors, especially NFκB. Association to SLE has further been demonstrated

326 for the type I IFN signalling via the IFNAR, specifically for *TYK2* and *STAT4*, as well as
327 the IFN-regulated genes *IFIH1*, *IRF5*, *IRF7* and *IL8*. Consequently, a large number of
328 genes, located in different functional parts of the type I IFN system, are associated with
329 SLE indicating a general role for the type I IFN system genes in autoimmunity.

330

331 **CONFLICT OF INTEREST**

332 Robert R. Graham and Timothy W. Behrens are employees of Genentech corp. The other
333 authors declare no conflict of interest.

334

335 **ACKNOWLEDGEMENTS**

336 This study was supported by a Target Identification in Lupus (TIL) grant from the Alliance
337 for Lupus Research, US, the Swedish Research Council for Medicine, the Knut and Alice
338 Wallenberg Foundation, the Swedish Rheumatism Foundation, the King Gustaf V 80-year
339 Foundation, COMBINE, the European Community 6th Framework Program [LSHM-CT-
340 2007-037273], the Swedish Heart-Lung Foundation, the Torsten and Ragnar Söderberg
341 Foundation, the “Visare Norr” Fund for Northern County Councils of Sweden, The Åke
342 Wiberg Foundation, ALF funding from Stockholm County Council and Karolinska
343 Institutet, the National Institutes of Health [R01 AR44804, K24 AR02175], the National
344 Center for Research Resources [5 M01 RR00079], and a Kirkland Scholar Award to
345 L.A.C. We thank Rezvan Kiani Dehkordi at the Rheumatology Clinic, Uppsala University
346 Hospital, for assistance with DNA sample collection. Professor Göran Hallmans, Head of
347 the Medical Biobank of Northern Sweden for providing blood samples. Ann-Christin

348 Wiman, Caisa Pöntinen, Molecular Medicine, Department of Medical Sciences, Uppsala
349 University, for assistance with genotyping. Genotyping was performed using equipment
350 available at the SNP Technology Platform in Uppsala, Sweden (www.genotyping.se).
351
352 Supplementary information is available at the European Journal of Human genetics
353 website.

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LEGENDS TO FIGURES

Figure 1 Association with SLE for the 92 genes tested in the discovery phase (482 Swedish cases and 536 controls). The negative logarithm of the P value from a Chi square test is plotted against chromosomal location. The gray line represents $P = 0.05$, and the black line $P = 0.01$.

Figure 2 A schematic illustration of signaling within the type I interferon (IFN) system. Circled gene names have confirmed association to SLE: *STAT4*, *IRF5*, *IRF7*, *IRF8*, *TNFAIP3* (A20), *TNFSF4* (OX40L), *FCGR2A*, *TYK2*, *IFIH1* (MDA5), *IRAK1* as well as *IKBKE* and *IL8* identified in this study. Genes with dashed circles are *TANK* and *TRAF6*, which we find associated with SLE in the Swedish but not US cohorts, and *STAT1* that has an association signal which cannot be distinguished from that of *STAT4*.

Left: Induction of inflammatory cytokines and type I IFN genes in response to viral RNA/DNA by the helicases *IFIH1* (MDA5), *DDX58* (RIG-I) and the DNA sensor *ZBP1* (DAI), or by bacterial LPS via toll-like receptor 4. These pathways signal using *TANK* that interacts with *IKBKE* or *TBK1*, which together can mediate phosphorylation of the interferon regulatory factors *IRF3* and *7*, leading to the transcription of type I IFN genes. Activation of this pathway can be inhibited by *TNFAIP3* (A20).

Middle: Induction of IFN production in plasmacytoid dendritic cells (pDCs) by endogenous immune complexes. Immune complexes are endocytosed via *FCGR2A* which leads to activation of *TLR7/9*. After a signaling cascade, which includes *IRAK1* and *TRAF6*, the interferon regulatory factors *IRF5* and *IRF7* are activated leading to the transcription of type I IFN genes. Also *IRF8* can contribute to this activation.

Right: IFN signaling via the type I IFN receptor *IFNAR*. Association of type I IFN to the receptor activates the kinases *TYK2* and *JAK1*, which signal via *STAT1* and *STAT2*. Also other STATs, including *STAT4*, are activated in this process. The *STAT1/STAT2* complex associates with *IRF9*, which in turn binds to interferon stimulated response elements (ISREs) and induces expression of IFN induced genes, such as *IRF5*, *IRF7* and indirectly *IL8*.

Far right: Membrane proteins expressed on pDCs. *TNFSF4* (OX40L) together with endogenous type I IFNs provide a means for the pDCs to control the T cell response, specifically to profile it towards a T_H1 response.

Activation of the $\text{NF}\kappa\text{B}$ pathway, and thus production of inflammatory cytokines, also occurs via *TLR7/9* (not shown). Proteins are denoted by their corresponding gene names.

Table 1 Combined analysis of association with SLE in the Swedish discovery and confirmation cohorts¹

Gene	CHR	SNP ²	bp	Minor/major alleles	MAF cases	MAF controls	P ³	Risk allele	OR (95% CI)
<i>IKBKE</i>	1	rs1539243	204714410	T/C	0.16	0.18	0.031	C	1.19 (1.02-1.39)
<i>IKBKE</i>	1	rs17433930	204719362	G/A	0.07	0.09	0.013	A	1.33 (1.06-1.67)
<i>TANK</i>	2	rs3754974	161721201	G/A	0.05	0.04	0.0087	G	1.44 (1.09-1.89)
<i>TANK</i>	2	rs1267075	161768063	G/A	0.19	0.18	0.75	G	1.02 (0.88-1.19)
<i>STAT1</i>	2	rs2030171	191577408	A/G	0.35	0.29	1.2E-05	A	1.31 (1.16-1.49)
<i>STAT1</i>	2	rs16833172	191584314	A/G	0.04	0.03	0.015	A	1.51 (1.09-2.09)
<i>IL8</i>	4	rs4694178	74831552	C/A	0.52	0.46	5.2E-05	C	1.26 (1.12-1.42)
<i>NRP1</i>	10	rs734187	33524702	A/G	0.24	0.24	0.67	A	1.03 (0.90-1.18)
<i>NRP1</i>	10	rs1331314	33564687	G/C	0.11	0.10	0.32	G	1.11 (0.92-1.34)
<i>NRP1</i>	10	rs2073320	33593263	A/G	0.38	0.37	0.42	A	1.05 (0.93-1.18)
<i>TRAF6</i>	11	rs5030482	36466602	C/T	0.12	0.14	0.0094	T	1.25 (1.05-1.49)
<i>TRAF6</i>	11	rs5030472	36470362	T/C	0.09	0.12	0.013	C	1.28 (1.05-1.55)
<i>STAT5B</i>	17	rs6503691	37647616	T/C	0.09	0.10	0.44	C	1.08 (0.88-1.32)
<i>PIAS4</i>	19	rs2289863	3979783	C/T	0.27	0.29	0.19	T	1.09 (0.96-1.25)

¹826 Swedish cases and 1 835 controls genotyped in the discovery and replication phases including controls genotyped on the 1M chip. ²Genotypes for rs17433930, rs3754974, rs2030171, rs16833172, rs4694178, rs734187 and rs1331314 were imputed in the Swedish controls genotyped on the 1M chip.

³P-values calculated using SNPtest. MAF = Minor Allele Frequency, NA = Not Available, CI = Confidence Interval

Table 2 Meta-analysis of association with SLE for the Swedish and US cohorts

Gene	SNP	Sweden ¹		US ²		Risk allele	P	Meta-analysis ⁵	
		P ³	OR (95% CI)	P ³	OR (95% CI) ⁴			Corrected P ⁶	OR (95% CI) ⁴
<i>IKBKE</i>	rs1539243	0.031	1.19 (1.02-1.39)	0.0028	1.20 (1.07-1.35)	C	0.00026	0.0054	1.19 (1.09-1.31)
<i>IKBKE</i>	rs17433930	0.013	1.33 (1.06-1.67)	0.0021	1.33 (1.11-1.58)	A	0.00010	0.0022	1.33 (1.16-1.53)
<i>TANK</i>	rs3754974	0.0087	1.44 (1.09-1.89)	0.98	0.99 (0.80-1.22)	NA	0.22	1.0	1.13 (0.96-1.34)
<i>TANK</i>	rs1267075	0.75	1.02 (0.88-1.19)	0.87	0.99 (0.89-1.11)	NA	0.99	1.0	1.00 (0.92-1.10)
<i>STAT1</i>	rs2030171	1.2E-05	1.31 (1.16-1.49)	0.018	1.11 (1.02-1.21)	A	3.3E-05	0.00069	1.17 (1.09-1.26)
<i>STAT1</i>	rs16833172	0.015	1.51 (1.09-2.09)	0.16	1.23 (0.95-1.59)	A	0.016	0.34	1.33 (1.09-1.63)
<i>IL8</i>	rs4694178	5.2E-05	1.26 (1.12-1.42)	0.064	1.13 (1.04-1.23)	C	0.00040	0.0084	1.17 (1.10-1.26)
<i>NRPI</i>	rs734187	0.67	1.03 (0.90-1.18)	0.66	1.01 (0.92-1.12)	A	0.56	1.0	1.02 (0.94-1.10)
<i>NRPI</i>	rs1331314	0.32	1.11 (0.92-1.34)	0.11	1.11 (0.97-1.26)	G	0.061	1.0	1.11 (1.00-1.23)
<i>NRPI</i>	rs2073320	0.42	1.05 (0.93-1.18)	0.61	0.98 (0.90-1.06)	NA	0.94	1.0	1.00 (0.94-1.07)
<i>TRAF6</i>	rs5030482	0.0094	1.25 (1.05-1.49)	0.12	1.10 (0.97-1.24)	T	0.0097	0.20	1.14 (1.03-1.26)
<i>TRAF6</i>	rs5030472	0.013	1.28 (1.05-1.55)	0.19	1.09 (0.96-1.25)	C	0.020	0.42	1.15 (1.03-1.28)
<i>STAT5B</i>	rs6503691	0.44	1.08 (0.88-1.32)	0.78	0.98 (0.86-1.12)	NA	0.91	1.0	1.01 (0.90-1.13)
<i>PIAS4</i>	rs2289863	0.19	1.09 (0.96-1.25)	0.72	0.98 (0.89-1.08)	NA	0.76	1.0	1.02 (0.95-1.10)

¹826 Swedish cases and 1 835 controls genotyped in the discovery and replication phases including controls genotyped on the 1M chip. ²1 310 US SLE cases and 7 859 controls genotyped in a GWAS. Genotypes for rs17433930, rs3754974, rs2030171, rs16833172, rs4694178, rs734187, rs1331314 and rs5030482 were imputed in this dataset as described in Gateva *et al.* ³P-values calculated using SNPtest. ⁴Odds ratios are calculated relative to the risk allele defined in the analysis of the combined Swedish cohort. ⁵Meta-analysis including 2 136 SLE cases and 9 694 controls. ⁶P-value corrected for the 21 tested SNPs. MAF = Minor Allele Frequency, NA = Not Available, CI = Confidence Interval



