ARTHRITIS & RHEUMATOLOGY
Vol. 68, No. 9, September 2016, pp 2338–2344
DOI 10.1002/art.39730

BRIEF REPORT

IRF4 Newly Identified as a Common Susceptibility Locus for Systemic Sclerosis and Rheumatoid Arthritis in a Cross-Disease Meta-Analysis of Genome-Wide Association Studies

Elena López-Isac,[1] Jose-Ezequiel Martín,[1] Shervin Assassi,[2] Carmen P. Simeón,[3] Patricia Carreira,[4] Norberto Ortego-Centeno,[5] Mayka Freire,[6] Emma Beltrán,[7] Javier Narváez,[8] Juan J. Alegre-Sancho,[9] the Spanish Scleroderma Group, Benjamín Fernández-Gutiérrez,[10] Alejandro Balsa,[11] Ana M. Ortiz,[12] Miguel A. González-Gay,[13] Lorenzo Beretta,[14] Alessandro Santaniello,[14] Chiara Bellocchi,[14] Claudio Lunardi,[15] Gianluca Moroncini,[16] Armando Gabrielli,[16] Torsten Witte,[17] Nicolas Hunzelmann,[18] Jörg H. W. Distler,[19] Gabriella Riekemasten,[20] Annette H. van der Helm-van Mil,[21] Jeska de Vries-Bouwstra,[21] Cesar Magro-Checa,[21] Alexandre E. Voskuyl,[22] Madelon C. Vonk,[23] Øyvind Molberg,[24] Tony Merriman,[25] Roger Hesselstrand,[26] Annika Nordin,[27] Leonid Padyukov,[27] Ariane Herrick,[28] Steve Eyre,[28] Bobby P. C. Koeleman,[29] Christopher P. Denton,[30] Carmen Fonseca,[30] Timothy R. D. J. Radstake,[31] Jane Worthington,[28] Maureen D. Mayes,[2] and Javier Martín[1]

Objective. **Systemic sclerosis (SSc) and rheumatoid arthritis (RA) are autoimmune diseases that have similar clinical and immunologic characteristics. To date, several shared SSc–RA genetic loci have been identified independently. The aim of the current study was to systematically search for new common SSc–RA loci through an interdisease meta–genome-wide association (meta-GWAS) strategy.**

Methods. **The study was designed as a meta-analysis combining GWAS data sets of patients with SSc and patients with RA, using a strategy that allowed identification of loci with both same-direction and opposite-direction allelic effects. The top single-nucleotide**

Supported by a grant from the Ministerio de Educación, Cultura y Deporte through the program FPU (to Dr. López-Isac), grant 115565 from the EU/EFPIA Innovative Medicines Initiative Joint Undertaking PRECISESADS (ref. no. 115565) and BIO-1395 from the Junta de Andalucía, grant PI-0590-2010 from the Consejería de Salud y Bienestar Social, Junta de Andalucía, Spain (to Dr. Ortego-Centeno), a VIDI laureate from the Dutch Association of Research and Dutch Arthritis Foundation (to Dr. Radstake), and grant SAF2012-34435 from the Spanish Ministry of Economy and Competitiveness (to Dr. J. Martín). Dr. Assassi's work was supported by grants KL2-RR-024149-04 and K23-AR-061436 from the NIH, grant 3-UL1-RR-024148 from the NIH National Center for Research Resources, and grant U01-1U01AI09090 from the NIH National Institute of Allergy and Infectious Diseases. Dr. Mayes' work was supported by grant P50-AR-054144 from the NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) Centers of Research Translation, grant N01-AR-0-2251 from the NIAMS SSc Family Registry and DNA Repository, grant PR-1206877 from the Department of Defense, and grant R01-AR-055258 from the NIAMS.

[1]Elena López-Isac, MSc, Jose-Ezequiel Martín, PhD, Javier Martín, MD, PhD: Institute of Parasitology and Biomedicine López-Neyra CSIC, Granada, Spain; [2]Shervin Assassi, MD, Maureen D. Mayes, MD, MPH: University of Texas Health Science Center, Houston; [3]Carmen P. Simeón, MD, PhD: Valle de Hebrón Hospital, Barcelona, Spain; [4]Patricia Carreira, MD: 12 de Octubre University Hospital, Madrid, Spain; [5]Norberto Ortego-Centeno, MD: Clinic University Hospital, Granada, Spain; [6]Mayka Freire, MD, PhD: Complexo Hospitalario Universitario de Vigo, Vigo, Spain; [7]Emma Beltrán, MD: Hospital General Universitario de Valencia, Valencia, Spain; [8]Javier Narváez, MD, PhD: Hospital Universitari de Bellvitge, Barcelona, Spain; [9]Juan J. Alegre-Sancho, MD, PhD: Hospital Universitari Doctor Peset, Valencia, Spain; [10]Benjamín Fernández-Gutiérrez, MD, PhD: Hospital Clínico San Carlos, Madrid, Spain; [11]Alejandro Balsa, MD, PhD: Hospital Universitario La Paz, Instituto de Investigación Sanitaria La Paz, Madrid, Spain; [12]Ana M. Ortiz, MD, PhD: Hospital Universitario La Princesa, Instituto de Investigación Sanitaria La Princesa, Madrid, Spain; [13]Miguel A. González-Gay, MD, PhD: University of Cantabria, Santander, Spain; [14]Lorenzo Beretta, MD, Alessandro Santaniello, MD, Chiara Bellocchi, MD: Referral Center for Systemic Autoimmune Diseases, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Milan, Italy; [15]Claudio Lunardi, MD: Università degli Studi di Verona, Verona, Italy; [16]Gianluca Moroncini, MD, PhD, Armando Gabrielli, MD, PhD: Università Politecnica delle Marche and Ospedali Riuniti, Ancona, Italy; [17]Torsten Witte, MD, PhD: Hannover Medical School, Hannover, Germany; [18]Nicolas Hunzelmann, MD: University of Cologne, Cologne, Germany; [19]Jörg H. W. Distler, MD: University of Erlangen–Nuremberg, Erlangen, Germany; [20]Gabriella Riekemasten, MD, PhD: University of Lübeck, Lübeck, Germany; [21]Annette H. van der Helm-van Mil, MD, PhD, Jeska de Vries-Bouwstra, MD, PhD, Cesar Magro-Checa, MD: Leiden University Medical Center, Leiden, The Netherlands; [22]Alexandre E. Voskuyl, MD: VU University Medical Center, Amsterdam, The Netherlands; [23]Madelon C. Vonk, MD, PhD: Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; [24]Øyvind Molberg, MD, PhD: Oslo University Hospital Rikshospitalet and Institute of Clinical Medicine, University of Oslo, Oslo, Norway; [25]Tony Merriman, PhD: University of Otago, Otago, New Zealand; [26]Roger Hesselstrand, MD, PhD: Lund University,

polymorphisms were followed up in independent SSc and RA case–control cohorts. This allowed an increase in the sample size to a total of 8,830 patients with SSc, 16,870 patients with RA, and 43,393 healthy controls.

Results. **This cross-disease meta-analysis of the GWAS data sets identified several loci with nominal association signals ($P < 5 \times 10^{-6}$) that also showed evidence of association in the disease-specific GWAS scans. These loci included several genomic regions not previously reported as shared loci, as well as several risk factors that were previously found to be associated with both diseases. Follow-up analyses of the putatively new SSc–RA loci identified *IRF4* as a shared risk factor for these 2 diseases ($P_{combined} = 3.29 \times 10^{-12}$). Analysis of the biologic relevance of the known SSc–RA shared loci identified the type I interferon and interleukin-12 signaling pathways as the main common etiologic factors.**

Conclusion. **This study identified a novel shared locus, *IRF4*, for the risk of SSc and RA, and highlighted the usefulness of a cross-disease GWAS meta-analysis strategy in the identification of common risk loci.**

Genome-wide association studies (GWAS) and immune-focused fine-mapping studies have revolutionized our understanding of the genetic component of complex autoimmune diseases by facilitating the identification of thousands of susceptibility loci associated with autoimmunity (1). The vast majority of these loci are shared risk factors for at least 2 autoimmune diseases, pointing to a common genetic background underlying these autoimmune processes. This genetic overlap was suspected some time ago, given the high rate of co-occurrence of autoimmune diseases and the well-established familial aggregation reported for these immune disorders (1).

Systemic sclerosis (SSc) and rheumatoid arthritis (RA) are complex autoimmune diseases that have similar clinical and immunologic features. Both diseases are rheumatic connective tissue disorders, characterized by an exacerbated inflammatory response, deregulation of innate and adaptive immunity, including autoantibody production, and systemic complications. Because of the establishment of large consortiums and international collaborations, the number of confirmed RA susceptibility factors has increased up to a total of 101 loci associated with the disease at the genome-wide significance level (2). With regard to SSc, GWAS, Immunochip, and candidate gene studies have clearly identified various genetic regions involved in susceptibility to SSc (3). However, the knowledge of the genetic predisposition to this disease is relatively limited, in part due to its low prevalence, which impairs the recruitment of large cohorts required to reach a high statistical power and to effectively detect association signals. Interestingly, a considerable proportion of the SSc susceptibility factors also represent RA risk loci (2,3). In addition, although not very common, co-familiarity and co-occurrence between these 2 rheumatic conditions have been observed (4). These observations provide evidence of a genetic overlap of both diseases. Thus, it is expected that additional shared risk factors remain to be discovered.

One approach that has been developed for the identification of common loci in a cost-effective manner is to perform a combined-phenotype GWAS, that is, to combine genome-wide genotype data for 2 autoimmune diseases. This strategy has been successfully applied to the study of not only closely related phenotypes but also nonrelated phenotypes, and thus far the results have been encouraging (5).

Taking into account all of these considerations, the purpose of the present study was to systematically identify new common risk loci for SSc and RA by applying the combined-phenotype GWAS strategy, followed by replication testing in independent case–control data sets.

PATIENTS AND METHODS

Study population. The first stage of the present study, the discovery phase, included 6,537 patients with either SSc or RA and 8,741 healthy controls. The SSc GWAS panel comprised 4 case–control sets from Spain, Germany, The Netherlands, and the US (2,716 cases and 5,666 controls), whose data had been obtained in previous studies (5–7). The RA case–control GWAS panel included 2 previously published RA GWAS cohorts (the Wellcome Trust Case Control Consortium [WTCCC] and the Epidemiological Investigation of Rheumatoid Arthritis study cohort) from the UK and Sweden (3,821 cases and 3,075 controls) (8).

Subjects included in the second stage of the study, the replication phase, were drawn from independent SSc and RA case–control sets of individuals European ancestry. The SSc

Lund, Sweden; [27]Annika Nordin, MD, PhD, Leonid Padyukov, PhD: Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden; [28]Ariane Herrick, MD, PhD, Steve Eyre, MD, PhD, Jane Worthington, MD, PhD: University of Manchester, Manchester Academic Health Science Centre, Manchester, UK; [29]Bobby P. C. Koeleman, PhD: University Medical Center Utrecht, Utrecht, The Netherlands; [30]Christopher P. Denton, PhD, FRCP, Carmen Fonseca, MD, PhD: Centre for Rheumatology, Royal Free and University College Medical School, London, UK; [31]Timothy R. D. J. Radstake, MD, PhD: University Medical Center Utrecht, Utrecht, The Netherlands. Members of the Spanish Scleroderma Group are shown in Appendix A.

Address correspondence to Elena López-Isac, MSc, or to Javier Martín, MD, PhD, IPBLN-CSIC, Parque Tecnológico Ciencias de la Salud, Avenida del Conocimiento s/n 18016-Armilla, Granada, Spain. E-mail: elenalopezisac@ipb.csic.es or martin@ipb.csic.es.

Submitted for publication March 30, 2016; accepted in revised form April 19, 2016.

replication cohort included 6,114 cases and 8,744 healthy controls from 8 different countries (Spain, Germany, Italy, the UK, The Netherlands, Sweden, Norway, and the US). The healthy controls from the UK and US partially overlapped with the control sets of previously published cohorts (the WTCCC and the second North American Rheumatoid Arthritis Consortium [NARAC2]) (8). The RA replication cohort included 9 case–control collections from North America (US and Canada), Spain, The Netherlands, the UK, Sweden, France, and New Zealand, and comprised a total of 13,049 RA cases and 25,908 healthy controls. Of these, 9,711 cases and 24,253 healthy controls were obtained from several previously published studies, including the Brigham Rheumatoid Arthritis Sequential Study, NARAC1, CANADA, studies from the Rheumatoid Arthritis Consortium International (RACI-US, RACI-i2b2, RACI-UK, RACI-SE-U, and RACI-NL), Consortium of Rheumatology Researchers of North America, Vanderbilt, Dutch studies (Amsterdam Medical Center, Treatment Strategies for RA [BeSt Study], Leiden University Medical Center, and Dutch Rheumatoid Arthritis Monitoring Registry), Research in Active Rheumatoid Arthritis, and the Anti-TNF Response to Therapy collection (ACR-REF: BRAGGSS, BRAGGSS2, ERA, KI, and TEAR) (2). All of the patients with SSc and patients with RA fulfilled previously described classification criteria for each disease (2,5). All individuals enrolled in the present study provided written informed consent, and approval from the local ethics committees was obtained from all of the centers in accordance with the tenets of the Declaration of Helsinki.

Study design. We performed a 2-stage study to systematically identify SSc–RA shared risk factors, with the first stage being the discovery phase for GWAS meta-analysis of each disease separately and a combined-phenotype GWAS meta-analysis, and the second stage being the replication phase (Figure 1).

Discovery phase. We performed GWAS analysis for each disease separately and in a combined-phenotype GWAS analysis. Two different tests were considered for the combined analysis (5). In the first test, in order to detect common signals for SSc and RA with same-direction allelic effects, the meta-analysis considering both diseases was performed as usual. Those SNPs that showed an association at $P < 5 \times 10^{-6}$ in the combined-phenotype analysis (referred to as P_{combined}) and also showed nominal significance in the association study for each disease ($P < 0.05$) were selected for follow-up in the replication phase.

In the second test, in order to identify common signals with opposite-direction allelic effects, we flipped the direction of association (1/odds ratio [OR]) in the RA data set for the combined-phenotype meta-analysis. To select SNPs for replication, the same selection criteria as stated above were followed.

For both sorts of meta-analyses, we only considered for follow-up those SNPs that had not been previously reported as genetic risk factors for SSc and RA, or those that had been reported for one disease but not reported for the other.

Replication phase. The SNPs selected were followed-up in independent replication cohorts. Subsequently, we performed a meta-analysis of the initial GWAS screening and replication stages. The SNP signals that 1) reached the genome-wide significance level for association ($P_{\text{combined}} < 5 \times 10^{-8}$) in the combined-phenotype meta-analysis (GWAS + Replication phases), and that 2) showed, for each disease separately, nominally significant associations ($P < 0.05$) in the replication phase as well as significant associations ($P < 5 \times 10^{-3}$) in the GWAS + Replication meta-analysis were considered shared risk factors for the 2 analyzed diseases.

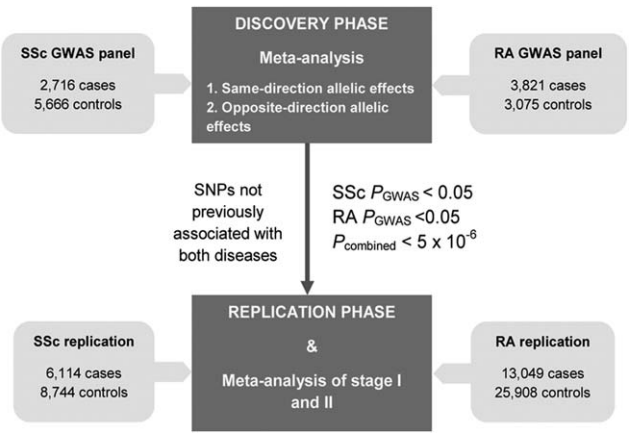

Figure 1. Overall work flow of the present study. SSc = systemic sclerosis; GWAS = genome-wide association study; RA = rheumatoid arthritis; SNPs = single-nucleotide polymorphisms.

Quality control and genotype imputation of GWAS data. We applied stringent quality control criteria in all of the GWAS data sets. Cutoff values for the sample call rate and the SNP call rate were set as 95%. Markers with allele distributions deviating from Hardy-Weinberg equilibrium (HWE) ($P < 0.001$) in controls from any of the populations analyzed separately were excluded. Markers with minor allele frequencies lower than 1% were filtered out. After quality control, we performed whole-genome genotype imputation with IMPUTE2 software (9) using as reference panels the CEU (Utah residents with northern and western European ancestry from the CEPH collection) and TSI (Toscani in Italy) populations of the HapMap Phase 3 project (available at http://www.hapmap.org). Imputed SNP quality was assessed by establishing a probability threshold for merging genotypes at 0.9. Subsequently, stringent quality control was applied to the imputed data using the same criteria as stated above. Thereafter, genome-wide genotyping data were available for a total of 219,756 SNPs.

The first 5 principal components were estimated, and individuals deviating more than 6 SDs from the cluster centroids were considered outliers. In addition, duplicate pairs or highly related individuals among data sets were also removed on the basis of pairwise comparisons, using the Genome function in Plink version 1.7 (see http://pngu.mgh.harvard.edu/purcell/plink/) (Pi-HAT threshold of 0.5).

Follow-up genotyping. The genotyping of the replication cohorts was performed with either TaqMan SNP genotyping technology in a LightCycler 480 Real-Time polymerase chain reaction system (Roche Applied Science) or the GWAS and Immunochip platforms.

For the SSc study, all cases were genotyped using the TaqMan genotyping system, with TaqMan 5′ allele discrimination predesigned assays from Applied Biosystems. The genotyping call rate was >95% for the 3 SNPs. The control samples were also genotyped using this technology, with the exception of the UK and US cohorts. For these 2 control

cohorts, genotyping data were obtained from previously published genome-wide genotyping data sets (from the WTCCC and NARAC2) (8).

RA cases from Spain and New Zealand and the Spanish controls were genotyped by TaqMan technology. Genotype data for the New Zealand healthy controls partially overlapped with those from a previous GWAS report (10). For the remaining RA case–control sets, genotype frequencies and association data were obtained from a previously published study (2). The genotype methods used in these studies were described in detail in the study by Okada et al (2). For those cohorts in which genotyping was performed using the Illumina Immunochip platform, only data for the rs9328192 SNP of the interferon regulatory factor 4 (IRF-4) gene (*IRF4*) were available.

Statistical analysis. All data were analyzed using Plink software. To test for association, we performed logistic regression analysis in each of the SSc and RA GWAS cohorts separately. The first 5 principal components were included as covariates to control for any potential population stratification effects. The replication cohorts were also analyzed by logistic regression analysis. The meta-analyses were performed with the inverse-variance method based on population-specific logistic regression results. Heterogeneity of the ORs across studies was assessed using Cochran's Q test. HWE was tested in all of the validation cohorts genotyped by TaqMan technology (in HWE analyses, $P < 0.01$ was considered to show significant deviation from equilibrium). None of the included control cohorts showed significant deviation from HWE, with the exception of *HNF1A* rs10774577. The cohorts in which HWE was not observed were excluded from the analysis of this specific SNP. The statistical power of the combined-phenotype analysis and the analysis for each disease separately is shown in Supplementary Table 1 (available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39730/abstract).

RESULTS

Discovery analysis. In the first phase of this study, we conducted a cross-disease meta-analysis in order to systematically identify new putatively shared loci between SSc and RA. The overall workflow of the study is illustrated in Figure 1.

The meta-analysis combining both data sets identified various SNPs from 7 distinct genomic regions that showed a significant association at the level of $P < 5 \times 10^{-6}$, as well as a nominal signal of association ($P < 0.05$) in the disease-specific analyses. The strongest association was found in the well-accepted SSc- and RA-associated locus *IRF5* ($P_{combined} = 8.44 \times 10^{-17}$; for SSc, $P_{GWAS} = 1.14 \times 10^{-16}$; for RA, $P_{GWAS} = 7.86 \times 10^{-4}$). Three additional known SSc–RA loci, namely *PTPN22*, *ATG5*, and *BLK*, were also identified at the initial discovery stage (Figure 2) (see also Supplementary Table 2 and Supplementary Figure 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39730/abstract). The remaining SNPs were located in 3 different loci, including *FBN2* and *HNF1A*, neither of which has been previously reported as a genetic risk factor for SSc and RA, and *IRF4*, which has been found to be associated with RA in previous studies (Table 1 and Figure 2) (see also Supplementary Figure 2 on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39730/abstract). Interestingly, the regional association plots of the *FBN2*, *IRF4*, and *HNF1A* loci showed that the top SNPs in the combined-phenotype analysis were also the top SNPs in the analyses for SSc and RA separately, or at least were in high linkage disequilibrium with the top signal observed for each disease (see Supplementary Figure 2).

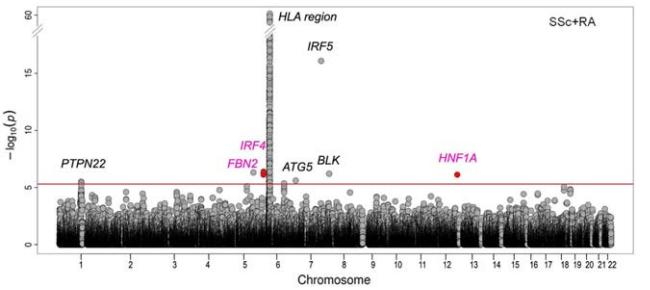

Figure 2. Manhattan plot showing the results of the cross-disease meta–genome-wide association study. The $-\log_{10}$ of the combined-phenotype meta-analysis P values are plotted against their physical chromosomal position. The plot displays the $-\log_{10} P$ values from the same-direction meta-analysis of associations with systemic sclerosis (SSc) and rheumatoid arthritis (RA). The signals from the opposite-direction meta-analysis that reached the selection criteria are also plotted (red points). The red line represents the threshold of significance at $P < 5 \times 10^{-6}$. Those loci with single-nucleotide polymorphisms that reached the selection criteria for the replication phase are plotted (loci selected for follow-up are highlighted in pink).

These new putatively shared SNPs were selected for follow-up in additional SSc and RA replication cohorts. For *IRF4*, 3 SNPs met our criteria for being selected for validation in the replication phase. In this case, we selected the SNP with the lowest P value for association (see Supplementary Table 2).

Replication phase and meta-analysis. According to the established thresholds discussed above in Patients and Methods, we identified 1 new association signal shared between SSc and RA at SNP rs9328192 of *IRF4* ($P_{combined} = 3.29 \times 10^{-12}$). Furthermore, this *IRF4* SNP almost reached genome-wide significance in the meta-analysis for each disease separately (for SSc, $P_{GWAS + Replication} = 2.78 \times 10^{-7}$, OR 0.90; for RA, $P_{GWAS + Replication} = 1.44 \times 10^{-6}$, OR 1.08) (Table 1).

Regarding the *HNF1A* and *FBN2* genetic variants, despite the initial suggestive association signals found in the first stage, these loci did not show genome-wide significance in our combined-phenotype meta-analysis.

Table 1. Results of the cross-disease meta-GWAS of disease associations with 3 selected SNPs*

Locus (Chr.)	SNP	Ref. allele	*P* for SSc–RA GWAS	SSc						RA						SSc–RA GWAS + Replication	
				GWAS		Replication		GWAS + Replication (meta-GWAS)		GWAS		Replication		GWAS + Replication (meta-GWAS)			
				P	OR†	*P*	OR†	*P*	OR†	*P*	OR†	*P*	OR†	*P*	OR†	*P*	Current status
FBN2 (5)	rs6897611	T	4.79×10^{-7}	2.85×10^{-3}	1.16	0.641	0.98	0.165	1.04	3.15×10^{-5}	1.24	0.684	0.99	0.650‡	1.02‡	0.018	–
IRF4 (6)	rs9328192	G	4.06×10^{-7}	8.86×10^{-6}	0.86	1.89×10^{-3}	0.93	2.78×10^{-7}	0.90	7.26×10^{-3}	1.10	5.22×10^{-5}	1.07	1.44×10^{-6}	1.08	3.29×10^{-12}	SSc–RA; RA
HNF1A (12)	rs10774577§	T	7.53×10^{-7}	8.62×10^{-4}	0.89	0.036	0.94	1.64×10^{-4}	0.91	2.50×10^{-4}	1.14	0.290	1.03	0.208‡	1.05‡	1.59×10^{-6}	–

* GWAS = genome-wide association study; SNPs = single-nucleotide polymorphisms; Chr. = chromosome.
† Represents the odds ratio (OR) for the reference (Ref.) allele.
‡ *P* values and ORs were derived from meta-analysis under random effects due to heterogeneity of the ORs among cohorts.
§ The rheumatoid arthritis (RA) and systemic sclerosis (SSc) replication cohorts from Spain, and the SSc replication cohorts from Italy and The Netherlands were excluded from the analysis of rs10774577 due to issues with Hardy-Weinberg equilibrium.

Nevertheless, *HNF1A* rs10774577 showed suggestive evidence of association in the meta-analysis performed in the SSc data set ($P_{\text{Replication}} = 0.036$, OR 0.94; $P_{\text{GWAS + Replication}} = 1.64 \times 10^{-4}$, OR 0.91), and showed an association at the level of $P = 1.59 \times 10^{-6}$ in the combined-phenotype meta-analysis. Considering that this SNP was not included in those cohorts that were genotyped with Immunochip, the present study had a lower statistical power for the analysis of this genomic region. Therefore, the possibility of a slight or modest genetic effect of *HNF1A* rs10774577 on both diseases cannot be ruled out, and further studies will be required to establish whether this locus is a shared SSc–RA risk factor.

DISCUSSION

In the present study, we identified a novel non-HLA susceptibility locus that is shared between SSc and RA, namely *IRF4*, using a combined-phenotype GWAS strategy in large case–control cohorts of patients with SSc and those with RA. This locus, *IRF4*, was already reported to be involved in RA susceptibility, but had not been previously associated with SSc (2).

The cross-disease meta-analysis performed with the SSc and RA GWAS data sets identified various SNPs from 7 different loci that met our stringent selection criteria for the replication phase ($P_{\text{combined}} < 5 \times 10^{-6}$; for SSc and for RA, each $P_{\text{GWAS}} < 0.05$). Four of the 7 SNPs were already known risk factors for SSc and RA (*PTPN22*, *ATG5*, *IRF5*, and *BLK*), thus providing support for the effectiveness of this strategy in the identification of shared risk loci (2,3). It is worth mentioning that these loci were detected by the 2 different tests used in the first phase, which were performed in order to detect both same-direction and opposite-direction allelic effects. In fact, the shared *IRF4* SNP newly identified in this study showed opposite effects for SSc and for RA (protective effect and risk effect, respectively). This discrepancy might be attributable to the fact that the actual causal variants for the associations in each disease could be different, and that *IRF4* rs9328192 is tagging them. This discordant phenomenon is particularly common between autoimmune diseases (1). However, to completely understand these discordant effects, the interaction with other genetic variants contributing to disease susceptibility should be considered, in addition to analyzing the precise biologic impact of the associations.

The associated *IRF4* SNP (rs9328192) showed modest effect sizes for SSc and RA. However, we were able to capture this association in our meta-analysis because of the large cohort used in this study, together with the combined-phenotype approach, which allowed us to increase the statistical power. This highlights the capability of the combined-phenotype approach in the identification of shared variants with low penetrance, whose associations might have been missed in disease-specific GWAS due to a lack of power (11).

IRF-4 belongs to the IRF family of transcription factors and plays a pivotal role in the development and function of several autoimmune-associated cells (12). Various genetic and functional studies have pointed to IRF-4 as a master regulator for autoimmunity (12,13). It has been demonstrated that IRF-4 is a crucial factor for the editing and L-chain rearrangements of the B cell receptor, and for pre–B cell expansion, both of which are processes directly related to the development of autoimmunity (14). In addition, IRF-4 is a critical controller of Th17 cell differentiation and the production of interleukin-17 (IL-17) and IL-21 (12), which are components of the immune system that play a key role in the pathogenesis of SSc and RA.

The results of the present study add another *IRF* to the list of *IRF*s associated with SSc (*IRF4*, *IRF5*, *IRF7*, and *IRF8*) and RA (*IRF4*, *IRF5*, and *IRF8*) (2,3), thus providing genetic support for the type I interferon (IFN) signature described in patients with SSc and those with RA (15). Moreover, our pathway enrichment analysis also identified the type I IFN signaling pathway as one of the most relevant common pathways between SSc and RA on the basis of their common genetic background (see Supplementary Methods, Supplementary Table 3, and Supplementary Figure 3, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39730/abstract). Therefore, deregulation of this signaling pathway might be a biologic process that underlies the onset of these 2 autoimmune rheumatic conditions.

In summary, through a cross-disease meta-analysis of GWAS for SSc and RA, we were able to identify *IRF4* as a new shared susceptibility locus for these 2 autoimmune diseases. The results of the present study, taken together with the findings from previous studies, reinforce the idea of a common genetic background between SSc and RA. The identification of these pleiotropic autoimmunity loci may point to common pathogenic pathways, which ultimately may represent a clinical advantage in that it may provide support for drug repositioning on the basis of the true understanding of the pathogenic mechanisms of SSc and RA.

ACKNOWLEDGMENTS

We thank Sofia Vargas, Sonia García, and Gema Robledo for their excellent technical assistance, and all of the

patients and control donors for their essential collaboration. We thank National DNA Bank Carlos III (University of Salamanca, Spain) for supplying part of the control DNA samples. We also would like to thank the following organizations: the European League Against Rheumatism Scleroderma Trials and Research Group, the German Network of Systemic Sclerosis, the US Scleroderma Foundation, and the US Raynauds & Scleroderma Association.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Drs. López-Isac and J. Martín had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Fonseca, Radstake, Worthington, Mayes, J. Martín.

Acquisition of data. Assassi, Simeón, Carreira, Ortego-Centeno, Freire, Beltrán, Narváez, Alegre-Sancho, Fernández-Gutiérrez, Balsa, Ortiz, González-Gay, Beretta, Santaniello, Bellocchi, Lunardi, Moroncini, Gabrielli, Witte, Hunzelmann, Distler, Riekemasten, van der Helm-van Mil, de Vries-Bouwstra, Magro-Checa, Voskuyl, Vonk, Molberg, Merriman, Hesselstrand, Nordin, Padyukov, Herrick, Eyre, Koeleman, Denton, Fonseca, Radstake, Worthington, Mayes, J. Martín.

Analysis and interpretation of data. López-Isac, J.-E. Martín.

REFERENCES

1. Parkes M, Cortes A, van Heel DA, Brown MA. Genetic insights into common pathways and complex relationships among immune-mediated diseases. Nat Rev Genet 2013;14:661–73.
2. Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 2014;506:376–81.
3. Bossini-Castillo L, Lopez-Isac E, Martin J. Immunogenetics of systemic sclerosis: defining heritability, functional variants and shared-autoimmunity pathways. J Autoimmun 2015;64:53–65.
4. Elhai M, Avouac J, Kahan A, Allanore Y. Systemic sclerosis at the crossroad of polyautoimmunity. Autoimmun Rev 2013;12: 1052–7.
5. Martin JE, Assassi S, Diaz-Gallo LM, Broen JC, Simeon CP, Castellvi I, et al. A systemic sclerosis and systemic lupus erythematosus pan-meta-GWAS reveals new shared susceptibility loci. Hum Mol Genet 2013;22:4021–9.
6. Radstake TR, Gorlova O, Rueda B, Martin JE, Alizadeh BZ, Palomino-Morales R, et al. Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. Nat Genet 2010;42:426–9.
7. Martin JE, Broen JC, Carmona FD, Teruel M, Simeon CP, Vonk MC, et al. Identification of CSK as a systemic sclerosis genetic risk factor through genome wide association study follow-up. Hum Mol Genet 2012;21:2825–35.
8. Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP, et al. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat Genet 2010;42:508–14.
9. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 2009;5:e1000529.
10. Consortium TAaNZMSG. Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. Nat Genet 2009;41:824–8.
11. Festen EA, Goyette P, Green T, Boucher G, Beauchamp C, Trynka G, et al. A meta-analysis of genome-wide association scans identifies IL18RAP, PTPN2, TAGAP, and PUS10 as shared risk loci for Crohn's disease and celiac disease. PLoS Genet 2011;7:e1001283.
12. Xu WD, Pan HF, Ye DQ, Xu Y. Targeting IRF4 in autoimmune diseases. Autoimmun Rev 2012;11:918–24.
13. Biswas PS, Gupta S, Stirzaker RA, Kumar V, Jessberger R, Lu TT, et al. Dual regulation of IRF4 function in T and B cells is required for the coordination of T-B cell interactions and the prevention of autoimmunity. J Exp Med 2012;209:581–96.
14. Zouali M. Receptor editing and receptor revision in rheumatic autoimmune diseases. Trends Immunol 2008;29:103–9.
15. Ronnblom L, Eloranta ML. The interferon signature in autoimmune diseases. Curr Opin Rheumatol 2013;25:248–53.

APPENDIX A: MEMBERS OF THE SPANISH SCLERODERMA GROUP

Members of the Spanish Scleroderma Group include the following: Raquel Ríos and Jose Luis Callejas (Hospital Clínico Universitario San Cecilio, Granada), José Antonio Vargas Hitos (Hospital Virgen de las Nieves, Granada), Rosa García Portales (Hospital Virgen de la Victoria, Málaga), María Teresa Camps (Hospital Carlos Haya, Málaga), Antonio Fernández-Nebro (Hospital Carlos Haya, Málaga), María F. González-Escribano (Hospital Virgen del Rocío, Seville), Francisco José García-Hernández and M[a] Jesús Castillo (Hospital Virgen del Rocío, Seville), M[a] Ángeles Aguirre and Inmaculada Gómez-Gracia (Hospital Reina Sofía/IMIBIC, Córdoba), Luis Rodríguez-Rodríguez (Hospital Clínico San Carlos, Madrid), Paloma García de la Peña (Madrid Norte Sanchinarro Hospital, Madrid), Esther Vicente (Hospital La Princesa, Madrid), José Luis Andreu and Mónica Fernández de Castro (Hospital Puerta de Hierro Majadahonda, Madrid), Francisco Javier López-Longo and Lina Martínez (Hospital General Universitario Gregorio Marañón, Madrid), Vicente Fonollosa and Alfredo Guillén (Hospital Valle de Hebrón, Barcelona), Iván Castellví (Santa Creu i Sant Pau University Hospital, Barcelona), Gerard Espinosa (Hospital Clinic, Barcelona), Carlos Tolosa (Hospital Parc Tauli, Sabadell), Anna Pros (Hospital Del Mar, Barcelona), Mónica Rodríguez Carballeira (Hospital Universitari Mútua Terrasa, Barcelona), Francisco Javier Narváez (Hospital Universitari de Bellvitge, Barcelona), Manel Rubio Rivas (Hospital Universitari de Bellvitge, Barcelona), Vera Ortiz-Santamaría (Hospital General de Granollers, Granollers), Ana Belén Madroñero (Hospital General San Jorge, Huesca), Bernardino Díaz and Luis Trapiella (Hospital Central de Asturias, Oviedo), Adrián Sousa (Hospital Xeral-Complexo Hospitalario Universitario de Vigo, Vigo), María Victoria Egurbide (Hospital Universitario Cruces, Barakaldo), Patricia Fanlo Mateo (Hospital Virgen del Camino, Pamplona), Luis Sáez-Comet (Hospital Universitario Miguel Servet, Zaragoza), Federico Díaz and Vanesa Hernández (Hospital Universitario de Canarias, Tenerife), Emma Beltrán (Hospital General Universitario de Valencia, Valencia), José Andrés Román-Ivorra and Elena Grau (Hospital Universitari i Politecnic La Fe, Valencia), Juan José Alegre-Sancho (Hospital Universitari Doctor Peset, Valencia), Francisco J. Blanco García and Natividad Oreiro (INIBIC-Hospital Universitario A Coruña, La Coruña).