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Evidence for Gene–Gene Epistatic Interactions Among Susceptibility Loci for Systemic Lupus Erythematosus

Travis Hughes,[1] Adam Adler,[1] Jennifer A. Kelly,[1] Kenneth M. Kaufman,[2] Adrienne H. Williams,[3] Carl D. Langefeld,[3] Elizabeth E. Brown,[4] Graciela S. Alarcón,[4] Robert P. Kimberly,[4] Jeffrey C. Edberg,[4] Rosalind Ramsey-Goldman,[5] Michelle Petri,[6] Susan A. Boackle,[7] Anne M. Stevens,[8] John D. Reveille,[9] Elena Sanchez,[1] Javier Martín,[10] Timothy B. Niewold,[11] Luis M. Vilá,[12] R. Hal Scofield,[2] Gary S. Gilkeson,[13] Patrick M. Gaffney,[1] Lindsey A. Criswell,[14] Kathy L. Moser,[1] Joan T. Merrill,[15] Chaim O. Jacob,[16] Betty P. Tsao,[17] Judith A. James,[15] Timothy J. Vyse,[18] Marta E. Alarcón-Riquelme[19] on behalf of the BIOLUPUS Network, John B. Harley,[20] Bruce C. Richardson,[21] and Amr H. Sawalha[2]

Objective. **Several confirmed genetic susceptibility loci for lupus have been described. To date, no clear evidence for genetic epistasis in lupus has been established. The aim of this study was to test for gene–gene interactions in a number of known lupus susceptibility loci.**

Methods. **Eighteen single-nucleotide polymorphisms tagging independent and confirmed lupus susceptibility loci were genotyped in a set of 4,248 patients with lupus and 3,818 normal healthy control subjects of European descent. Epistasis was tested by a 2-step approach using both parametric and nonparametric**

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[1]Travis Hughes, BS, Adam Adler, BS, Jennifer A. Kelly, MPH, Elena Sanchez, PhD, Patrick M. Gaffney, MD, Kathy L. Moser, PhD: Oklahoma Medical Research Foundation, Oklahoma City; [2]Kenneth M. Kaufman, PhD, R. Hal Scofield, MD, Amr H. Sawalha, MD: Oklahoma Medical Research Foundation, University of Oklahoma Health Sciences Center, and Oklahoma City VA Medical Center, Oklahoma City; [3]Adrienne H. Williams, MA, Carl D. Langefeld, PhD: Wake Forest University Health Sciences Center, Winston-Salem, North Carolina; [4]Elizabeth E. Brown, PhD, MPH, Graciela S. Alarcón, MD, MPH, Robert P. Kimberly, MD, Jeffrey C. Edberg, PhD: University of Alabama at Birmingham; [5]Rosalind Ramsey-Goldman, MD, DrPH: Northwestern University Feinberg School of Medicine, Chicago, Illinois; [6]Michelle Petri, MD, MPH: Johns Hopkins University School of Medicine, Baltimore, Maryland; [7]Susan A. Boackle, MD: University of Colorado Denver School of Medicine, Aurora; [8]Anne M. Stevens, MD, PhD: University of Washington and Seattle Children's Research Institute, Seattle; [9]John D. Reveille, MD: University of Texas Health Science Center at Houston; [10]Javier Martín, MD, PhD: Instituto de Parasitología y Biomedicina López-Neyra, CSIC, Granada, Spain; [11]Timothy B. Niewold, MD: University of Chicago, Chicago, Illinois; [12]Luis M. Vilá, MD: University of Puerto Rico, San Juan, Puerto Rico; [13]Gary S. Gilkeson, MD: Medical University of South Carolina, Charleston; [14]Lindsey A. Criswell, MD, MPH, DSc: University of California, San Francisco; [15]Joan T. Merrill, MD, Judith A. James, MD, PhD: Oklahoma Medical Research Foundation and University of Oklahoma Health Sciences Center, Oklahoma City; [16]Chaim O. Jacob, MD, PhD: University of Southern California, Los Angeles; [17]Betty P. Tsao, PhD: University of California, Los Angeles; [18]Timothy J. Vyse, MBBS, MA, MRCP, PhD: King's College London and Guy's Hospital, London, UK; [19]Marta E. Alarcón-Riquelme, MD, PhD: Oklahoma Medical Research Foundation, Oklahoma City, and Center for Genomics and Oncological Research Pfizer–University of Granada–Junta de Andalucia, Granada, Spain; [20]John B. Harley, MD, PhD: Cincinnati Children's Hospital Medical Center and Cincinnati VA Medical Center, Cincinnati, Ohio; [21]Bruce C. Richardson, MD, PhD: University of Michigan and Ann Arbor VA Medical Center, Ann Arbor.

Address correspondence to Amr H. Sawalha, MD, Oklahoma Medical Research Foundation, 825 Northeast 13th Street, MS#24, Oklahoma City, OK 73104. E-mail: amr-sawalha@omrf.ouhsc.edu.

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methods. The false discovery rate (FDR) method was used to correct for multiple testing.

***Results.* We detected and confirmed gene–gene interactions between the HLA region and *CTLA4*, *IRF5*, and *ITGAM* and between *PDCD1* and *IL21* in patients with lupus. The most significant interaction detected by parametric analysis was between rs3131379 in the HLA region and rs231775 in *CTLA4* (interaction odds ratio 1.19, Z = 3.95, $P = 7.8 \times 10^{-5}$ [FDR ≤0.05], *P* for multifactor dimensionality reduction = 5.9×10^{-45}). Importantly, our data suggest that in patients with lupus, the presence of the HLA lupus risk alleles in rs1270942 and rs3131379 increases the odds of also carrying the lupus risk allele in *IRF5* (rs2070197) by 17% and 16%, respectively ($P = 0.0028$ and $P = 0.0047$, respectively).**

***Conclusion.* We provide evidence for gene–gene epistasis in systemic lupus erythematosus. These findings support a role for genetic interaction contributing to the complexity of lupus heritability.**

Recent candidate gene and genome-wide association studies led to the discovery and validation of multiple susceptibility loci for systemic lupus erythematosus (SLE) (1). However, the heritability of lupus cannot be completely explained by the susceptibility loci already discovered. We suggest that the missing heritability in lupus can be explained by 3 potential mechanisms: a heritable epigenetic component, common and rare disease susceptibility variants yet to be discovered, and gene–gene interactions involving known and perhaps yet to be discovered genetic variants for disease susceptibility. The data regarding gene–gene interaction (epistasis) in lupus (2,3) are very limited and controversial. Consequently, it is widely accepted that the known lupus susceptibility loci operate additively rather than epistatically to increase the risk of lupus.

Herein, we sought to examine gene–gene interactions in some of the previously established and confirmed susceptibility loci for lupus, using a large set of patients with lupus and control subjects. We identified and confirmed 6 novel gene–gene interactions for lupus, using both parametric and nonparametric statistical methods.

PATIENTS AND METHODS

Study participants and genotyping. A total of 4,248 patients with lupus and 3,818 normal healthy control subjects of European descent were included in this study. Eighteen single-nucleotide polymorphisms (SNPs) representing previously confirmed and independent autosomal lupus susceptibility loci were genotyped (Table 1). A summary of the allelic association results in these loci, based on the patients and controls included in this study, is shown in Table 2.

Two tag SNPs in the HLA region were genotyped. These 2 SNPs were selected because they were recently shown

Table 1. Previously reported lupus susceptibility loci analyzed for gene–gene interaction in this study*

Gene/region	Chromosome	Associated SNP	Risk allele	OR†	Reference
BANK1	4q24	rs10516487	G	1.38	26
C8orf13-BLK	8p22–23	rs13277113	A	1.39	27
CTLA4	2q33	rs231775	G	1.23	28
FCGR2A	1q23	rs1801274	C	1.35	29
HLA region 1	6p21.33	rs3131379	A	2.36	4
HLA region 2	6p21.32	rs1270942	G	2.35	4
IL21	4q26	rs907715	G	1.29	30
IRF5	7q32	rs2070197	C	1.85‡	5
IRF5	7q32	rs729302	A	1.39‡	5
IRF5	7q32	rs10954213	A	1.25‡	5
ITGAM	16p11.2	rs1143679	A	1.78	31
KIAA1542	11p15.5	rs4963128	C	1.28	4
MBL	10q11	rs1800450	A	1.41	32
PDCD1	2q37.3	rs11568821	A	2.85	33
PTPN22	1p13	rs2476601	A	1.53	34
PXK	3p14.3	rs6445975	C	1.25	4
STAT4	2q32.2	rs7574865	T	1.55	35
TNFSF4	1q25	rs2205960	T	1.28	36

* SNP = single-nucleotide polymorphism; OR = odds ratio.
† Patients with systemic lupus erythematosus versus healthy control subjects, as reported in previous studies.
‡ Transmitted:untransmitted ratio, based on trio and family studies.

Table 2. Genetic association analysis for each locus included in the gene–gene interaction analysis, using patients and controls included in this study*

Gene	SNP	Associated allele	Frequency		P	OR (95% CI)
			Patients	Controls		
BANK1	rs10516487	G	0.738	0.693	1.66×10^{-9}	1.25 (1.16–1.34)
C8orf13-BLK	rs13277113	A	0.291	0.238	6.73×10^{-13}	1.32 (1.22–1.42)
CTLA4	rs231775	G	0.361	0.347	0.074	1.06 (0.99–1.14)
FCGR2A	rs1801274	G	0.541	0.508	6.59×10^{-5}	1.14 (1.07–1.22)
HLA region 1	rs3131379	A	0.177	0.094	1.04×10^{-47}	2.06 (1.87–2.27)
HLA region 2	rs1270942	G	0.177	0.094	1.45×10^{-48}	2.08 (1.88–2.29)
IL21	rs907715	G	0.686	0.656	8.78×10^{-5}	1.15 (1.07–1.23)
IRF5	rs2070197	G	0.175	0.104	3.73×10^{-35}	1.83 (1.66–2.01)
IRF5	rs729302	A	0.743	0.678	1.70×10^{-18}	1.38 (1.28–1.48)
IRF5	rs10954213	A	0.680	0.625	2.53×10^{-12}	1.28 (1.19–1.36)
ITGAM	rs1143679	A	0.194	0.126	5.30×10^{-29}	1.67 (1.52–1.83)
KIAA1542	rs4963128	G	0.708	0.667	1.13×10^{-7}	1.21 (1.13–1.30)
MBL	rs1800450	A	0.145	0.139	0.305	1.05 (0.96–1.15)
PDCD1	rs11568821	G	0.888	0.882	0.213	1.07 (0.96–1.18)
PTPN22	rs2476601	A	0.109	0.081	4.66×10^{-9}	1.40 (1.25–1.56)
PXK	rs6445975	C	0.293	0.264	0.000105	1.15 (1.07–1.24)
STAT4	rs7574865	A	0.307	0.225	1.60×10^{-28}	1.53 (1.42–1.65)
TNFSF4	rs2205960	A	0.269	0.214	6.89×10^{-15}	1.35 (1.25–1.46)

* SNP = single-nucleotide polymorphism; OR = odds ratio; 95% CI = 95% confidence interval.

to have independent genetic effects, by logistic regression analysis of a large number of lupus-associated SNPs in the HLA region (4). Likewise, 3 tag SNPs representing independent genetic susceptibility effects in *IRF5* were genotyped (5). All lupus patients fulfilled the American College of Rheumatology criteria for the classification of SLE (6,7). Genotyping was performed using an Illumina custom bead system on an iScan instrument as part of a large lupus candidate gene association study, to reduce the cost of genotyping and maximize sample size. We genotyped 347 ancestry-informative markers in all of the samples included in this study (8–11).

Individuals with a genotype success rate of <90% (361 samples) were excluded from the analysis. The remaining samples were then evaluated for duplicates or related individuals, and one individual from each pair was removed (117 samples) if the proportion of alleles shared identical by descent was >0.4. Samples were assessed for mismatches between the reported sex of the individual and his or her genetic data. One hundred twelve samples were removed from the analysis because they did not meet the following criteria: an assigned male subject was required to have chromosome X heterozygosity of ≤10% and to be heterozygous at rs2557524, and an assigned female subject was required to have chromosome X heterozygosity of >10% and to be homozygous at rs2557524. The SNP rs2557524 is mapped on a region on chromosomes X and Y that is identical except for this one base. Because of this one-base difference, male subjects generate a heterozygous genotype (due to the presence of both X and Y chromosomes), and female subjects generate a homozygous genotype (due to the presence of only X chromosomes).

Next, samples with increased heterozygosity (>5 SDs from the mean) were removed from the analysis (n = 5). Finally, 42 genetic outliers, as determined by principal components analysis, were removed from further analysis. An additional 2 outlier samples identified by admixture proportions calculated using AdmixMap were also removed. After the quality control measures detailed above were applied, samples from the following individuals were included in our analysis: 3,936 lupus patients of European descent (3,592 women and 344 men), and 3,491 healthy control subjects of European descent (2,340 women and 1,151 men).

Detection of gene–gene interaction. Testing for gene–gene interaction was performed sequentially, using 2 independent statistical approaches. First, a parametric analysis for epistasis was applied as implemented in Plink (12). Epistatic interactions detected using Plink were validated using allelic 2×2 tables among lupus patients to calculate interaction odds ratios (ORs) and identify the specific alleles in each SNP pair that contributed to the interaction detected. Allelic 2×2 tables (Figure 1) were obtained from 3×3 genotypic tables (Figure 2) for each interaction tested. The allelic 2×2 tables are based on 4n allele counts, where n is the total number of individuals, with each individual contributing a total of 4 independent alleles. Z scores were calculated as the natural logarithm of the OR divided by the square root of the variance, and associated P values were assigned from the Z scores for each interaction. Chi-square statistics for pairwise interaction were calculated as were chi-square–derived P values. Second, a pairwise nonparametric epistasis test was applied utilizing multifactor dimensionality reduction (MDR) analysis (13,14). The false discovery rate (FDR) method as described by Benjamini and Hochberg was used to correct for multiple comparisons (15,16).

RESULTS

To test for gene–gene interactions within the known lupus susceptibility loci examined, we performed a 2-step epistasis analysis using a parametric approach,

rs231775		rs3131379 A	rs3131379 G
	G	1086	4556
	A	1670	8308

rs231775		rs1270942 G	rs1270942 A
	G	1083	4553
	A	1671	8305

rs2070197		rs1270942 G	rs1270942 A
	G	541	2211
	A	2239	10737

rs2070197		rs313179 A	rs313179 G
	G	538	2214
	A	2244	10740

rs3131379		rs1143679 A	rs1143679 G
	G	2554	10356
	A	488	2290

rs907715		rs11568821 A	rs11568821 G
	A	576	4158
	G	1107	9230

Figure 1. Allelic 2 × 2 tables used to calculate interaction odds ratios and identify the specific alleles in each single-nucleotide polymorphism pair that contributed to the interaction detected.

followed by a nonparametric analysis. This 2-step approach has the strength of examining and confirming epistatic interactions using 2 independent statistical methods. This is necessary, because the best methodology for detecting gene–gene interaction remains controversial.

We first used a case-only pairwise epistasis analysis implemented in Plink. The case-only analysis was selected because it was shown to be a more powerful test for epistasis compared with case–control analysis (17,18). Interactions with an FDR of ≤0.05 were considered established, and those with an FDR of >0.05

rs231775		rs3131379 AA	rs3131379 AG	rs3131379 GG
	GG	20	183	316
	AG	54	532	1197
	AA	34	447	1122

rs231775		rs1270942 GG	rs1270942 AG	rs1270942 AA
	GG	19	185	314
	AG	54	529	1199
	AA	34	449	1120

rs2070197		rs1270942 GG	rs1270942 AG	rs1270942 AA
	GG	8	36	81
	AG	32	373	721
	AA	68	765	1848

rs2070197		rs3131379 AA	rs3131379 AG	rs3131379 GG
	GG	8	36	81
	AG	32	370	724
	AA	69	767	1847

rs3131379		rs1143679 AA	rs1143679 AG	rs1143679 GG
	GG	113	852	1677
	AG	32	334	805
	AA	5	35	69

rs907715		rs11568821 AA	rs11568821 AG	rs11568821 GG
	AA	5	97	279
	AG	18	326	1261
	GG	22	329	1431

Figure 2. Genotypic 3 × 3 tables used to generate the allelic 2 × 2 tables shown in Figure 1.

Table 3. Gene–gene interaction results in 18 known independent lupus susceptibility loci, using logistic regression analysis implemented in Plink*

Locus	Polymorphism	Risk allele	Interacting alleles	Interaction OR	Z score	*P* for Z score	χ^2	*P* for χ^2
CTLA4	rs231775 (A/G)	G						
HLA	rs3131379 (A/G)	A	GXA	1.19	3.95	7.8×10^{-5}	15.19	9.7×10^{-5}
CTLA4	rs231775 (A/G)	G						
HLA	rs1270942 (A/G)	G	GXG	1.18	3.88	1.0×10^{-4}	14.87	1.0×10^{-4}
IRF5	rs2070197 (A/G)	G						
HLA	rs1270942 (A/G)	G	GXG	1.17	2.99	0.0028	8.93	0.0028
IRF5	rs2070197 (A/G)	G						
HLA	rs3131379 (A/G)	A	GXA	1.16	2.83	0.0047	7.98	0.0047
HLA	rs3131379 (A/G)	A						
ITGAM	rs1143679 (A/G)	A	GXA	1.16	2.67	0.0075	6.93	0.0085
IL21	rs907715 (A/G)	G						
PDCD1	rs11568821 (A/G)	A	AXA	1.16	2.64	0.0084	6.80	0.0091

* Only interactions with a false discovery rate of ≤0.25 are shown. Z scores were calculated as the natural logarithm of the odds ratio (OR) divided by the square root of the variance.

and ≤0.25 were considered suggestive interactions that require confirmation. A high FDR was used in the initial screening for suggestive interactions to avoid excluding true gene–gene interactions from confirmatory analyses.

We identified 6 gene–gene interactions using parametric analysis (Table 3). The 2 most significant interactions were between *CTLA4* and the 2 SNPs representing 2 independent genetic effects within the HLA region (FDR ≤0.05). The detected epistasis signal between the risk alleles in *CTLA4* and rs3131379 (HLA region 1) and *CTLA4* and rs1270942 (HLA region 2) showed interaction ORs of 1.19 and 1.18, respectively (Z = 3.95 [$P = 7.8 \times 10^{-5}$] and Z = 3.88 [$P = 1.0 \times 10^{-4}$], respectively). These data indicated that in lupus patients, the presence of the lupus risk allele in *CTLA4* increases the odds of carrying the risk allele in either of the HLA lupus-associated loci by ~20%, and vice versa (Figure 1). Four additional suggestive gene–gene interactions (FDR ≤0.25) were observed between the HLA region and *IRF5*, the HLA region and *ITGAM*, and *IL21* and *PDCD1* (Table 3). The presence of the risk allele in the 2 HLA lupus-associated loci examined (rs1270942 and rs3131379) increased the odds of carrying the lupus risk allele in *IRF5* (rs2070197) by 17% and 16%, respectively, and vice versa ($P = 0.0028$ and $P = 0.0047$, respectively). Interestingly, our data suggested that the presence of the risk allele in *ITGAM* increases the odds of carrying the protective allele in rs3131379 (HLA) by 16% ($P = 0.0075$).

Next, and in order to confirm the 2 gene–gene interactions that we established using parametric tests, and to test whether the other 4 suggestive gene–gene interactions can be established, we applied MDR analysis to the interactions initially identified using parametric analysis. The MDR is a nonparametric test for nonlinear epistasis. A pairwise MDR analysis was applied to test the specific interactions detected using parametric analysis. It should be noted, however, that

Table 4. Multifactor dimensionality reduction (MDR) analysis for pairwise interactions detected using parametric analysis in lupus patients and controls*

Interaction	Cross-validation consistency	Balanced accuracy	χ^2	*P*, 3df
CTLA4 (rs231775) × *HLA* (rs3131379)	10/10	0.5737	208.57	5.9×10^{-45}
CTLA4 (rs231775) × *HLA* (rs1270942)	10/10	0.5744	212.76	7.4×10^{-46}
HLA (rs1270942) × *IRF5* (rs2070197)	10/10	0.5949	270.60	2.3×10^{-58}
HLA (rs3131379) × *IRF5* (rs2070197)	10/10	0.5946	268.81	5.6×10^{-58}
HLA (rs3131379) × *ITGAM* (rs1143679)	10/10	0.5985	287.71	4.6×10^{-62}
PDCD1 (rs11568821) × *IL21* (rs907715)	10/10	0.5235	17.44	5.7×10^{-4}

* Cross-validation consistency reflects the number of times MDR analysis identified the same model as the data were divided into different segments. Balanced accuracy is defined as (sensitivity + specificity)/2, where sensitivity = true positives/(true positives + false negatives), and specificity = true negatives/(false positives + true negatives). This gives an accuracy estimate that is not biased by the larger class (37).

results obtained using the MDR nonparametric analysis reflect a joint effect consisting of the main genetic association effect in the loci examined and the interaction effect. These results are presented in Table 4 (also see Supplementary Table 1 and Supplementary Figure 1, available on the *Arthritis & Rheumatism* Web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131).

DISCUSSION

The nature and very existence of genetic epistasis in lupus have been elusive. We combined the strengths of 2 independent approaches to test for genetic epistasis in lupus and identified several novel gene–gene interactions, using a large sample derived from individuals of European descent. The most significant interaction identified was between the HLA region and *CTLA4*. Indeed, 2 independent lupus-associated SNPs within the HLA region (rs3131379 and rs1270942) showed evidence for significant interaction with rs231775 in *CTLA4* (Tables 3 and 4). The HLA–*CTLA4* interaction in lupus underscores antigen presentation and T cell stimulation as important processes involved in the pathogenesis of lupus. This interaction is biologically logical, because CTLA-4 is up-regulated on T cells following T cell activation by antigen-presenting cells (19). Following T cell activation via binding of the major histocompatibility complex–antigen complex to the T cell receptor (signal 1), the binding of CD80/CD86 on antigen-presenting cells to CD28 on the surface of T cells (signal 2) ensures T cell activation and interleukin-2 (IL-2) production (19). CTLA-4 competes with CD28 to bind CD80/CD86 and provides a negative signal that suppresses T cell activation. This process is thought to be important to control T cell activation and prevent autoimmunity.

A role for antigen-presenting cells in lupus is highlighted again with the HLA–*ITGAM* gene–gene interaction, although this interaction is between the risk and protective alleles in these 2 loci. *ITGAM* (integrin, alpha M) encodes for CD11b, the α chain in the integrin molecule CD11b/CD18 (Mac-1, CR3). It is expressed on the surface of antigen-presenting cells and neutrophils and plays a role in cell–cell adhesions, leukocyte extravasation, and in complement-mediated phagocytosis of C3bi-opsonized antigens (20,21).

We also showed evidence for gene–gene interaction between the 2 independent lupus-associated SNPs within the HLA region and rs2070197 in *IRF5*. This interaction emphasizes the role of the interferon pathway in the pathogenesis of lupus.

The other gene–gene interaction that we identified was between rs907715 in *IL21* and rs11568821 in *PDCD1*. This interaction is very interesting, because it highlights a role for follicular helper T (Tfh) cells in lupus. High *PDCD1* expression and IL-21 production are hallmarks of Tfh cells (22). Tfh cells promote germinal center formation, plasma cell differentiation, and antibody isotype switching (23). *PDCD1* deficiency results in impaired germinal center B cell survival and diminished production of long-lived plasma cells (24). Indeed, the production of IL-21 is reduced in Tfh cells from *Pdcd1*$^{-/-}$ mice (24). IL-21 deficiency results in impaired germinal center formation, plasma cell differentiation, and isotype class switching (23), emphasizing a central role for IL-21 in Tfh cell function. Of interest, a higher fraction of circulating Tfh cells was detected in the peripheral blood of patients with lupus compared with normal control subjects (25).

In summary, we provided strong evidence that in patients with lupus, the presence of one risk allele can influence the presence or absence of other risk alleles, across different loci. We have identified novel gene–gene epistatic interactions in lupus. Gene–gene interactions might help explain at least part of the "missing heritability" in complex diseases. Our findings provide evidence against a simple "additive" genetic model in autoimmunity and highlight antigen presentation and T cell activation, the interferon pathway, and Tfh cells as important contributors to the pathogenesis of lupus.

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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. Dr. Sawalha had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Hughes, Kaufman, Langefeld, Gaffney, Harley, Sawalha.

Acquisition of data. Hughes, Adler, Kelly, Kaufman, Brown, Alarcón, Kimberly, Edberg, Ramsey-Goldman, Petri, Boackle, Stevens, Reveille, Martín, Niewold, Vilá, Scofield, Gilkeson, Gaffney, Criswell, Moser, Merrill, Jacob, Tsao, James, Vyse, Alarcón-Riquelme, Harley, Richardson, Sawalha.

Analysis and interpretation of data. Hughes, Williams, Langefeld, Sanchez, Gaffney, Sawalha.

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