Published in final edited form as:
Arthritis Rheum. 2009 August ; 60(8): 2402–2407. doi:10.1002/art.24658.

A polymorphism within interleukin-21 receptor (*IL21R*) confers risk for systemic lupus erythematosus

Ryan Webb, BS[1,2], **Joan T. Merrill, MD**[3,4], **Jennifer A. Kelly, MPH**[1], **Andrea Sestak, MD, PhD**[1], **Kenneth M. Kaufman, PhD**[1,4,5], **Carl D. Langefeld, PhD**[6], **Julie Ziegler, MS**[6], **Robert P. Kimberly, MD**[7], **Jeffrey C. Edberg, PhD**[7], **Rosalind Ramsey-Goldman, MD**[8], **Michelle Petri, MD, MPH**[9], **John D. Reveille, MD**[10], **Graciela S. Alarcón, MD, MPH**[7], **Luis M. Vilá, MD**[11], **Marta E. Alarcón-Riquelme, MD, PhD**[1,12], **Judith A. James, MD, PhD**[1,4], **Gary S. Gilkeson, MD**[13], **Chaim O. Jacob, MD, PhD**[14], **Kathy L. Moser, PhD**[1], **Patrick M. Gaffney, MD**[1], **Timothy J. Vyse, MD**[15], **Swapan K. Nath, PhD**[1], **Peter Lipsky, MD**[16], **John B. Harley, MD, PhD**[1,4,5], and **Amr H. Sawalha, MD**[1,4,5]

[1] Arthritis & Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

[2] College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

[3] Clinical Pharmacology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

[4] Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

[5] US Department of Veterans Affairs Medical Center, Oklahoma City, OK, USA

[6] Department of Biostatistical Sciences, Wake Forest University Health Sciences, Medical Center Blvd, Winston-Salem, NC, USA

[7] Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

[8] Department of Medicine, Northwestern University, Chicago, IL, USA

[9] Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[10] Department of Medicine, University of Texas-Houston Health Science Center, Houston, TX, USA

[11] Department of Medicine, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico, USA

[12] Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden

[13] Department of Medicine, Division of Rheumatology, Medical University of South Carolina, Charleston, SC, USA

[14] Department of Medicine, University of Southern California School of Medicine, Los Angeles, CA, USA

[15] Imperial College, Rheumatology Section, Hammersmith Hospital, London, UK

[16] Autoimmunity Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD, USA

Please address correspondence to Amr H. Sawalha MD; 825 N.E. 13th Street, MS#24, Oklahoma City, Oklahoma 73104. Phone: (405) 271-7977. Fax: (405) 271-4110. amr-sawalha@omrf.ouhsc.edu.

Abstract

Objective—Interleukin (IL) 21 is a member of the type I cytokine superfamily that exerts a variety of effects on the immune system including B cell activation, plasma cell differentiation, and immunoglobulin production. The expression of IL21R is reduced in B cells from lupus patients, while IL21 serum levels are increased in both lupus patients and some lupus-murine models. We recently reported that polymorphisms within the *IL21* gene are associated with increased susceptibility to lupus. Herein, we examined the genetic association between SNPs within *IL21R* and lupus.

Methods—We genotyped 17 SNPs in the *IL21R* gene in two large cohorts of lupus patients and ethnically-matched healthy controls. Genotyping was performed with the Illumina BeadStation 500GX instrument using Illumina Infinum II genotyping assays.

Results—We identified and confirmed the association between rs3093301 within the *IL21R* gene and lupus in two independent European-derived and Hispanic cohorts (meta analysis odds ratio= 1.16, 95% CI= 1.08-1.25, meta analysis $p=1.0\times10^{-4}$).

Conclusion—We identified *IL21R* as a novel susceptibility gene for lupus.

Introduction

Systemic lupus erythematosus (SLE or lupus) is a chronic systemic autoimmune disease that is associated with multiple organ involvement. The etiology and pathogenesis of lupus involves genetic, epigenetic, and environmental factors. Lupus is a heterogeneous disease with highly variable presentation. In 1982, the American College of Rheumatology (ACR) published 11 classification criteria (at least four of which must be met) used to aid in the classification of lupus. The one criterion common in nearly all lupus patients is the presence of anti-nuclear autoantibodies, considered a hallmark of the disease (1).

A large body of evidence supports the role of genetics in lupus. More than 100 possible genetic risk factors for lupus have been identified through case studies, linkage analyses of multiplex families, and candidate gene case-control studies (2). Epidemiological studies have shown familial clustering in lupus and increased risk for family members of someone with lupus to develop this disease (3,4). There is, for example, more than a ten-fold increase in the concordance rate of lupus in monozygotic twins compared to dizygotic twins (5).

Interleukin (IL) 21 is a recently discovered member of the type I cytokine superfamily and is expressed primarily by activated CD4+ T cells and NK cells (6). Its receptor, IL21 receptor (IL21R), is expressed on a variety of cells including B cells, T cells, NK cells, and monocyte-derived dendritic cells (7-9). IL21 exerts a variety of effects on the immune system, and is important for B cell responsiveness, proliferation, plasma cell differentiation, and immunoglobulin production (10,11). Further, IL21 is involved in Th17 cell differentiation (12) and modulates the function of both dendritic cells and NK cells (13).

IL21 overproduction has been reported in several murine lupus models including the BXSB-*Yaa* mouse (11). Excessive production of IL21 and a severe lupus-like autoimmune phenotype has been reported in the *sanroque* mouse strain, which has a mutation in a RING-type ubiquitin ligase protein family member (*roquin*) (14). In addition, an IL21R-Fc fusion protein significantly improves disease in MRL/*lpr* lupus-prone mice, suggesting that blocking IL21 might be a potential therapeutic approach in lupus patients (15).

In lupus patients, IL21R is underexpressed on total, naïve and memory B cells, and plasmablasts compared to controls, while there is no difference in the expression levels of IL21R in T cells (8). Decreased expression of IL21R is also associated with nephritis and a

high titer of anti-dsDNA antibody in lupus patients (8). Lupus patients have higher concentrations of IL21 in the plasma, though plasma IL21 levels do not appear to correlate with lupus disease activity (6).

We recently reported that polymorphisms within *IL21* are associated with lupus in two independent lupus cohorts (16). Herein, we evaluate genetic polymorphisms in *IL21R* in a large group of lupus patients and controls. We identify a genetic association between a single nucleotide polymorphism (SNP) within the *IL21R* gene and lupus in a European-derived cohort, which is confirmed in a second independent cohort of Hispanic lupus patients and controls.

Methods

Patients and controls

Lupus patients and healthy controls were recruited and enrolled in the lupus genetics studies at the Oklahoma Medical Research Foundation and at collaborating sites in the US, UK, and Sweden. A total of 2,573 independent lupus patients and 3,075 healthy unrelated ethnically-matched controls were included in our European-derived cohort. A total of 657 independent lupus patients and 265 healthy unrelated ethnically-matched controls were included in our Hispanic cohort. All lupus patients met the 1997 ACR classification criteria for lupus. Clinical and serological data were acquired as previously described (17).

Genotyping

Genotyping was performed on DNA isolated from peripheral blood mononuclear cells, lymphoblastoid cell lines, or buccal cell swabs as previously described (18). We genotyped 17 SNPs located within the *IL21R* gene in two independent, ethnically divergent populations of lupus patients and controls. These SNPs were selected from the published SNP databases (http://www.ncbi.nlm.nih.gov/projects/SNP/) to cover the entire length of the *IL21R* gene. They were all validated SNPs, and were previously tested successfully using the Illumina genotyping platform that we utilized in this study. Genotyping was performed with the Illumina BeadStation 500GX instrument using Illumina Infinum II genotyping assays following manufacturer's recommendations.

Statistical Analysis

Principal component analyses were used to examine sample homogeneity in both cohorts. To further exclude population substratification, we used the Genomic Control (GC) method, and calculated an inflation factor (λ) using 2218 “null” SNPs in the European-derived cohort and 2196 “null” SNPs in the Hispanic cohort.

This study was conducted utilizing a population-based case-control study design. Pearson's chi-square measures were calculated to determine whether allele frequencies were different between cases and controls. Uncorrected p values are reported with 95 percent confidence intervals. Permutation test was used to correct allele frequency differences for multiple testing, using Haploview 4.1 (19). Meta analysis was performed using the Cochran-Mantel-Haenszel test included in SAS ®. Logistic regression analysis was used to test for gene-gene interaction between the lupus-associated *IL21* and *IL21R* SNPs.

Results

We genotyped 17 SNPs located within the *IL21R* gene in two independent, ethnically divergent populations of lupus patients and controls. Our European-derived cohort included 2,573 independent lupus cases and 3,075 healthy controls while our Hispanic cohort

included 657 independent lupus cases and 265 healthy controls. Principal component analyses were used to examine sample homogeneity in both cohorts. A total of 234 and 156 samples were identified as outliers in the European-derived and Hispanic cohorts, respectively, and were excluded from further analysis. Subsequently, GC analysis was performed and revealed no significant population substratification (λ= 1.13 and 1.17, in the European-derived and Hispanic cohorts, respectively). Fifteen of the 17 SNPs genotyped had a minor allele frequency of at least 1%, which we required for analysis. Genotyping success rate was ≥99.2%, and ≥97.8% in the European-derived and Hispanic cohorts, respectively.

We found a genetic association between rs3093301 (A/G) and lupus in both the European-derived and the Hispanic cohorts. The lupus-associated allele (A) had a frequency of 66.6% among European-derived lupus cases compared to 63.7% among controls (OR= 1.13, χ^2 = 9.42, p=0.0022) (Table 1, Figure 1A). This association was confirmed in the Hispanic cohort, which had an associated allele frequency of 64.4% in lupus cases compared to 55.9% in controls (OR= 1.43, χ^2 = 9.16, p=0.0025) (Table 2, Figure 1B). Using Cochran-Mantel-Haenszel test, the meta-analysis odds ratio for the associated allele in rs3093301 in lupus patients compared to controls was 1.16 (95%CI= 1.08-1.25, meta-analysis p value= 1.0×10^{-4}) in the combined European-derived and Hispanic samples.

IL21R is located in proximity to the *IL4R* gene, another important candidate in autoimmunity. However, *IL21R* and *IL4R* are located in separate haplotype blocks in the European-derived samples (CEU) genotyped in the International HapMap Project (Figure 1). To further confirm that the genetic effect we describe in rs3093301 is not a surrogate for a genetic association in *IL4R*, we genotyped 5 SNPs within the *IL4R* gene in both our European-derived and Hispanic cohorts. None of the genotyped SNPs in *IL4R* (rs2107356, rs2234895, rs1805011, rs1805013, and rs2074570) showed an association with lupus in either cohort (p>0.05).

To determine whether the lupus-risk allele in rs3093301 is more frequently associated with any clinical or serological manifestation in lupus patients, we determined the frequency of the various lupus manifestations in female lupus patients homozygous for the risk allele (A/A) (n=197) compared to patients homozygous for the protective allele (G/G) (n=62) in the European-derived cohort. Variables tested included malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, pericarditis, pleuritis, proteinuria, urine cellular casts, seizures, psychosis, hemolytic anemia, leucopenia, lymphopenia, thrombocytopenia, the presence of anti-dsDNA, anti-Sm, anti-nRNP, anti-Ro, anti-La, and anti-ribosomal P antibodies. We found that the A/A genotype in rs3093301 is associated with the presence of malar rash in European-derived female lupus patients compared to the G/G genotype (60.9% versus 35.5%, OR=2.83, CI = 1.56-5.13, χ^2 = 12.31, p=0.00045). This association remains significant after correcting for multiple testing using Bonferroni correction (corrected p= 0.0095). It was previously reported that reduced expression of IL21R in peripheral blood B cells is associated with nephritis in lupus patients (8). We found no difference in the frequency of proteinuria or urine cellular casts between lupus patients with the A/A genotype compared to the G/G genotype in rs3093301 (p= 0.51 and 0.14, respectively).

We have previously reported a genetic association between *IL21* and lupus. The SNP rs907715 within the *IL21* gene was the most significantly associated SNP in our European-derived cohort (20). To test if there is gene-gene interaction between *IL21* and *IL21R* upon lupus susceptibility, we performed logistic regression analysis to determine if the simultaneous presence of the risk alleles in the *IL21* lupus-associated SNP (rs907715) and the *IL21R* lupus associated SNP (rs3093301) could explain the increased susceptibility to lupus. We find no evidence for a synergistic effect for the presence of the risk alleles in

rs907715 and rs3093301 (interaction p=0.58), suggesting no genetic epistasis between the *IL21* and *IL21R* loci upon lupus susceptibility.

Discussion

IL21 is a pleiotropic cytokine that has multiple effects on a number of immunocompetent cells. It is critically involved in B cell activation, differentiation, and immunoglobulin production (6). We report on the genetic association between a SNP in *IL21R* (rs3093301) and lupus in two ethnically divergent lupus cohorts. This SNP is located on chromosome 16p11 in the first intron of *IL21R* just upstream of the translational start site (Figure 2).

European-derived lupus patients who are homozygous for the rs3093301 risk allele (A/A) have approximately 3 times the odds of suffering from malar rash compared to lupus patients homozygous for the protective *IL21R* allele (G/G). However, this finding should be considered preliminary and requires further replication in other independent cohorts of lupus patients. Plasma IL21 levels were elevated in a cohort of lupus patients, nonetheless, there was no correlation between IL21 levels and malar rash or any other symptom or sign of lupus in that cohort (unpublished data; reviewed in (6)).

IL21R is located on chromosome 16p11, which is close to 16p12, a region that has been previously linked to lupus susceptibility using linkage analysis studies in familial lupus (20). The 16p11 region also harbors the *ITGAM* gene which is a confirmed genetic association for lupus (21,22). The *ITGAM* and *IL21R* genes are not in linkage disequilibrium. The *IL21R* gene is therefore a likely candidate gene contributing to the genetic linkage effect on chromosome 16p12 along with the *ITGAM* gene.

In summary, we report and confirm for the first time the genetic association between lupus and *IL21R*. Further studies are needed to identify the causal SNP for this association, and to determine the functional consequences of this polymorphism upon lupus susceptibility. These findings, along with our previous report on *IL21* genetic association with lupus, indicate that the IL21/IL21R pathway is important in understanding the pathogenesis of this disease. However, there is no evidence for gene-gene interaction (epistasis) between the lupus associated SNPs in *IL21* and *IL21R* upon the susceptibility to lupus.

Acknowledgments

We are thankful to Dr. Peter Gregersen for providing DNA control samples for our study.

This work was made possible by NIH Grant Number R03AI076729 from the National Institute of Allergy and Infectious Diseases, NIH Grant Number P20-RR015577 from the National Center for Research Resources, funding from the Arthritis National Research Foundation and the University of Oklahoma College of Medicine (AHS); NIH Grants Number AR42460, AI024717, AI31584, AR62277, AR048940, AR0490084, Kirkland Scholar award, Alliance for Lupus Research, and US Department of Veterans Affairs (JBH); NIH grant number P01-AR049084 (RPK); NIH grant number AR043274 (KLM); and NIH grant number AI063274 (PMG).

References

1. Sawalha AH, Harley JB. Antinuclear autoantibodies in systemic lupus erythematosus. Current Opinion in Rheumatology. 2004; 16(5):534–540. [PubMed: 15314490]
2. Sestak A, Nath S, Sawalha A, Harley J. Current status of lupus genetics. Arthritis Research & Therapy. 2007; 9(3):210. [PubMed: 17509159]
3. Vyse TJ, Todd JA. Genetic analysis of autoimmune disease. Cell. 1996; 85(3):311–8. [PubMed: 8616887]

4. Sestak AL, Shaver TS, Moser KL, Neas BR, Harley JB. Familial aggregation of lupus and autoimmunity in an unusual multiplex pedigree. J Rheumatol. 1999; 26(7):1495–9. [PubMed: 10405936]
5. Reichlin M, Harley JB, Lockshin MD. Serologic studies of monozygotic twins with systemic lupus erythematosus. Arthritis Rheum. 1992; 35(4):457–64. [PubMed: 1567495]
6. Ettinger R, Kuchen S, Lipsky PE. The role of IL-21 in regulating B-cell function in health and disease. Immunol Rev. 2008; 223:60–86. [PubMed: 18613830]
7. Parrish-Novak J, Dillon SR, Nelson A, Hammond A, Sprecher C, Gross JA, et al. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. Nature. 2000; 408(6808):57–63. [PubMed: 11081504]
8. Mitoma H, Horiuchi T, Kimoto Y, Tsukamoto H, Uchino A, Tamimoto Y, et al. Decreased expression of interleukin-21 receptor on peripheral B lymphocytes in systemic lupus erythematosus. Int J Mol Med. 2005; 16(4):609–15. [PubMed: 16142394]
9. Brandt K, Bulfone-Paus S, Foster DC, Ruckert R. Interleukin-21 inhibits dendritic cell activation and maturation. Blood. 2003; 102(12):4090–8. [PubMed: 12893770]
10. Kuchen S, Robbins R, Sims GP, Sheng C, Phillips TM, Lipsky PE, et al. Essential role of IL-21 in B cell activation, expansion, and plasma cell generation during CD4+ T cell-B cell collaboration. J Immunol. 2007; 179(9):5886–96. [PubMed: 17947662]
11. Ozaki K, Spolski R, Ettinger R, Kim HP, Wang G, Qi CF, et al. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. J Immunol. 2004; 173(9):5361–71. [PubMed: 15494482]
12. Yang L, Anderson DE, Baecher-Allan C, Hastings WD, Bettelli E, Oukka M, et al. IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. Nature. 2008; 454(7202):350–2. [PubMed: 18469800]
13. Maeda M, Yanagawa Y, Iwabuchi K, Minami K, Nakamaru Y, Takagi D, et al. IL-21 enhances dendritic cell ability to induce interferon-gamma production by natural killer T cells. Immunobiology. 2007; 212(7):537–47. [PubMed: 17678711]
14. Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM, et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature. 2005; 435(7041):452–8. [PubMed: 15917799]
15. Herber D, Brown TP, Liang S, Young DA, Collins M, Dunussi-Joannopoulos K. IL-21 Has a Pathogenic Role in a Lupus-Prone Mouse Model and Its Blockade with IL-21R.Fc Reduces Disease Progression. J Immunol. 2007; 178(6):3822–3830. [PubMed: 17339481]
16. Sawalha AH, Kaufman KM, Kelly JA, Adler AJ, Aberle T, Kilpatrick J, et al. Genetic association of interleukin-21 polymorphisms with systemic lupus erythematosus. Ann Rheum Dis. 2008; 67(4):458–461. [PubMed: 17720724]
17. Jeffries M, Hamadeh F, Aberle T, Glenn S, Kamen DL, Kelly JA, et al. Haemolytic anaemia in a multi-ethnic cohort of lupus patients: a clinical and serological perspective. Lupus. 2008; 17(8): 739–43. [PubMed: 18625652]
18. Gray-McGuire C, Moser KL, Gaffney PM, Kelly J, Yu H, Olson JM, et al. Genome scan of human systemic lupus erythematosus by regression modeling: evidence of linkage and epistasis at 4p16-15.2. Am J Hum Genet. 2000; 67(6):1460–9. [PubMed: 11078476]
19. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–5. [PubMed: 15297300]
20. Lee YH, Nath SK. Systemic lupus erythematosus susceptibility loci defined by genome scan meta-analysis. Hum Genet. 2005; 118(3-4):434–43. [PubMed: 16208513]
21. Nath SK, Han S, Kim-Howard X, Kelly JA, Viswanathan P, Gilkeson GS, et al. A nonsynonymous functional variant in integrin-alpha(M) (encoded by ITGAM) is associated with systemic lupus erythematosus. Nat Genet. 2008; 40(2):152–4. [PubMed: 18204448]
22. Han S, Kim-Howard X, Deshmukh H, Kamatani Y, Viswanathan P, Guthridge JM, et al. Evaluation of Imputation-based Association in and around the Integrin-{alpha}-M (ITGAM) gene and Replication of Robust Association between a Non-synonymous Functional variant within ITGAM and Systemic lupus erythematosus (SLE). Hum Mol Genet. 2009

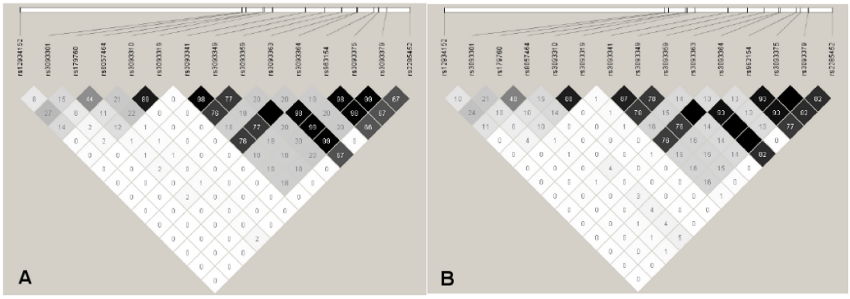

Figure 1.
Linkage disequilibrium (LD) plots for the *IL21R* SNPs analyzed in the European-derived (A), and the Hispanic (B) lupus patients and controls. Values depicted represent pair-wise correlation coefficient (r^2).

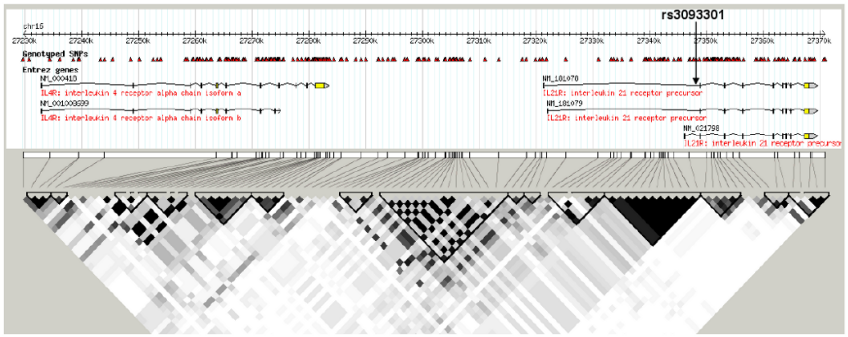

Figure 2.

Linkage disequilibrium (LD) plot of the *IL21R-IL4R* region depicting the haplotype structure in the CEU samples genotyped in the International HapMap Project. The arrow points to the location of the *IL21R* SNP rs3093301 that was associated with lupus in the two cohorts included in this study.

Table 1

Genetic association between SNPs within *IL21R* and lupus in a cohort of European-derived lupus patients and controls. Cases analyzed included 2,245 females and 239 males, and controls analyzed included 2,009 females and 921 males. Only SNPs with minor allele frequencies of ≥1% were analyzed.

SNP	Associated Allele	Associated Allele Frequency		Chi2	OR	95 % Confidence Interval		P Value	Permutation P Value	HWE P Value
		Cases	Controls							
		n (%)	n (%)			LL	UL			
rs12934152	G	1254 (25.6)	1481 (25.3)	0.09	1.01	0.93	1.11	0.76	1.00	0.98
rs3093301	A	3264 (66.6)	3696 (63.7)	9.42	1.13	1.05	1.23	0.0022	0.015	0.06
rs179760	A	1168 (23.6)	1333 (22.7)	0.99	1.05	0.96	1.14	0.32	0.93	0.79
rs8057464	A	1102 (22.2)	1282 (21.9)	0.16	1.02	0.93	1.12	0.69	1.00	0.75
rs3093310	G	2845 (57.4)	3329 (56.8)	0.37	1.02	0.95	1.11	0.55	1.00	0.65
rs3093319	G	2183 (44.2)	2587 (44.2)	0.00	1.00	0.93	1.08	1.00	1.00	0.15
rs3093341	G	479 (9.7)	554 (9.5)	0.20	1.03	0.91	1.17	0.65	1.00	0.90
rs3093349	G	472 (9.5)	552 (9.4)	0.03	1.01	0.89	1.15	0.86	1.00	0.98
rs3093359	A	428 (8.6)	504 (8.6)	0.00	1.00	0.88	1.15	0.98	1.00	0.55
rs3093363	G	1597 (32.5)	1811 (30.9)	3.01	1.07	0.99	1.17	0.08	0.46	0.88
rs3093364	G	425 (8.6)	501 (8.6)	0.00	1.00	0.88	1.15	0.97	1.00	0.59
rs963154	C	1570 (31.9)	1781 (30.5)	2.26	1.06	0.98	1.16	0.13	0.63	0.82
rs3093375	C	1602 (32.6)	1814 (31.0)	3.16	1.08	0.99	1.17	0.08	0.43	0.80
rs3093379	A	1592 (32.3)	1807 (30.9)	2.36	1.07	0.98	1.16	0.12	0.61	0.94
rs2285452	A	1229 (24.8)	1351 (23.1)	4.27	1.10	1.00	1.20	0.039	0.24	0.76

OR, odds ratio; LL, lower limit; UL, upper limit; HWE, Hardy-Weinberg equilibrium

Table 2

Genetic association between SNPs within *IL21R* and lupus in a cohort of Hispanic lupus patients and controls. Cases analyzed included 494 females and 63 males, and controls analyzed included 167 females and 42 males. Only SNPs with minor allele frequencies of ≥1% were analyzed.

SNP	Associated Allele	Associated Allele Frequency		Chi2	OR	95 % Confidence Interval		p Value	Permutation P Value	HWE P Value
		Cases	Controls							
		n (%)	n (%)			LL	UL			
rs12934152	G	202 (18.2)	58 (14)	3.74	1.37	0.99	1.70	0.053	0.37	0.38
rs3093301	A	702 (64.4)	228 (55.9)	9.16	1.43	1.13	1.80	0.0025	0.021	0.76
rs179760	A	292 (26.3)	100 (23.9)	0.87	1.13	0.87	1.47	0.35	0.96	0.49
rs8057464	A	268 (24.1)	87 (20.8)	1.80	1.21	0.92	1.58	0.18	0.78	0.76
rs3093310	A	382 (34.4)	125 (29.9)	2.71	1.23	0.96	1.56	0.10	0.57	0.08
rs3093319	G	376 (33.8)	129 (30.9)	1.20	1.14	0.90	1.46	0.27	0.91	0.13
rs3093341	G	75 (6.7)	21 (5)	1.51	1.36	0.83	2.24	0.22	0.84	0.37
rs3093349	G	68 (6.1)	19 (4.5)	1.41	1.37	0.81	2.31	0.23	0.87	0.56
rs3093359	A	62 (5.6)	16 (3.8)	1.90	1.48	0.84	2.60	0.17	0.77	0.81
rs3093363	G	307 (27.7)	105 (25.1)	0.99	1.14	0.88	1.47	0.32	0.94	0.57
rs3093364	G	55 (5)	16 (3.8)	0.96	1.33	0.75	2.34	0.33	0.95	1.00
rs963154	C	295 (26.7)	94 (22.7)	2.55	1.24	0.95	1.62	0.11	0.59	0.60
rs3093375	C	308 (27.7)	105 (25.1)	1.03	1.14	0.88	1.48	0.31	0.94	0.60
rs3093379	A	308 (27.6)	105 (25.1)	0.99	1.14	0.88	1.47	0.32	0.94	0.59
rs2285452	A	266 (23.9)	92 (22)	0.59	1.11	0.85	1.46	0.44	0.98	0.45

OR, odds ratio; LL, lower limit; UL, upper limit; HWE, Hardy-Weinberg equilibrium