

CONCISE COMMUNICATION

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The inflammatory disease–associated variants in *IL12B* and *IL23R* are not associated with rheumatoid arthritis

There is increasing evidence that common genetic variants can contribute to general immune dysregulation and susceptibility to noninfectious inflammatory diseases. *CTLA4* has been associated with Graves' disease, type 1 diabetes mellitus (type 1 DM), Addison's disease, celiac disease, and rheumatoid arthritis (RA), with odds ratios (ORs) ranging from 1.1 to 1.5 (1). In addition, a missense single-nucleotide polymorphism (SNP), rs2476601, in *PTPN22* has been shown to be a risk factor (OR 1.4–2.0) for several autoimmune diseases, including RA, type 1 DM, autoimmune thyroid disease, and systemic lupus erythematosus (SLE) (1), while variants in the third intron of the transcription factor *STAT4* have been associated with the risk of both RA and SLE (OR 1.2–1.8) (2). Finally, the recent plethora of whole-genome association studies suggest that common variants in other genes such as *PTPN2* and *IL2RA* may contribute to the risk of multiple noninfectious inflammatory diseases (3,4).

We recently observed that common haplotypes in both *IL12B* and *IL23R*, marked by the *IL12B* SNPs rs3212227 and rs6887695 and the *IL23R* SNPs rs7530511 and rs11209026, were associated with psoriasis (OR 1.4–1.5) (5), and association of these genes with psoriasis has now been replicated in other studies (6,7). Independently, Duerr and colleagues (8), and subsequently other investigators (3,9), showed that the *IL23R* SNP, rs11209026, which encodes an arginine-to-glutamine change at position 381, was also strongly associated with inflammatory bowel disease (OR 2.2–3.8). Interestingly, the psoriasis-associated *IL12B* SNP, rs6887695, has also been associated with Crohn's disease (9), and a large association scan of 14,500 nonsynonymous SNPs identified the *IL23R* missense SNP, rs11209026, as a risk factor for ankylosing spondylitis (AS) (10).

IL12B and *IL23R* encode proteins that are members of the interleukin-12 (IL-12) superfamily of cytokines (for review, see ref. 11). *IL12B* encodes the IL-12 p40 subunit of both the IL-12 and IL-23 cytokines, and insertions and deletions in this gene have been associated with a rare recessive form of Mendelian susceptibility to mycobacterial infection, while *IL23R* encodes one of the two subunits of the IL-23 receptor. The IL-12 and IL-23 cytokines, which play critical and unique roles in bridging the innate and adaptive immune systems, are produced primarily by activated dendritic cells and macrophages in response to microbial stimulation. IL-12, a proinflammatory cytokine, plays a central role in promoting the differentiation of naive CD4⁺ T cells into mature interferon- γ (IFN γ)–producing Th1 effector cells, and it is a potent stimulus of natural killer and CD8⁺ T cells to produce IFN γ . In contrast, IL-23 is required for the generation of effector memory T cells and drives the expansion and maintenance of the newly defined IL-17–secreting Th cells that appear to play a fundamental role in autoimmunity.

We sought to determine whether the *IL12B* and *IL23R*

SNPs associated with psoriasis and other noninfectious inflammatory diseases were also associated with RA and/or specific subphenotypes of this disease, by genotyping the 4 implicated SNPs (rs3212227, rs6887695, rs7530511, and rs11209026) in 3 large, independent, RA case–control sample sets (1,732 patients and 2,502 control subjects). All patients included in this study were white, met the 1987 American College of Rheumatology (formerly, the American Rheumatism Association) criteria for a diagnosis of RA (12), and have been described in detail elsewhere (13,14). Written informed consent was obtained from every subject, and national and/or local institutional review boards approved all protocols and recruitment sites.

Briefly, sample set 1, obtained by Genomics Collaborative, Inc., comprised 475 white rheumatoid factor (RF)–positive patients with RA and 475 white control subjects individually matched for sex, age \pm 5 years, and grandparents' country of origin. All individuals in sample set 1 were from North America, 66% were female, and the average age at disease onset was 47 years. Sample set 2, obtained by the North American Rheumatoid Arthritis Consortium, comprised 661 unrelated patients with RA (536 RF-positive patients and 125 RF-negative patients) from white multiplex families from across North America and 1,322 healthy white control subjects from the New York Cancer Project. In sample set 2, control subjects were matched with patients (2-to-1 ratio) for sex, age (decade of birth), and ethnicity (grandparents' country/region of origin). The average age at disease onset was 38.6 years, and 81% of the individuals were female. Sample set 3, from the Leiden University Medical Centre, included 596 unrelated Dutch white patients and 705 unrelated white control subjects. Complete phenotype data were not available for all of the Dutch patients; however, 362 (65%) of 558 patients were female, and 317 (72%) of 440 were RF positive. The average age at disease onset was 54.6 years (data were available for 306 patients). Information on carriage of the *PTPN22* C1858T risk allele was available for all 3 sample sets. Complete information on *HLA-DRB1* shared epitope (SE) status was available for sample sets 1 and 2.

Individuals in sample set 1 were genotyped using a multiplexed conventional polymerase chain reaction (PCR) followed by a flow cytometry–based oligonucleotide ligation assay (15); individuals in sample sets 2 and 3 were genotyped for all 4 SNPs using kinetic PCR with allele-specific primers (16) (primer and probe sequences are available upon request). Genotypes were automatically called using custom software followed by hand curation, without knowledge of case–control status. Previous cross-validation studies of these 2 platforms suggest a genotyping concordance of >99.8% (5). Carriage of the *HLA-DRB1* SE was determined as previously described (13).

An exact test of Hardy-Weinberg equilibrium (HWE) on the genotype data, performing the analysis separately for patients and control subjects in each sample set, provided no evidence for deviation from HWE at any of the 4 SNPs tested ($P \geq 0.05$ for all analyses) (Table 1). Case and control allele frequencies for all 4 SNPs were similar across all 3 studies and were consistent with control frequencies from 3 psoriasis

Table 1. Association of the *IL12B* and *IL23R* SNPs with rheumatoid arthritis*

SNP (allele 1/allele 2)	Genotype			HWE <i>P</i> †	MAF	Allelic, 1 vs. 2		Genotypic, HWE <i>P</i> ¶
	11	12	22			OR (95% CI)‡	<i>P</i> §	
rs3212227 (C/A)								
Sample set 1								
Case	27	142	301	0.069	0.209			
Control	23	145	306	0.316	0.201	1.04 (0.84–1.31)	0.732	0.830
Sample set 2								
Case	27	195	437	0.374	0.189			
Control	61	433	823	0.679	0.211	0.87 (0.74–1.03)	0.111	0.248
Sample set 3								
Case	24	169	398	0.263	0.184			
Control	30	191	478	0.055	0.180	1.03 (0.84–1.26)	0.798	0.871
Combined sample sets						0.96 (0.86–1.07)		
rs6887695 (C/G)								
Sample set 1								
Case	52	194	223	0.338	0.318			
Control	51	194	228	0.336	0.313	1.02 (0.84–1.24)	0.843	0.976
Sample set 2								
Case	58	278	323	0.926	0.299			
Control	137	578	601	0.950	0.324	0.89 (0.77–1.03)	0.119	0.284
Sample set 3								
Case	58	237	296	0.299	0.299			
Control	66	262	371	0.050	0.282	1.09 (0.91–1.29)	0.360	0.558
Combined sample sets						0.98 (0.89–1.08)		
rs7530511 (T/C)								
Sample set 1								
Case	9	120	342	0.854	0.146			
Control	6	101	368	1.000	0.119	1.27 (0.97–1.66)	0.078	0.210
Sample set 2								
Case	6	151	501	0.205	0.124			
Control	14	267	1,037	0.580	0.112	1.12 (0.91–1.38)	0.269	0.383
Sample set 3								
Case	11	144	436	0.823	0.140			
Control	11	182	508	0.243	0.146	0.96 (0.77–1.20)	0.735	0.243
Combined sample sets						1.12 (0.94–1.33)		
rs11209026 (A/G)								
Sample set 1								
Case	3	54	414	0.423	0.064			
Control	0	60	415	0.243	0.063	1.01 (0.70–1.46)	1	0.128
Sample set 2								
Case	7	88	563	0.100	0.078			
Control	4	182	1,132	0.305	0.072	1.08 (0.84–1.39)	0.562	0.125
Sample set 3								
Case	1	84	505	0.194	0.073			
Control	2	77	621	1.000	0.060	1.28 (0.94–1.75)	0.127	0.227
Combined						1.09 (0.96–1.25)		

* SNP = single-nucleotide polymorphism; RA = rheumatoid arthritis; MAF = minor allele frequency; 95% CI = 95% confidence interval.

† Calculated using Weir's exact test for Hardy-Weinberg equilibrium (HWE).

‡ Combined odds ratios (ORs) were calculated using a Mantel-Haenszel common OR; results are reported for the minor allele.

§ By Fisher's exact test.

¶ By G-test with Williams' correction.

case-control sample sets of white North Americans (5). No significant association between any of the 4 SNPs and RA was detected by either allele or genotype analyses (Table 1). Stratifying by the presence of RF, sex, age at disease onset, carriage of *HLA-DRB1* SE-positive alleles, or carriage of the known *PTPN22* 1858T risk allele also revealed no consistent evidence for association across sample sets (data not shown).

The power to detect a disease model with a continuity-corrected Mantel-Haenszel test (17) on allelic data was deter-

mined for each SNP, using a Monte Carlo simulation (10,000 replicates for each data point), with the significance level set at $\alpha = 0.05$ for all runs. Simulations were performed to estimate the disease model allelic OR necessary to achieve 80% power, given the observed allele frequencies and sample sizes in our RA data sets. These results indicate that our study had 80% power to detect allelic ORs of 1.17 for rs3212227, 1.14 for rs6887695, 1.21 for rs7530511, and 1.27 for rs11209026.

Two SNP haplotypes were estimated for each gene

Table 2. Two marker haplotypes for *IL12B* and *IL23R**

Haplotype	Sample set 1				Sample set 2				Sample set 3			
	Cases (n = 475)	Controls (n = 475)	OR	P	Cases (n = 661)	Controls (n = 1,322)	OR	P	Cases (n = 596)	Controls (n = 705)	OR	P
<i>IL12B</i>												
rs3212227/rs6887695												
A/G	600 (0.635)	604 (0.644)	0.98	0.731	874 (0.661)	1,668 (0.632)	1.14	0.074	788 (0.668)	958 (0.683)	0.93	0.399
A/C	146 (0.155)	146 (0.155)	1.00	0.984	198 (0.150)	416 (0.158)	0.94	0.573	176 (0.149)	192 (0.137)	1.10	0.369
C/C	152 (0.162)	152 (0.161)	1.00	0.916	196 (0.149)	438 (0.166)	0.88	0.143	176 (0.149)	200 (0.143)	1.05	0.683
C/G	44 (0.048)	38 (0.040)	1.16	0.472	54 (0.040)	118 (0.045)	0.91	0.527	40 (0.034)	52 (0.037)	0.91	0.741
<i>IL23R</i>												
rs7530511/rs11209026												
C/G	746 (0.789)	772 (0.822)	0.81	0.108	1,056 (0.798)	2,162 (0.818)	0.88	0.182	930 (0.787)	1,116 (0.796)	0.95	0.528
T/G	138 (0.147)	106 (0.114)	1.34	0.057	164 (0.124)	290 (0.110)	1.15	0.252	166 (0.140)	204 (0.146)	0.96	0.712
C/A	60 (0.064)	56 (0.061)	1.07	0.990	102 (0.078)	184 (0.070)	1.12	0.527	86 (0.073)	82 (0.058)	1.26	0.117
T/A	0 (0)	4 (0.003)	-	-	0 (0)	6 (0.002)	-	-	0 (0)	0 (0)	-	-

* Values are the number (%). The Haplo.Stats package was used to estimate haplotypes and test for association with disease status. P values and odds ratios (ORs) are for the indicated haplotype versus all others. For *IL12B*, global P = 0.909 for sample set 1, 0.327 for sample set 2, and 0.773 for sample set 3. For *IL23R*, global P = 0.162 for sample set 1, 0.559 for sample set 2, and 0.286 for sample set 3.

(Table 2), using the Haplo.Stats package (18). Case and control haplotype frequencies for both genes were similar across all 3 sample sets and were consistent with control frequencies estimated from 3 psoriasis case-control sample sets of white North Americans (5). No significant association was observed between these predicted *IL12B* and *IL23R* haplotypes and RA.

Lack of association of the *IL12B* 3'-untranslated region SNP, rs3212227, with RA has been reported in case-control studies of Greek individuals (179 patients and 159 control subjects), British individuals (258 patients and 255 control subjects), and Spanish individuals (545 patients and 393 control subjects) (19,20). Our results confirm the lack of association of rs3212227 with RA and extend these findings by showing that the psoriasis-associated 5' *IL12B* SNP, rs6887695, and the haplotypes predicted by it and rs3212227 also are not associated with RA. In addition, Orozco and colleagues (21) recently characterized 8 *IL23R* SNPs, including rs11209026, in 322 patients with RA and 243 healthy control subjects from Southern Spain and did not observe any of these SNPs to be associated with RA predisposition. Haplotypes were not tested (nor was rs7530511), and, according to CEU HapMap data, none of the 8 genotyped SNPs was in substantial linkage disequilibrium with this SNP ($r^2 \leq 0.153$ for all). Consequently, our data confirm lack of association of the *IL23R* R381Q missense SNP with RA and extend these results by showing that rs7530511 and the rs7530511-rs11209026 haplotypes do not appear to be associated with RA.

Because inflammatory diseases associated with the same genetic variant may share a common underlying mechanism, a thorough understanding of the diseases associated with a particular variant may shed light on disease pathogenesis. Consequently, the finding that a subset of psoriasis-associated *IL12B* and *IL23R* SNPs is also associated with Crohn's disease and AS led us to carefully evaluate the role of these same SNPs in other inflammatory diseases. We recently reported the results of a well-powered, family-based association analysis of 910 multiple sclerosis (MS) nuclear families (3,132 individuals), which provided no evidence that any of these 4 SNPs

were associated with MS risk, even after the data were stratified by sex of the patient, presence/absence of the risk *HLA-DRB1*1501* allele, and disease severity (22). Our analyses of 3 RA case-control sample sets (1,732 patients and 2,502 control subjects) presented here suggest that there is also no evidence of association between these same SNPs/haplotypes and RA, even after the data were stratified for sex of the patient, age at disease onset, presence/absence of the risk *HLA-DRB1 SE*, presence/absence of the risk *PTPN22* 1858T allele, and RF status. These studies were well powered to detect modest effect sizes, allowing us to rule out strong RA effects at these 4 SNPs; however, it is still possible that these SNPs could play a minor role in the risk of RA. This will be uncovered by using much larger sample sets.

These disease-association patterns are interesting in light of the recent proposal by McGonagle and McDermott (23) that all noninfectious inflammatory diseases lie on a spectrum from autoimmune (those mediated by the adaptive immune system) to autoinflammatory (those mediated by the innate immune system), and that each disease can be defined by its relative contributions. All of the inflammatory diseases associated with the *IL12B* and *IL23R* variants reported here lie toward the autoinflammatory end of the spectrum; Crohn's disease and AS are thought to be classic autoinflammatory diseases, while psoriasis is proposed to have a mixed pattern of both autoimmune and autoinflammatory characteristics. In contrast, RA is primarily autoimmune in nature. Interestingly, RA and several other classic autoimmune diseases, such as SLE and type 1 DM, are associated with the R620W variant in *PTPN22*, while Crohn's disease, AS, psoriasis, and other autoinflammatory diseases are not (1).

In summary, in our data sets, the *IL12B* and *IL23R* SNPs and haplotypes associated with the risk of psoriasis and other inflammatory diseases do not appear to play a major role in RA risk, suggesting that these variants in the IL-12/IL-23 pathway may not differentially influence RA pathogenesis. Analyses of the role of these genetic variants in additional inflammatory disorders should provide hints about the underlying mechanisms of these complex diseases as well as a

framework with which to begin to understand why certain inflammatory diseases often show familial aggregation.

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AUTHOR CONTRIBUTIONS

Dr. Begovich 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.

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1. Gregersen PK, Behrens TW. Genetics of autoimmune diseases: disorders of immune homeostasis [review]. *Nat Rev Genet* 2006; 7:917–28.
2. Remmers EF, Plenge RM, Lee AT, Graham RR, Horn G, Behrens TW, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N Engl J Med* 2007;357:977–86.
3. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–78.
4. Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis: a genomewide study. *N Engl J Med* 2007;357:1199–209.
5. Cargill M, Schrodi SJ, Chang M, Garcia VE, Brandon R, Callis KP, et al. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet* 2007;80:273–90.
6. Capon F, Di Meglio P, Szaub J, Prescott NJ, Dunster C, Baumber L, et al. Sequence variants in the genes for the interleukin-23 receptor (IL23R) and its ligand (IL12B) confer protection against psoriasis. *Hum Genet* 2007;122:201–6.
7. Smith RL, Warren RB, Eyre S, Ho P, Ke X, Young HS, et al. Polymorphisms in the IL-12 β and IL-23R genes are associated with psoriasis of early onset in a UK cohort. *J Invest Dermatol* 2007. E-pub ahead of print.
8. Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, et al. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006;314:1461–3.
9. Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA, et al. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. *Nat Genet* 2007;39:830–2.
10. Wellcome Trust Case Control Consortium; Australo-Anglo-American Spondylitis Consortium (TASC), Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A, et al. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat Genet* 2007;39:1329–37.
11. Kastelein RA, Hunter CA, Cua DJ. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation [review]. *Annu Rev Immunol* 2007;25:221–42.
12. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
13. Carlton VE, Hu X, Chokkalingam AP, Schrodi SJ, Brandon R, Alexander HC, et al. PTPN22 genetic variation: evidence for multiple variants associated with rheumatoid arthritis. *Am J Hum Genet* 2005;77:567–81.
14. Kurreeman FA, Padyukov L, Marques RB, Schrodi SJ, Seddighzadeh M, Stoeken-Rijsbergen G, et al. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis. *PLoS Med* 2007;4:e278.
15. Iannone MA, Taylor JD, Chen J, Li MS, Rivers P, Slentz-Kesler KA, et al. Multiplexed single nucleotide polymorphism genotyping by oligonucleotide ligation and flow cytometry. *Cytometry* 2000; 39:131–40.
16. Germer S, Holland MJ, Higuchi R. High-throughput SNP allele-frequency determination in pooled DNA samples by kinetic PCR. *Genome Res* 2000;10:258–66.
17. Sokal RR, Rohlf FJ. *Biometry: the principles and practice of statistics in biological research*. 3rd ed. New York: WH Freeman; 1995. p. 760–7.
18. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 2002;70: 425–34.

19. Orozco G, Gonzalez-Gay MA, Paco L, Lopez-Nevot MA, Guzman M, Pascual-Salcedo D, et al. Interleukin 12 (IL12B) and interleukin 12 receptor (IL12RB1) gene polymorphisms in rheumatoid arthritis. *Hum Immunol* 2005;66:710–5.
20. Hall MA, McGlenn E, Coakley G, Fisher SA, Boki K, Middleton D, et al. Genetic polymorphism of IL-12 p40 gene in immune-mediated disease. *Genes Immun* 2000;1:219–24.
21. Orozco G, Rueda B, Robledo G, Garcia A, Martin J. Investigation of the IL23R gene in a Spanish rheumatoid arthritis cohort. *Hum Immunol* 2007;68:681–4.
22. Begovich AB, Chang M, Caillier SJ, Lew D, Catanese JJ, Wang J, et al. The autoimmune disease-associated IL12B and IL23R polymorphisms in multiple sclerosis. *Hum Immunol* 2007;68:934–7.
23. McGonagle D, McDermott MF. A proposed classification of the immunological diseases. *PLoS Med* 2006;3:e297.

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Clinical Image: Lipoma arborescens: a rare cause of recurrent knee effusion



The patient, a 47-year-old man, presented with recurrent knee effusions. Magnetic resonance imaging of the knee (sagittal view) showed a large effusion associated with multiple ovoid filling defects of fat density throughout the knee joint space, but more in the suprapatellar pouch (**arrow**). This appearance is typical of lipoma arborescens, a rare benign synovial lipoma that predominantly affects the knee and can cause synovitis and recurrent effusions in joints. Lipoma arborescens should be considered in the differential diagnosis of patients with recurrent knee effusion of unknown etiology.

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