Genome-Wide Association Meta-Analysis Reveals Novel Juvenile Idiopathic Arthritis Susceptibility Loci

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Objective. Juvenile idiopathic arthritis (JIA) is the most common childhood rheumatic disease and has a strong genomic component. To date, JIA genetic association studies have had limited sample sizes, used heterogeneous patient populations, or included only candidate regions. The aim of this study was to identify new associations between JIA patients with oligoarticular disease and those with IgM rheumatoid factor (RF)−negative polyarticular disease, which are clinically similar and the most prevalent JIA disease subtypes.

Methods. Three cohorts comprising 2,751 patients with oligoarticular or RF-negative polyarticular JIA were genotyped using the Affymetrix Genome-Wide SNP Array 6.0 or the Illumina HumanCoreExome-12+ Array. Overall, 15,886 local and out-of-study controls, typed on these platforms or the Illumina HumanOmni2.5, were used for association analyses. High-quality single-nucleotide polymorphisms (SNPs) were used for imputation to 1000 Genomes prior to SNP association analysis.

Results. Meta-analysis showed evidence of association ($P < 1 \times 10^{-6}$) at 9 regions: *PRR9_LOR* ($P = 5.12 \times 10^{-8}$), *ILDR1_CD86* ($P = 6.73 \times 10^{-8}$), *WDFY4* ($P = 1.79 \times 10^{-7}$), *PTH1R* ($P = 1.87 \times 10^{-7}$), *RNF215* ($P = 3.09 \times 10^{-7}$), *AHI1_LINC00271* ($P = 3.48 \times 10^{-7}$), *JAK1* ($P = 4.18 \times 10^{-7}$), *LINC00951* ($P = 5.80 \times 10^{-7}$), and *HBP1* ($P = 7.29 \times 10^{-7}$). Of these, *PRR9_LOR, ILDR1_CD86, RNF215, LINC00951,* and *HBP1* were shown, for the first time, to be autoimmune disease susceptibility loci. Furthermore, associated SNPs included *cis* expression quantitative trait loci for *WDFY4, CCDC12, MTP18, SF3A1, AHI1, COG5, HBP1,* and *GPR22.*

Conclusion. This study provides evidence of both unique JIA risk loci and risk loci overlapping between JIA and other autoimmune diseases. These newly associated SNPs are shown to influence gene expression,

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and their bounding regions tie into molecular pathways of immunologic relevance. Thus, they likely represent regions that contribute to the pathology of oligoarticular JIA and RF-negative polyarticular JIA.

Juvenile idiopathic arthritis (JIA) is the most common childhood rheumatic disease, with a prevalence of ~1 per 1,000 children (1). It is a disabling, complex disorder characterized by inflammation of the joints and other tissues that persists for at least 6 weeks. Although there is ethnic diversity in JIA, it is a disease that predominately affects children of European descent (2). There is a strong genetic component to JIA, with numerous established susceptibility loci (3−5) and a sibling recurrence risk ratio (λ_s) of 11.6 (6). Additionally, the prevalence of other autoimmune diseases is increased in relatives of patients with JIA (7).

The International League of Associations for Rheumatology (ILAR) categorizes JIA into 7 subtypes (8). Two of these subtypes, oligoarticular JIA (persistent and extended forms) and IgM rheumatoid factor (RF)−negative polyarticular JIA, account for ~70% of cases of JIA and are the focus of the current study. These 2 subtypes present similarly in the clinic, are distinguished only by the number of affected joints after a disease duration of 6 months, and share HLA associations (9,10).

Genome-wide association studies (GWAS), while widely used across autoimmune diseases, have been somewhat limited in scope for JIA. Previous high-density array−based studies in patients with oligoarticular JIA or RF-negative polyarticular JIA have provided evidence of association for single-nucleotide polymorphisms (SNPs) corresponding to loci near or including *PTPN22, PTPN2, IL2RA, TNFAIP3, COG6, ADAD1/IL2/IL21, STAT4,* chromosome 3q13 within *C3orf1* and near *CD80,* and chromosome 10q21 near *JMJD1C* (4,5). Other GWAS included all JIA subtypes and showed associations with *TRAF1/C5* or *VTCN1* loci but were limited by either small sample sizes (11) or the number of markers assayed (12).

More recently, the Juvenile Arthritis Consortium for Immunochip studied patients and controls of European ancestry, using the Illumina Infinium Immunochip genotyping array, which provides dense SNP coverage in the HLA region and is limited to 186 non-HLA regions identified in 12 early studies of autoimmune disease association (not including JIA) (3). Therefore, it does not reflect the current catalog of autoimmune disease findings. Results from the Immunochip analyses provided convincing evidence of association for a number of JIA risk loci that are also risk loci for other autoimmune diseases, including the HLA region (10) and 27 non-HLA loci (3). However, a significant proportion of JIA heritability risk remains unexplained. Although there is a partial overlap between the patient and control samples used in this study and the Immunochip studies (3,10), the current study extends findings to a genome-wide level to further delineate JIA genetic risk factors and allows the findings to be translated to JIA disease mechanisms.

PATIENTS AND METHODS

Subjects. Three cohorts comprising 2,751 JIA patients of European ancestry with oligoarticular disease or RF-negative polyarticular disease and 15,886 controls (cohort I, 814 cases and 3,058 controls; cohort II, 1,057 cases and 11,843 controls; cohort III, 880 cases and 985 controls) were used for association analyses. Subjects in cohort I were primarily recruited from the Cincinnati Children's Hospital Medical Center (CCHMC) or as part of a National Institute of Arthritis and Musculoskeletal and Skin Diseases−supported registry of JIA-affected sibpairs. Collaborating centers including Children's Hospital of Wisconsin, Schneider Children's Hospital, and Children's Hospital of Philadelphia provided additional samples (4,5). JIA patients in cohort II have been described previously as a validation cohort (4,5). Clinics enrolling JIA patients for cohort III were located in Cincinnati, OH, Atlanta, GA, Charlotte, NC, Columbus, OH, Little Rock, AR, Long Island, NY, Chicago, IL, Salt Lake City, UT, Cleveland, OH, Nashville, TN, and Charleston, SC. Additional DNA samples, split between cohorts II and III, were collected in Cincinnati, OH (n = 105) or were collected as part of or obtained from the Gene Expression in Pediatric Arthritis Study (National Institute of Arthritis and Musculoskeletal and Skin Diseases [NIAMS] grant P01-AR-048929) (n = 117), Children's Mercy Hospital, Kansas City (n = 75), the Improved Understanding of the Biology and Use of TNF Inhibition in Children with JIA Study (ClinicalTrials.gov identifier: NCT00792233) (n = 40), Nemours/Alfred I. duPont Hospital for Children (n = 38), the Boston Children's Hospital JIA Registry (n = 26), the Trial of Early Aggressive Therapy in JIA Study (TREAT) (ClinicalTrials.gov identifier: NCT00443431) (n = 25), Emory University School of Medicine (n = 19), and Cohen Children's Medical Center (n = 4). Members of the consortia are shown in Appendix A.

All cases met the ILAR or American College of Rheumatology (13) classification criteria for JIA or juvenile rheumatoid arthritis (RA). Both regional and out-of-study controls obtained from the dbGaP database were included in the control cohorts. Controls for cohort I included regional controls recruited from the geographic region served by CCHMC (4,5) and 2,400 out-of-study controls from the Molecular Genetics of Schizophrenia nonGAIN Sample (MGS_nonGAIN; phs000167.v1vp1). Controls for cohort II included "Texas," "Utah," and "German" regional controls (4,5), 7,324 controls from the Atherosclerosis Risk in Communities Study cohort (phs000280.v3.p1), 2,555 controls from the Genetic Association Information Network (GAIN; phs000021.v3.p2 and phs000017.v3.p1), and 1,792 controls from the Cooperative Health Research in the Region

Augsburg (KORA) study (14). Cohort III contained only regional controls recruited in Denver. Use of these DNA collections has been approved by the Institutional Review Board at all participating centers, and participants or their parents provided written consent prior to study enrollment. Cohorts I and II have been used in previous association studies (4,5), and overall, ~65% of the JIA samples from the current study were used for Immunochip analyses (3,10).

Genotyping and quality control. Cohorts I and II were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0. Cohort I was genotyped at the Affymetrix Service Center, while cohort II was genotyped at Expression Analysis/Quintiles; cohort III was genotyped with the Illumina HumanCoreExome-12+ Array (Exome Array), which included 2,508 custom SNP assays derived from initial analyses in cohort I, at CCHMC. All out-of-study controls were genotyped on SNP Array 6.0, with the exception of the KORA cohort, which was genotyped on the HumanOmni2.5 BeadChip (Infinium). Samples were excluded if their call rates were <98% across the SNPs that passed quality control filters. Duplicates and first-degree relatives were identified using the software package KING (15), retaining the sample with the highest call rate. Self-reported and genetically determined sex were compared using chromosome X genotype data. The program ADMIXTURE (16) was used to compute admixture estimates from a subset of SNPs that met quality control criteria and were pruned to have low linkage disequilibrium (LD) ($r^2 < 0.2$). This study was limited to individuals who self-reported European ancestry, and individuals whose admixture estimates were outliers were removed. Primary inference was based on SNPs that showed no significant evidence of departure from expectation in Hardy-Weinberg equilibrium proportions ($P < 1 \times 10^{-6}$ and $P < 0.01$ in cases and controls, respectively), significant differential missingness between cases and controls ($P < 0.05$), a minor allele frequency of ≥0.01, and a call rate of >95%.

Statistical analysis. *Imputation*. Because the 3 cohorts were genotyped on 2 different arrays at 3 different times, imputation was performed separately for each cohort, using IMPUTE2 with the 1000 Genomes phase 1 integrated reference panel (17). Imputed SNPs were retained if their information score was >0.5 and their confidence score was >0.9. To validate imputation, a representative subset of subjects (96 subjects from each of the 3 JIA cohorts and 96 control subjects) were genotyped for 16 SNPs, including 7 of the 9 SNPs shown in Table 1, using TaqMan SNP Genotyping Assays (Life Technologies) and evaluated for concordance between imputed and TaqMan-generated genotypes. Reactions were performed on a ViiA 7 real-time polymerase chain reaction system (Applied Biosystems).

Tests of association. Tests of association were performed on the imputed data using SNPTEST under a logistic model, taking imputed genotype uncertainty into account (16). Admixture proportions were included as covariates. A weighted inverse normal meta-analysis was conducted to combine results across cohorts. Due to the discrepancy in the control-to-case ratio across cohorts, evidence was weighted by size of the case-only sample. For each cohort, a SNP was included in the meta-analysis if it passed the quality control criteria described previously and the additional requirement of 30 and 10 homozygotes for the minor allele for the additive and recessive models, respectively. For the dominant model, a total of 10 minor allele genotypes (heterozygote or homozygote) was required. Inference was based on the set of SNPs for which the meta-analysis contained data from at least 2 of the 3 cohorts and where the direction of the effect was consistent across contributing cohorts. This additional requirement reduces the Type I error rate.

Functional annotation analysis. The functional potential of the SNPs in the region of association ($r^2 \geq 0.8$) were examined using HaploReg version 2 (18) and RegulomeDB, a database that annotates SNPs with known and predicted regulatory elements, expression quantitative trait loci (eQTLs), DNase hypersensitivity, and binding sites of transcription factors in the intergenic regions of the human genome (19). Histone data were evaluated using the positions of the original SNPs as well as proxy SNPs in LD ($r^2 \geq 0.8$). Three well-studied epigenetic marks (H3K4me1, H3K4me3, and H3K27ac) from the ENCODE and Roadmap Epigenomic projects were evaluated. For the ENCODE data, tables for the 3 marks for each of the tier 1 cell lines (GM12878 cells, H1-hESC cells, human skeletal muscle myoblasts [HSMMs], human umbilical vein endothelial cells [HUVECs], K562 cells, normal HEK cells, and normal human lung fibroblasts [HLFs]) were downloaded from the UCSC Genome Browser (genome.ucsc.edu) using SNP positions. The Roadmap Epigenomic data were downloaded similarly, using the EpiGenome Browser (www.epigenomebrowser.org) for available cell types with probable relevance to JIA (CD14, CD15, CD19, primary peripheral blood mononuclear cells [PBMCs], CD3, primary memory CD4 cells (CD4M), primary CD4-naive cells (CD4N), and primary CD4+CD25+CD127− Treg cells [CCCTreg]).

Pathway analysis. Relationships between gene products were analyzed using IPA (Qiagen; www.qiagen.com/ingenuity). The genes analyzed were from 2 sources: all genes from the current JIA GWAS ($P < 1 \times 10^{-6}$) and all genes associated with oligoarticular and RF-negative polyarticular JIA using the Immunochip ($P < 5 \times 10^{-8}$) (3). Genes included in the analysis were *JAK1, PRR9, LOR, PTH1R, CD86, LINC00951 (FLJ41649), AHI1, LINC00271, HBP1, WDFY4, RNF215, HLA−DRB1, PTPN22, ATP8B2, IL6R, STAT4, IL2, IL21, ERAP2, LNPEP, C5orf56, IRF1, IL2RA, PRR5L, COG6, PTPN2, ANKRD55, TYK2, SH2B3, ATXN2, UBE2L3, RUNX1, IL2RB, FAS, ZFP36L1,* and *LTBR. LINC00951 (FLJ41649)* was not mapped using IPA at the time of analysis (version release date: December 2016). Only experimentally validated interactions were considered.

RESULTS

Demographics. The 3 JIA cohorts in this study were restricted to patients with either oligoarticular or RF-negative polyarticular disease, in order to reduce phenotypic heterogeneity. Across cohorts, 2,751 patients met individual-level quality control criteria (see Supplementary Table 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40216/abstract). Of these, 1,581 patients had a diagnosis of oligoarticular JIA, and 1,170 patients had a diagnosis of RF-negative polyarticular JIA. Overall, there were 629 male JIA patients (22.9%)

and 2,122 female JIA patients (77.1%). The mean ± SD age at the onset of JIA was 4.37 ± 3.59 years in female patients with oligoarticular JIA, 6.59 ± 4.51 years in female patients with RF-negative polyarticular JIA, 5.93 ± 3.71 years in male patients with oligoarticular JIA, and 7.35 ± 4.08 years in male patients with RF-negative polyarticular JIA. A group of 15,886 genetically well-matched controls was used, which included local controls from the US and Germany as well as out-of-study controls (see Patients and Methods) (details are shown in Supplementary Table 1). The current study is powered to detect associations with odds ratios (ORs) of 1.20, assuming an allele frequency between 0.30 and 0.40 (see Supplementary Figure 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40216/abstract).

Inferential SNP data set. The association analysis accounted for imputation uncertainty and included admixture proportions in the logistic model as covariates. For comparability, scaling the genomic inflation factors (λ) to the equivalent of 1,000 cases and 1,000 controls within each cohort yielded $\lambda_{1,000}$ values of 1.04, 1.09, and 1.03, respectively, for the 3 cohorts. A total of 622,740 SNPs (SNP Array 6.0) for cohort I, 535,078 SNPs (SNP Array 6.0) for cohort II, and 256,455 SNPs (Exome Array) for cohort III passed the genotyping quality control measures described in Patients and Methods. Including the HLA region, 4,710,143 SNPs passed imputation meta-analysis quality control filtering.

Discovery of new oligoarticular and RF-negative polyarticular JIA loci. The association results ($P < 1 \times 10^{-6}$) of the meta-analysis of 3 cohorts (2,751 patients and 15,886 controls) are shown in Figure 1. The 9 newly identified oligoarticular and RF-negative polyarticular JIA loci include *JAK1*, *PRR9_LOR*, *PTH1R*, *ILDR1_CD86*, *LINC00951 (FLJ41649)*, *AHI1_LINC00271*, *HBP1*, *WDFY4*, and *RNF215*. Regional plots of association (LocusZoom) are shown in Supplementary Figure 2, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40216/abstract. Lead SNPs representing novel associations with oligoarticular and RF-negative polyarticular JIA are shown in Table 1. For each region shown in Table 1, no signal remained after conditioning on the lead SNP. The strongest associations included *PRR9_LOR* (rs873234 [$P = 5.12 \times 10^{-8}$, OR 1.43, 95% CI 1.25−1.63]) and *ILDR1_CD86* (rs111700762 [$P = 6.73 \times 10^{-8}$, OR 1.45, 95% CI 1.26−1.66]). In addition, 36 previously unidentified loci achieved suggestive levels of significance ($1 \times 10^{-6} < P < 1 \times 10^{-5}$) in the meta-analysis and are shown in Supplementary Table 2 (available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40216/abstract).

A subset of imputed SNPs from Table 1 and Supplementary Table 2 were technically validated by direct genotyping (n = 16). Concordance of >98% between imputed and genotyped SNPs was observed in all SNPs evaluated (>99% concordance in 11 of the SNPs). All remaining SNPs meeting a false discovery rate−corrected P value less than 0.05 (n = 1,782) are shown in Supplementary Table 3 (available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40216/abstract).

Autoimmune disease loci overlap. Support for the 9 newly associated oligoarticular and RF-negative polyarticular JIA loci was gathered using the National Human Genome Research Institute and the European

Table 1. SNPs representing top statistical associations ($P < 1 \times 10^{-6}$) with oligoarticular JIA and RF-negative polyarticular JIA*

| Lead SNP (gene region) | Chr.:position† | Minor allele | MAF | | P | Model | OR (95% CI) | SNP position |
			Cases	Controls				
rs10889504 (*JAK1*)	1:65390503	C	0.11	0.13	4.18×10^{-7}	Add	0.78 (0.71−0.86)	Intron
rs873234 (*PRR9_LOR*)	1:153227177	A	0.40	0.37	5.12×10^{-8}	Rec	1.43 (1.25−1.63)	Intergenic
rs1138518 (*PTH1R*)‡	3:46944274	T	0.42	0.37	1.87×10^{-7}	Add	1.23 (1.14−1.34)	Coding (synonymous)
rs111700762 (*ILDR1_CD86*)	3:121780807	A	0.08	0.06	6.73×10^{-8}	Dom	1.45 (1.26−1.66)	Intron
rs10807228 (*LINC00951*)	6:40188351	T	0.36	0.33	5.80×10^{-7}	Rec	1.42 (1.23−1.65)	Intergenic
rs9321502 (*AHI1_LINC00271*)	6:135656252	C	0.43	0.40	3.48×10^{-7}	Add	1.18 (1.11−1.26)	Intron
rs11865019 (*HBP1*)	7:106812246	G	0.24	0.27	7.29×10^{-7}	Add	0.84 (0.78−0.90)	Intron
rs1904603 (*WDFY4*)	10:50013840	G	0.29	0.25	1.79×10^{-7}	Dom	1.27 (1.16−1.39)	Intron
rs5753109 (*RNF215*)	22:30777888	C	0.31	0.28	3.09×10^{-7}	Add	1.19 (1.11−1.28)	Intron

* The 3 cohorts comprised 2,751 cases and 15,886 controls. The loci are named using the genes bounding the regions of association and do not necessarily reflect a functional link with a specific gene. SNPs = single-nucleotide polymorphisms; JIA = juvenile idiopathic arthritis; RF = rheumatoid factor; MAF = minor allele frequency; Chr. = chromosome; OR = odds ratio; 95% CI = 95% confidence interval; Add = additive; Rec = recessive; Dom = dominant.
† Coordinates are based on the GRCh37 assembly.
‡ Because rs1138518 did not pass the quality control criteria in all cohorts, the numbers of cases and controls were 1,937 and 12,828, respectively.
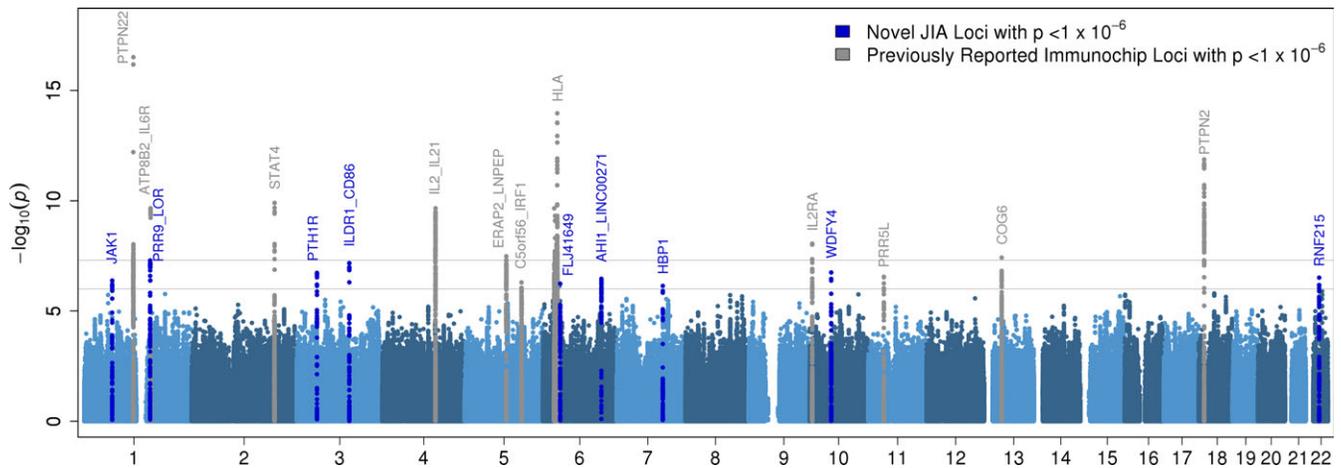
Figure 1. Manhattan plot of genome-wide genetic association statistics for oligoarticular and rheumatoid factor–negative polyarticular juvenile idiopathic arthritis (JIA) risk loci. The upper gray line indicates the genome-wide significance ($P < 5 \times 10^{-8}$) threshold. The lower gray line indicates the suggestive association ($P < 1 \times 10^{-6}$) threshold. Loci reaching this threshold and individual single-nucleotide polymorphisms mapping to these loci are shown in dark blue. Loci in gray have been reported for association by Hinks et al (see ref. 3). The loci are named using the genes bounding the regions of association and do not necessarily reflect a functional link with a specific gene.

Bioinformatics Institute GWAS Catalog (20) and Immunobase (https://www.immunobase.org). The lead SNP at each novel locus, as well as proxy SNPs in LD ($r^2 \geq 0.4$), were queried in the aforementioned databases to search for overlapping regions of association between oligoarticular and RF-negative polyarticular JIA and other autoimmune diseases. Four regions (*JAK1*, *PTH1R*, *AHI1_LINC00271*, and *WDFY4*) have been associated in autoimmune diseases (Table 2): *JAK1* in celiac disease (21) and multiple sclerosis (MS) (22); *PTH1R* in celiac disease (21); *AHI1_LINC00271* in celiac disease (21), MS (22), autoimmune thyroid disease (23), and type 1 diabetes mellitus (DM) (24); and *WDFY4* in systemic

lupus erythematosus (SLE) (25). Despite these shared associated loci, the causal variants of each region may not necessarily be the same among diseases.

Functional implications of oligoarticular JIA– and RF-negative polyarticular JIA–associated loci. In order to elucidate the impact of SNPs located in the oligoarticular JIA– and RF-negative polyarticular JIA–associated loci on gene transcription, each lead SNP and all proxy SNPs ($r^2 \geq 0.8$) were examined for eQTLs in tissues, using 2 public gene expression databases, the eQTL Browser from the University of Chicago (eqtl.uchicago.edu) and the Blood eQTL Browser (26). While both *cis* and *trans* eQTLs were queried, the

Table 2. Overlapping regions of association between oligoarticular and RF-negative polyarticular JIA and other autoimmune diseases*

JIA loci			Susceptibility loci for other autoimmune diseases†							
Lead SNP	Gene region	Chr.:position	Disease	SNP	Chr.:position‡	r^2§	P	OR	Risk allele	RAF
rs10889504	*JAK1*	1:65390503	CeD	rs12409333	1:65417839	0.90	3.80×10^{-5}	0.89	G	0.25
			MS	rs12409333	1:65417839	0.90	2.70×10^{-4}	0.91	G	0.25
rs1138518	*PTH1R*	3:46944274	CeD	rs2061197	3:47001350	0.41	8.55×10^{-6}	1.09	A	0.37
			MS	rs11154801	6:135739355	0.74	1.80×10^{-20}	1.12	A	0.37
rs9321502	*AHI1_LINC00271*	6:135656252	CeD	rs12206850	6:135797808	0.70	6.89×10^{-4}	1.07	C	0.27
			ATD	rs2179781	6:135719500	0.76	4.84×10^{-4}	NR	NR	NR
			Type 1 DM	rs11154801	6:135739355	0.74	2.55×10^{-5}	1.08	A	0.29
rs1904603	*WDFY4*	10:50013840	SLE	rs877819	10:50042951	0.47	8.00×10^{-9}	1.46	A	0.18

* The loci are named using the genes bounding the regions of association and do not necessarily reflect a functional link with a specific gene. RF = rheumatoid factor; Chr. = chromosome; OR = odds ratio; RAF = risk allele frequency; CeD = celiac disease; MS = multiple sclerosis; ATD = autoimmune thyroid disease; NR = not reported; DM = diabetes mellitus; SLE = systemic lupus erythematosus.
† Data were obtained from the Human Genome Research Institute and the European Bioinformatics Institute GWAS Catalog (https://www.ebi.ac.uk/gwas/) and Immunobase (https://www.immunobase.org).
‡ Coordinates are based on the GRCh37 assembly.
§ Correlation between lead single-nucleotide polymorphisms (SNPs) for juvenile idiopathic arthritis (JIA) loci.

Table 3. Expression of quantitative trait locus (eQTL) genes in novel oligoarticular- and RF-negative polyarticular JIA−associated regions*

Lead SNP (gene region), eQTL SNP†	Chr.:position‡	r^2§	eQTL gene
rs1138518 (*PTH1R*)			
rs2242116	3:46941116	1.00	*CCDC12*
rs9321502 (*AHI1_LINC00271*)			
rs2614276	6:135681704	0.83	*AHI1*
rs111865019 (*HBP1*)			
rs7790080	7:107031322	0.82	*COG5*
rs2301801	7:106870746	0.82	*HBP1*
rs2237659	7:106847492	0.82	*GPR22*
rs1904603 (*WDFY4*)			
rs2940707	10:49989792	0.95	*WDFY4*
rs5753109 (*RNF215*)			
rs757870	22:30776419	0.91	*MTP18*
rs4820003	22:30711624	0.91	*SF3A1*

* The loci are named using the genes bounding the regions of association and do not necessarily reflect a functional link with a specific gene. RF = rheumatoid factor; JIA = juvenile idiopathic arthritis; Chr. = chromosome.
† The single-nucleotide polymorphism (SNP) with the highest eQTL score for each eQTL gene is shown; data were obtained from the University of Chicago (eqtl.uchicago.edu) and Blood eQTL Browser (26).
‡ Coordinates are based on the GRCh37 assembly.
§ Correlation with lead SNP.

results identified evidence of *cis* eQTLs only. Strong eQTLs were identified for 5 oligoarticular and RF-negative polyarticular JIA loci: *PTH1R* SNPs for *CCDC12*; *AHI1_LINC00271* SNPs for *AHI1*; *HBP1* SNPs for *HBP1*, *COG5*, and *GPR22*; *WDFY4* SNPs for *WDFY4*; and *RNF215* SNPs for *MTP18* and *SF3A1* (Table 3).

Next, the novel oligoarticular JIA− and RF-negative polyarticular JIA−associated loci were analyzed for histone modifications. Using the 7 cell types in the ENCODE database for which the desired data were available (GM12878 cells, H1-hESC cells, HSMMs, HUVECs, K562 cells, normal HEK cells, and normal HLFs) and immunologically relevant cell types from the EpiGenome Browser (CD14, CD15, CD19, PBMCs, CD3, CD4M, CD4N, and CCCTreg [see Patients and Methods]), the SNP set described above for the eQTL analysis was evaluated for H3K4me1, H3K4me3, and H3K27ac modifications. H3K4me1 modifications tend to mark enhancer regions, whereas H3K4me3 modifications tend to mark promoter regions. Regions that are transcriptionally activated are associated with H3K27ac marks (27). Supplementary Table 4 (available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40216/abstract) summarizes the histone modification mark data for each of the 9 novel oligoarticular JIA and RF-negative polyarticular JIA associations, indicated by the enrichment score (number

of sequences) centered on a 25-bp window size. The maximum enrichment score among cell lines was determined, and the top 5 values for each histone mark were identified. Several SNPs were located in regions with enriched histone marks, mostly in isolated cell types. In addition, some SNPs were in regions with multiple marks (e.g., rs72922282 and rs10511408), indicating a high likelihood that these SNPs are in functionally active chromatin regions.

Comparison of RA- and type 1 DM−associated loci with oligoarticular and RF-negative polyarticular JIA. Many of the risk loci identified for oligoarticular JIA and RF-negative polyarticular JIA are shared with other autoimmune diseases, particularly RA and type 1 DM (3). However, individual variants identified may vary between diseases. To date, association studies have identified 101 loci for RA (28) and 50 loci for type 1 DM (24). Supplementary Tables 5 and 6 (available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40216/abstract) show the findings from this study for each SNP reported for RA and type 1 DM, respectively. Within the oligoarticular JIA and RF-negative polyarticular JIA data set, 86 RA and 39 type 1 DM SNPs met quality control metrics. In total, 9 RA and 6 type 1 DM SNPs, or only 10−15%, reached a Bonferroni-corrected P value less than 5.8×10^{-4} (calculated based on 86 tests) or $P < 1.2 \times 10^{-3}$ (calculated based on 39 tests), respectively. These significantly associated SNPs in oligoarticular and RF-negative polyarticular JIA are shown in Supplementary Tables 5 and 6.

Additionally, a power analysis for each of these RA-associated SNPs (see Supplementary Table 5) and type 1 DM−associated SNPs (see Supplementary Table 6) was computed, assuming the OR reported for RA or type 1 DM, the allele frequency in the JIA controls, the JIA patient sample size, and the Type I error rate defined by the Bonferroni corrections described above. The sum of the power across the SNPs is the statistical expectation of the number of associations a cohort of the size observed in this meta-analysis would detect. If the effect sizes in the JIA cohort were consistent with the RA or type 1 DM effect sizes for the respective SNPs, then the expected number of associations the JIA cohort should detect for RA is 25.9 (95% confidence interval [95% CI] 16.5−32.7) and for type 1 DM is 26.1 (95% CI 21.1−31.1).

DISCUSSION

This study includes the largest JIA cohort analyzed on genome-wide platforms to date (2,751 patients with oligoarticular JIA or RF-negative polyarticular

JIA and 15,886 controls) and identifies new oligoarticular and RF-negative polyarticular JIA associations (Figure 1). Nine of the 28 loci detected in oligoarticular and RF-negative polyarticular JIA Immunochip studies (3) remained significant ($P < 5 \times 10^{-8}$) in the current genome-wide analysis (*PTPN22, ATP8B2_IL6R, STAT4, IL2_IL21, ERAP2_LNPEP, HLA, IL2RA, COG6,* and *PTPN2*); 2 additional regions, *C5orf56_IRF1* and *PRR5L*, achieved suggestive levels of association ($P < 1 \times 10^{-6}$) (Figure 1). The higher-density SNP coverage provided by the Immunochip platform compared to genome-wide arrays may account for differences in the findings. However, there are comparable magnitudes of effect (Spearman's correlation coefficient = 0.89) between the OR reported for the most significant SNPs identified in the Immunochip study (3) and the current GWAS. Use of the Exome Array for the genotyping of cohort III was economically driven, but the lack of SNP inclusion in this array may contribute to these differential findings.

Although ~70% of the patient samples used for this study have been used in previous association studies (3−5), by using genome-wide data sets, we now extend the number of genetic loci associated with JIA. In spite of these efforts, the newly reported associations do not meet the generally used, but somewhat arbitrary, threshold of $P < 5 \times 10^{-8}$. Genome-wide thresholds assume 1 million independent tests and a genome-wide Type I error rate of 0.05. Yet, it is difficult to determine how many independent tests of association are computed in a GWAS, because LD pruning using $r^2 < 0.4$ reduces the number of loci in this European ancestral cohort to <1 million, and even these remain correlated. Nonetheless, the need remains to validate these findings in other cohorts, which are difficult to acquire for a rare disease affecting young children. Indeed, the patient cohorts reported were collected over the course of several decades.

Four of the newly identified oligoarticular JIA and RF-negative polyarticular JIA loci (*JAK1, PTH1R, AHI1_LINC00271,* and *WDFY4*) (see Table 2) reported here have been described as being associated with another autoimmune disease, according to the GWAS Catalog and ImmunoBase, suggesting common pathophysiologic mechanisms. Although the databases cataloging GWAS and Immunochip findings are incomplete, they begin to allow the findings of this study to be put in the context of other autoimmune diseases. Only a small percentage of the reported lead RA (28) and type 1 DM (24) SNPs reaching the Bonferroni-corrected P value cutoffs in our data set (see Supplementary Tables 5 and 6, available on the *Arthritis &*

Rheumatology web site at http://onlinelibrary.wiley. com/doi/10.1002/art.40216/abstract). This is markedly less than expected and well outside the confidence intervals. Given that the RA- and type 1 DM−associated SNPs were discovered in predominately European ancestral groups, it is unlikely that this deficit is merely due to differences in LD among the different disease populations. Rather, these results support the notion that although JIA (at least the oligoarticular and RF-negative polyarticular subtypes) shares some risk loci, it is genetically distinct from seropositive RA (despite similar clinical presentations) and type 1 DM (another autoimmune disease with childhood onset). The remaining 5 unique newly identified oligoarticular and RF-negative polyarticular JIA loci reveal additional regions of the genome that will require further investigation to fully delineate their importance in JIA and autoimmune diseases in general.

The *PRR9_LOR* locus was the most strongly associated novel region, approaching genome-wide significance ($P = 5.12 \times 10^{-8}$). *PRR9* encodes a protein of unknown function, with the highest expression levels in the skin (29), while *LOR* encodes loricrin, a component of the cornified cell envelope in terminally differentiated epidermal cells. Interestingly, association analyses in patients with psoriasis, an inflammatory skin disorder, have identified *PRR9* as a susceptibility locus (30,31). Although this may represent overlap of disease risk loci between JIA and psoriasis, there is also the possibility that the JIA cohorts include psoriasis patients, given that ~20−25% of patients present with arthritis before skin disease, which is difficult to define in younger children.

Biologic pathways that feature gene products suggested by the results of this study and previous Immunochip studies (3,10) may offer insight into disease pathophysiology (see Figure 2). While some of the associated regions observed in the current study may not have direct functional relationships with other loci, loci such as *CD86* and *JAK1* appear to be central to key signaling pathways involving T cell differentiation and proliferation. *JAK1* encodes a tyrosine kinase that interacts with the common γ-chain to elicit signals from cytokines, such as interleukin-2 (IL-2) and IL-21, to induce interferon-γ, IL-17, and IL-6 production (32). Further bioinformatic analysis revealed the presence of H3K4me1 histone modifications near *JAK1* in CD14+ cells and CD15+ cells, suggesting the presence of an enhancer region at this locus (see Supplementary Table 4, available on the *Arthritis & Rheumatology* web site at http:// onlinelibrary.wiley.com/doi/10.1002/art.40216/abstract). Notably, a JAK inhibitor, tofacitinib, is already used for

Figure 2. IPA showing interactions between products of genes located in the juvenile idiopathic arthritis–associated regions from the current study and previous Immunochip studies. Solid lines represent direct interactions, where 2 molecules make direct physical contact with each other. Dashed lines represent indirect interactions. Only experimentally validated interactions are shown. GWAS = genome-wide association study.

the treatment of RA. With clinical trials underway, it remains to be seen whether inhibitors of the *JAK1* pathway are efficacious in children with JIA.

Given that JIA is an autoimmune disease, it was expected that additional immune-related disease loci that were not found on Immunochip would be identified by genome-wide approaches. Specifically, CD86 is important in the immune regulation of multiple immune cell types (33). It is expressed on antigen-presenting cells and interacts with both CD28 and CTLA-4 on T cells, resulting in T cell stimulation or inhibition, respectively (34). CD86 signaling has been shown to lead to increased antibody production (35). Modulation of CD86 with CTLA-4Ig has been shown to down-regulate proinflammatory cytokine production (including IL-6 and tumor necrosis factor α) by synovial macrophages in vitro (36). Conditional analysis showed that the *CD86* association signal is independent of the previously reported signal at Chr3q13 that includes the nearby gene *CD80* (4).

As expected, most associated regions fall within noncoding, regulatory regions. An exception, the association in *PTH1R*, is represented by a synonymous coding region mutation (Table 1). Although this SNP does not

alter the amino acid sequence of the protein, it still may potentially contribute to disease through epigenetic (37−39) or other mechanisms, making it an interesting region for future investigation. Furthermore, *PTH1R* is expressed at high levels in the bone and activates *RANKL* expression, which promotes thymic tolerization and the dendritic cell−T cell interaction for T cell activation (40). In addition, a functional polymorphism in the *RANKL* promoter region (rs7984870) has been associated with early age at onset of RA (~50 years) (41), suggesting the potential mechanistic importance of *PTH1R* in JIA.

Gene expression and chromatin accessibility analyses suggest that many of the associated SNPs located in noncoding portions of the genome are functionally relevant and therefore could impact disease. Results of the current study appear to be consistent with this hypothesis. For example, SNPs near the *AHI1_LINC00271* locus can lead to alterations in *AHI1* expression and show evidence of H3K4me1 histone modifications in CD14+ cells (Table 3 and Supplementary Table 4, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40216/abstract). *AHI1* expression is critical to hematopoietic cell differentiation, and dysregulation may have significant consequences (42).

Other mechanisms are also probable. For example, several East Asian GWAS showed associations of *WDFY4* with SLE (43,44). Functional studies using peripheral blood mononuclear cells revealed decreased messenger RNA expression of *WDFY4* in SLE patients when compared to controls. This may be due to the fact that intronic SNP rs877819 has a decreased binding affinity to transcription factor Yin Yang 1 (YY1). Binding of YY1 to this intronic region was shown to directly influence *WDFY4* expression (45). This SNP is marginally associated with JIA ($P = 1.3 \times 10^{-3}$ [genotyped in cohorts I and II]) but is in LD with the lead JIA SNP in the *WDFY4* region (rs1904603; $r^2 = 0.47$). Although the functional relevance of these eQTLs and histone analyses is limited to the cell and tissue types available in public data sets, the information provided is, nonetheless, important to prioritizing future functional studies.

Expression QTL analysis also showed that SNP variations near *HBP1* and *RNF215* are related to the expression levels of nearby genes *COG5* and *MTP18/SF3A1*, respectively (Table 3 and Supplementary Table 4). Intriguingly, *COG5*, like *COG6*, is a member of the conserved oligomeric Golgi (COG) complex that regulates protein glycosylation and Golgi trafficking. We previously reported *COG6* genetic associations in JIA, and associations with the *COG6* region have also been described in RA and SLE (3,5,46). Thus, the findings that risk variants for multiple COG proteins in JIA were identified, that *COG6* associations have been reported for other autoimmune disease, and that COG defects result in systemic pathologies (47) suggests that Golgi complex pathways may be important in autoimmune disease. In addition, results of the eQTL analysis also implicate *MTP18*, which represents a plausible candidate because it is a mitochondrial protein downstream of phosphatidylinositol 3-kinase signaling, potentially playing a role in the induction of apoptosis. Although there is no direct support for the associated region nearest *LINC00951* (*FLJ41649*), the genetic data, including numerous directly genotyped SNPs, support inclusion as a JIA risk locus (see Supplementary Figure 2, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40216/abstract).

In conclusion, the results of this study further emphasize the role of common genetic variation and add to the understanding of the genomic architecture influencing the risk of oligoarticular and RF-negative polyarticular JIA. As seen in other autoimmune diseases, the majority of these JIA-associated SNPs are located in regulatory regions, supporting the notion that JIA is also a disease of disordered gene regulation. Further work, including evaluation of chromatin interactions and regulatory regions, is essential to understanding the contributions of associated SNPs to disease risk and the genomics influencing oligoarticular and RF-negative polyarticular JIA (48). There is also evidence of interplay among associated regions, suggesting dysregulated pathways as potential targets in clinical care.

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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. Thompson 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. Langefeld, Thompson.
Acquisition of data. McIntosh, Sudman, Becker, Bohnsack, Fingerlin, Griffin, Haas, Lovell, Maier, Nigrovic, Prahalad, Punaro, Rosé, Wallace, Wise, Langefeld, Thompson.
Analysis and interpretation of data. McIntosh, Marion, Sudman, Comeau, Moncrieffe, Howard, Langefeld, Thompson.

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APPENDIX A: CONSORTIA INVOLVED IN THE STUDY AND THEIR PARTICIPATING MEMBERS

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