Published in final edited form as:
Arthritis Rheum. 2009 July ; 60(7): 2124–2130. doi:10.1002/art.24618.

Variants in *TNFAIP3, STAT4* and *c12orf30* loci associated with multiple auto-immune diseases are also associated with Juvenile Idiopathic Arthritis

Sampath Prahalad[1], **Sterling Hansen**[2], **April Whiting**[3], **Stephen L. Guthery**[3], **Bronte Clifford**[3], **Bernadette McNally**[3], **Andrew S. Zeft**[3], **John F. Bohnsack**[3], and **Lynn B. Jorde**[4]

[1]Departments of Pediatrics and Human Genetics, Emory University School of Medicine, Atlanta, GA

[2]University of Utah School of Medicine Salt Lake city.

[3]Department of Pediatrics, University of Utah School of Medicine, Salt Lake city.

[4]Eccles Institute of Human Genetics, University of Utah, Salt Lake city.

Abstract

Objectives—Subtypes of juvenile idiopathic arthritis (JIA) share phenotypic features with other autoimmune disorders. We investigated several genetic variants associated with rheumatoid arthritis (RA) and other autoimmune disorders for association with JIA, to test the hypothesis that clinically distinct phenotypes share common genetic susceptibility factors.

Methods—Cases were 445 children with JIA, and controls were 643 healthy adults. Eight single nucleotide polymorphisms (SNPs) in 7 loci [*TNFAIP3* (rs10499194 and rs6920220), *RSBN1* (rs6679677), *C12ORF30* (rs17696736), *TRAF1* (rs3761847), *IL2RA* (rs2104286), *PTPN2* (rs2542151), and *STAT4* (rs7574865)] were genotyped by the TaqMan assay. Alleles and genotypes were analyzed for association with JIA and JIA subtypes. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated.

Results—The strongest associations were observed for *TNFAIP3* variants rs10499194 (OR: 0.74 (0.61-0.91); p <0.004), and *TNFAIP*3 rs6920220 (OR: 1.3 (1.05-1.61); p <0.02). We also observed associations between JIA and *STAT4* (OR: 1.24 (1.02-1.51); p <0.03) and *C12ORF30* (OR: 1.2 (1.01-1.43); p <0.04) variants. The *PTPN2* variant rs2542151 deviated from Hardy-Weinberg equilibrium and was excluded from analyses. Variants in *IL2RA, TRAF1, and RSBN1* were not associated with JIA. After stratification by JIA subtype, *TNFAIP3* and *C12ORF30* variants were associated with oligoarticular JIA, while the *STAT4* variant was associated primarily with polyarticular JIA.

Conclusions—We have demonstrated associations between JIA and variants in *TNFAIP3, STAT4* and C*12ORF30* regions that have previously shown associations with other autoimmune diseases, including RA and systemic lupus erythematosus. Our results suggest that clinically distinct autoimmune phenotypes share common genetic susceptibility factors.

Keywords

JRA; genetics; autoimmune; association; juvenile idiopathic arthritis; rheumatoid arthritis

Corresponding author: Sampath Prahalad, MD, MSc, Associate Professor of Pediatrics and Human Genetics, Emory University School of Medicine, 2015 Uppergate Drive NE, Atlanta, GA 30322; Ph: (404) 778 2400, fax 404 727 3757, sprahal@emory.edu.

Juvenile idiopathic arthritis (JIA) comprises a collection of chronic childhood arthritides (1). Although associations between JIA and variants encoding human leukocyte antigens (HLA) have been consistently described, the HLA-DR locus has been estimated to account for only ~17% of JIA susceptibility (2). This suggests that non-HLA loci likely play significant roles in predisposition to JIA. To date, most investigators have utilized candidate-gene association studies to find genes predisposing to JIA susceptibility. More than 100 genetic loci have been tested for associations with JIA, most of which have not demonstrated meaningful associations (3). Candidates have often been chosen *ad hoc*, and many studies have suffered from lack of statistical power. However, convincing associations have been demonstrated with several non-HLA variants such as *PTPN22, MIF, WISP3* using relatively large cohorts of children with JIA (3).

Children with JIA have an increased prevalence of other autoimmune disorders(4). Furthermore, relatives of children with JIA have an increased prevalence of autoimmune disorders (5). These observations suggest that clinically distinct autoimmune phenotypes may have common genetic susceptibility factors. Recently variants in genes encoding *PTPN22* and *CTLA4* have been shown to predispose to clinically distinct autoimmune phenotypes. Our objective was to test the hypothesis that genetic variants predisposing to multiple autoimmune phenotypes could also underlie susceptibility to JIA. We investigated 8 single nucleotide polymorphisms (SNPs) in seven loci associated with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), type 1 diabetes (T1D) and inflammatory bowel disease (IBD) in our cohort of children with JIA and healthy controls.

Patients and methods

Cases were 445 children with JIA from the Intermountain States Database of Childhood Rheumatic Diseases. Patients were diagnosed according to the ILAR criteria (1). The median onset age was 5.6 years, and 67% of the subjects were female. There were 201 children with oligoarticular JIA, 100 with rheumatoid factor (RF)-negative polyarticular JIA, 40 with enthesitis related arthritis, 35 with RF-positive polyarticular JIA, 36 with systemic JIA, and 33 with other subtypes. Controls were 643 healthy adults (60 % female) screened for several common autoimmune diseases ascertained from the same geographic region. A questionnaire was used to screen controls for autoimmune disorders. Controls who reported an autoimmune disorder, or had a first-degree relative with an autoimmune disorder were excluded. Cases and controls were very similar in ethnicity, and predominantly (>90%) were of Northern European ancestry._Subjects were enrolled under protocols approved by the Institutional Review Board at the University of Utah.

Genotyping

DNA was isolated from blood using the Puregene DNA purification kit from Qiagen (Valencia, CA). Subjects were genotyped for the eight SNPs in seven loci including rs10499194 and rs6920220 in the*TNFAIP3* locus, rs6679677 in the *RSBN1* locus, rs17696736 in the *C12orf30* locus, rs3761847 in the *TRAF1/C5* locus, rs2104286 in the *IL2RA* locus, rs7574865 in the *STAT4* locus, and rs2542151 in the *PTPN2* locus. These variants were chosen based on their reported associations with other autoimmune phenotypes including RA, SLE, T1D and IBD (6-9). Each of these variants has been shown to have an association with at least two autoimmune phenotypes. Genotyping was performed using Taqman Pre-designed SNP genotyping assays (Applied Biosystems, Foster City, CA) according to the manufacturer's protocols. For quality control twelve samples were typed in duplicate on different plates; the concordance was 100% for all samples.

Analysis

All variants were evaluated for deviation from Hardy-Weinberg equilibrium (HWE) by comparing observed and expected frequencies of genotypes. Alleles and genotypes were investigated for association with JIA. Since rs10499194 and rs6920220 were both in the *TNFAIP3* locus, haplotypes were also analyzed for an association with JIA. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for alleles, as well as genotypes using dominant and recessive models. Since JIA is a collection of several heterogeneous subtypes, analyses were repeated after stratifying subjects into onset types and gender. The polyarticular subtypes were analyzed both individually and together to improve statistical power.

The *STAT4* variant rs7574865 has been reported to be associated with several autoimmune phenotypes including RA(9-16), SLE (9-11, 15, 17), T1D (18, 19), Sjogren's syndrome (20), and IBD(18). Published case-control association studies of *STAT4* rs7574865 were identified using a PUBMED search. Allele frequency data were obtained from these studies. We performed meta-analyses of *STAT4* associations with RA and SLE using a fixed-effects model, weighting studies by number of subjects. Mantel-Haenszel Chi-square tests were performed for the individual studies and for the studies combined. The Breslow-Day test was performed for heterogeneity among studies with significance set at $p < 0.05$. All analyses were carried out using SAS 9.1.3. We did not perform meta-analyses of the other variants since their associations were mainly reported in genome-wide association studies, and there were only one or two published studies of these other variants.

RESULTS

The genotyping call rates ranged from 96.8 to 99.8% for the eight SNPs. SNP rs2542151 at the *PTPN2* locus deviated significantly from HWE in the controls ($p < 0.01$) and hence was not analyzed further. The allele and genotype frequencies of the seven remaining SNPs in cases and controls are shown in Table 1. The minor allele frequencies (MAF) among our controls did not differ by more than ~10% from MAF reported for individuals of European descent. The ancestral alleles were the major allele at SNP rs10499194, rs6920220, rs6679677, rs17696736, rs3761847, and rs2104286. However at rs7574865 in the *STAT4* locus, the ancestral allele, T, was the minor allele (MAF = 0.24 among controls).

At the *TNFAIP3* locus, the T allele at *TNFAIP3* SNP rs10499194 demonstrated a significant protective effect against JIA in the entire cohort (OR 0.74 (0.61-0.91), $p < 0.004$). A recessive model significantly improved the OR (0.49 (0.30-0.83), $p < 0.006$). The effect was also pronounced in female subjects (0.71 (0.55-0.91), $p < 0.007$). When analyses were repeated after stratification by subtypes, the association was significant in the oligoarticular JIA cohort (Table 2). In contrast, the minor allele (A) at *TNFAIP3* SNP rs6920220 conferred risk to the JIA cohort as a whole (OR 1.30 (1.05-0.61), $p < 0.02$). At this locus, a dominant model improved the OR (1.44 (1.12-1.86, $p < 0.004$). The association was also pronounced in female subjects (1.42 (1.09-2.66), $p < 0.009$). Similar to the other variant, when analyses were repeated after stratification by subtypes, the association was significant in the oligoarticular JIA cohort (Table 2).

Since both rs10499194 and rs6920220 are in the *TNFAIP3* region, we investigated haplotype combinations of these two SNPs for association with JIA. The haplotype carrying the major alleles at both SNPs (CG) was neutral, while haplotype TG was protective (case frequency 0.23, control frequency 0.28, $p < 0.006$) and haplotype CA conferred a risk (case frequency 0.22, control frequency 0.18, $p < 0.01$).

An association was also observed between JIA and the G allele of rs17696736 (allelic OR 1.2, p <0.04) at the *C12ORF30* locus. A recessive model improved the OR slightly in the entire JIA cohort (1.3 (1.00-1.68), p <0.05). This association was also pronounced in the oligoarticular JIA subset (Table 2). The minor allele (A) at SNP rs6679677 in the *RSBN1* locus demonstrated a marginal association with JIA in the entire JIA cohort (OR 1.28 (0.98-1.68), p <0.07). When analyses were repeated after stratification, there was significant risk conferred to male subjects (146 cases, 257 controls; OR = 1.9 (1.20-2.97), p <0.006). The variants at *TRAF1* and *IL2RA* did not show an association either with JIA or any of the JIA subtypes in our cohorts.

Finally, the T allele at the *STAT4* SNP rs7574865 demonstrated a significant association with JIA (OR 1.24 (1.02-1.51), p <0.03). The association was pronounced in the polyarticular JIA subtypes (Table 2). When the RF-negative and RF-positive polyarticular JIA subsets were combined to improve power, the magnitude of the association with the STAT4 variant improved (OR 1.5 (1.13-2.0), p <0.005).

Meta-analyses of *STAT* variant rs7574865

The rs7574865 variant has been investigated for an association with an autoimmune phenotype in 18 case-control comparisons (Figure 1). Statistically significant associations had been reported between rs7574865 and RA (OR 1.17-1.9), SLE (OR 1.55-1.71), T1D (OR 1.36-1.94) and Sjogren's syndrome (OR 1.47), as well as IBD (OR 1.29) (9-17, 19, 20). The subjects in these studies were predominantly of European ancestry, and most of the remainder were of Asian ancestry. There were 8 case-control associations of RA and *STAT4*. The pooled analysis confirmed an association between RA and *STAT4* with an OR of 1.26 (1.22-1.31), p $<10^{-7}$. When all 8 studies were included, the Breslow-Day statistic was significant (p <0.01), suggesting heterogeneity between studies. This was mainly due to the study by Zervou et al., which included a genetically homogeneous population from the Greek island of Crete(16). When we repeated the analysis excluding this study, there was no heterogeneity (Breslow-Day p < 0.17), and the OR estimate changed minimally, with an OR of 1.25 (1.21-1.30) p $<10^{-7}$. The pooled OR for the 5 investigations of SLE was 1.59 (1.49-1.70), p $<10^{-7}$. The Breslow-Day statistic for heterogeneity was not significant (p <0.97).

DISCUSSION

JIA is a collection of chronic arthropathies in children that shares phenotypic features with several autoimmune disorders(1). Relatives of children with JIA have an increased prevalence of autoimmune disorders, and individuals with JIA also appear to have an increased prevalence of other autoimmune disorders(4, 5). These observations suggest that clinically distinct autoimmune phenotypes could share common genetic susceptibility factors. This hypothesis is supported by genetic studies. Meta-analyses of genome-wide scans of immune mediated disorders demonstrated nonrandom clustering of susceptibility loci between different human diseases(21). The identification of *CTLA4* (22) and *PTPN22* (23) variants underlying susceptibility to clinically distinct autoimmune disorders also supports this hypothesis.

We tested the hypothesis that variants associated with autoimmune disorders will also be associated with JIA. Our results confirmed that variants in the *TNFAIP3* and *STAT4* loci associated with RA and SLE also demonstrate associations with JIA. In addition, we found evidence for an association between JIA and a variant on chromosome 12 previously shown to be associated with T1D (24). To our knowledge these variants have not previously been tested for association with JIA. While the size of our JIA cohort is modest, it is still larger than ~90% of published case-control comparisons of non-HLA variants in JIA(3). Our

combined case and control cohort of almost 1100 subjects has sufficient power to detect modest associations. We observed that the associations between *TNFAIP3* and *STAT4* variants and JIA were similar in magnitude and direction to the association between these variants and RA. While some of the subtypes did not show statistically significant associations with *TNFAIP3* and *STAT4* variants, it should be noted that in general the associations were in the same direction, suggesting that these cohorts might be underpowered to detect significant associations. These results suggest that *TNFAIP3* and *STAT4* variants might also be involved in susceptibility to multiple autoimmune phenotypes, similar to *PTPN22* and *CTLA4*.

We did not perform a correction for multiple testing. Since our hypothesis was that different autoimmune phenotypes share common susceptibility factors, we treated JIA as a combined phenotype for the primary analysis. The loci investigated in this study were chosen based on prior findings of association in other autoimmune phenotypes, and they have an increased prior probability of association. Given that the genetic basis of JIA is not in doubt, each gene that is potentially involved in the etiology of the trait represents an individual null hypothesis to assess.(25, 26) For these reasons, we believe that a standard multiple comparisons correction would be too conservative. Nevertheless, the association with *TNFAIP3* variant rs10499194 and JIA was significant after correcting for the 7 loci investigated. Ultimately, the best validation of these results will be replication in an independent cohort.

TNFAIP3 (also known as A20) is an important regulator of NF-κB signaling through ubiquitin modifications of adaptor proteins downstream of TNF-α and toll-like receptors(27, 28). Validated associations between RA and variants in the *TNFAIP3* region have been reported (6, 7, 29). The *TNFAIP3* SNPs investigated in our study have been shown to tag haplotypes that confer either susceptibility to or protection from RA(7). Our results confirmed that the same susceptibility and protective haplotypes are also associated with JIA. Interestingly, *TNFAIP3* SNP rs6920220 is also associated with susceptibility to SLE (30).

The *STAT4* gene encodes a transcription factor expressed on lymphocytes, macrophages, and dendritic cells. STAT4 is essential for IL-12 signaling and induces Th1 differentiation and production of interferon-γ, a pro-inflammatory cytokine(31). Since *STAT4* variants were first described to be associated with both RA and SLE by Remmers et al. (9), several authors have confirmed associations between *STAT4* variants and various autoimmune diseases(10-13, 17, 18, 20). Our demonstration of an association between the same *STAT4* variant (rs7574865) and JIA adds to this literature. Both RF-positive and RF-negative polyarticular JIA subtypes demonstrated an association with this variant. Together these observations strongly support a role for STAT4 in autoimmunity.

We also found an association between JIA and a variant in the *C12ORF30* region on chromosome 12. This variant shows strong association with type 1 diabetes(24). This variant also showed an association with autoimmune phenotypes analyzed together (RA, T1D, IBD) in the association study of complex traits performed by the Wellcome Trust Case Control Consortium(6). This suggests that this region might harbor genes involved in susceptibility to autoimmunity in general. Finally, there was a marginal association between the SNP rs6679677 in *RSBN1* locus and JIA, and this was pronounced in male subjects. This variant, located ~73 kb from a functional variant in *PTPN22* (rs2476601), is in a haplotype block suggesting that the association might be due to linkage disequilibrium with other SNPs.

We did not find associations with other autoimmunity-associated variants in the *TRAF1* or *IL2RA* loci. Two studies have reported associations between JIA and *TRAF1* variants. The

variant that we tested in our cohort, rs3761847, was described by Behrens et al. to be associated with JIA in their cohort of 67 White subjects (OR 1.45, p <0.03)(32). This is the SNP that shows the strongest associations with RA(8). Another *TRAF1* variant, rs10818488, which is ~14kb from rs3761847 and in linkage disequilibrium with it, was tested for an association with JIA in 338 cases and 511 controls(33). While the whole JIA cohort did not demonstrate an association with JIA, they did find an association with RF-negative polyarticular JIA subtype (OR 1.54), as well as with extended oligoarthritis and polyarticular JIA subtypes combined (OR 1.46). Our cohort had sufficient power (>80%) to detect an OR of 1.45 described in these two studies. It is possible that the actual effect sizes of the associations are lower than observed initially, and our cohort, although larger than both, is underpowered. It should also be noted that our JIA cohort was underpowered to detect associations of these variants with modest effects, given the inclusion of heterogeneous subgroups.

Our results suggest that *STAT4* and *TNFAIP3* variants are similar to *PTPN22* and *CTLA4* in predisposing to autoimmunity in general. The identification of common genetic factors underlying clinically distinct autoimmune phenotypes supports efforts to search for other variants that predispose to autoimmunity in general.

Acknowledgments

Supported by, The National Institute of Arthritis and Musculoskeletal and Skin Diseases (K23-AR50177), The National Institute of Diabetes and Digestive and Kidney Disorders (K23-DK069513) and The National Center for Research Resources (UL1-RR025764 and C06-RR11234), The Arthritis Foundation, and The Val A Browning Charitable Foundation, and The Primary Children's Medical Center Foundation, Salt Lake City, UT.

References

1. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol. 2004; 31:390–2. [PubMed: 14760812]
2. Prahalad S, Ryan MH, Shear ES, Thompson SD, Giannini EH, Glass DN. Juvenile rheumatoid arthritis: linkage to HLA demonstrated by allele sharing in affected sibpairs. Arthritis Rheum. 2000; 43:2335–8. [PubMed: 11037894]
3. Prahalad S, Glass DN. A comprehensive review of the genetics of juvenile idiopathic arthritis. Pediatr Rheumatol Online J. 2008; 6:11. [PubMed: 18644131]
4. Stagi S, Giani T, Simonini G, Falcini F. Thyroid function, autoimmune thyroiditis and coeliac disease in juvenile idiopathic arthritis. Rheumatology (Oxford). 2005; 44:517–20. [PubMed: 15695302]
5. Prahalad S, Shear ES, Thompson SD, Giannini EH, Glass DN. Increased prevalence of familial autoimmunity in simplex and multiplex families with juvenile rheumatoid arthritis. Arthritis Rheum. 2002; 46:1851–6. [PubMed: 12124869]
6. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447:661–78. [PubMed: 17554300]
7. Plenge RM, Cotsapas C, Davies L, Price AL, de Bakker PI, Maller J, et al. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. Nat Genet. 2007
8. 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. [PubMed: 17804836]
9. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. N Engl J Med. 2007; 357:977–86. [PubMed: 17804842]

10. Kobayashi S, Ikari K, Kaneko H, Kochi Y, Yamamoto K, Shimane K, et al. Association of STAT4 with susceptibility to rheumatoid arthritis and systemic lupus erythematosus in the Japanese population. Arthritis Rheum. 2008; 58:1940–6. [PubMed: 18576330]
11. Taylor KE, Remmers EF, Lee AT, Ortmann WA, Plenge RM, Tian C, et al. Specificity of the STAT4 genetic association for severe disease manifestations of systemic lupus erythematosus. PLoS Genet. 2008; 4:e1000084. [PubMed: 18516230]
12. Lee HS, Remmers EF, Le JM, Kastner DL, Bae SC, Gregersen PK. Association of STAT4 with rheumatoid arthritis in the Korean population. Mol Med. 2007; 13:455–60. [PubMed: 17932559]
13. Orozco G, Alizadeh BZ, Delgado-Vega AM, Gonzalez-Gay MA, Balsa A, Pascual-Salcedo D, et al. Association of STAT4 with rheumatoid arthritis: a replication study in three European populations. Arthritis Rheum. 2008; 58:1974–80. [PubMed: 18576336]
14. Barton A, Thomson W, Ke X, Eyre S, Hinks A, Bowes J, et al. Re-evaluation of putative rheumatoid arthritis susceptibility genes in the post-genome wide association study era and hypothesis of a key pathway underlying susceptibility. Hum Mol Genet. 2008; 17:2274–9. [PubMed: 18434327]
15. Palomino-Morales RJ, Rojas-Villarraga A, Gonzalez CI, Ramirez G, Anaya JM, Martin J. STAT4 but not TRAF1/C5 variants influence the risk of developing rheumatoid arthritis and systemic lupus erythematosus in Colombians. Genes Immun. 2008; 9:379–82. [PubMed: 18432273]
16. Zervou MI, Sidiropoulos P, Petraki E, Vazgiourakis V, Krasoudaki E, Raptopoulou A, et al. Association of a TRAF1 and a STAT4 gene polymorphism with increased risk for rheumatoid arthritis in a genetically homogeneous population. Hum Immunol. 2008; 69:567–71. [PubMed: 18625278]
17. Kawasaki A, Ito I, Hikami K, Ohashi J, Hayashi T, Goto D, et al. Role of STAT4 polymorphisms in systemic lupus erythematosus in a Japanese population: a case-control association study of the STAT1-STAT4 region. Arthritis Res Ther. 2008; 10:R113. [PubMed: 18803832]
18. Martinez A, Varade J, Marquez A, Cenit MC, Espino L, Perdigones N, et al. Association of the STAT4 gene with increased susceptibility for some immune-mediated diseases. Arthritis Rheum. 2008; 58:2598–602. [PubMed: 18759272]
19. Zervou MI, Mamoulakis D, Panierakis C, Boumpas DT, Goulielmos GN. STAT4: A risk factor for type 1 diabetes? Hum Immunol. 2008
20. Korman BD, Alba MI, Le JM, Alevizos I, Smith JA, Nikolov NP, et al. Variant form of STAT4 is associated with primary Sjogren's syndrome. Genes Immun. 2008; 9:267–70. [PubMed: 18273036]
21. Becker KG, Simon RM, Bailey-Wilson JE, Freidlin B, Biddison WE, McFarland HF, et al. Clustering of non-major histocompatibility complex susceptibility candidate loci in human autoimmune diseases. Proc Natl Acad Sci U S A. 1998; 95:9979–84. [PubMed: 9707586]
22. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature. 2003; 423:506–11. [PubMed: 12724780]
23. Criswell LA, Pfeiffer KA, Lum RF, Gonzales B, Novitzke J, Kern M, et al. Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. Am J Hum Genet. 2005; 76:561–71. [PubMed: 15719322]
24. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, et al. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet. 2007; 39:857–64. [PubMed: 17554260]
25. Boehringer S, Epplen JT, Krawczak M. Genetic association studies of bronchial asthma--a need for Bonferroni correction? Hum Genet. 2000; 107:197. [PubMed: 11030420]
26. Perneger TV. What's wrong with Bonferroni adjustments. BMJ. 1998; 316:1236–8. [PubMed: 9553006]
27. Boone DL, Turer EE, Lee EG, Ahmad RC, Wheeler MT, Tsui C, et al. The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. Nat Immunol. 2004; 5:1052–60. [PubMed: 15334086]

28. Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. Nature. 2004; 430:694–9. [PubMed: 15258597]

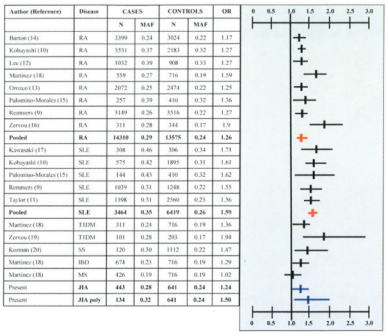
29. Thomson W, Barton A, Ke X, Eyre S, Hinks A, Bowes J, et al. Rheumatoid arthritis association at 6q23. Nat Genet. 2007

30. Graham RR, Cotsapas C, Davies L, Hackett R, Lessard CJ, Leon JM, et al. Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. Nat Genet. 2008

31. Watford WT, Hissong BD, Bream JH, Kanno Y, Muul L, O'Shea JJ. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. Immunol Rev. 2004; 202:139–56. [PubMed: 15546391]

32. Behrens EM, Finkel TH, Bradfield JP, Kim CE, Linton L, Casalunovo T, et al. Association of the TRAF1-C5 locus on chromosome 9 with juvenile idiopathic arthritis. Arthritis Rheum. 2008; 58:2206–7. [PubMed: 18576341]

33. Albers HM, Kurreeman FA, Houwing-Duistermaat JJ, Brinkman DM, Kamphuis SS, Girschick HJ, et al. The TRAF1/C5 region is a risk factor for polyarthritis in Juvenile Idiopathic Arthritis. Ann Rheum Dis. 2008



Author (Reference)	Disease	CASES		CONTROLS		OR
		N	MAF	N	MAF	
Barton (14)	RA	3399	0.24	3024	0.22	1.17
Kobayashi (10)	RA	3531	0.37	2181	0.32	1.27
Lee (12)	RA	1032	0.39	908	0.33	1.27
Martinez (18)	RA	559	0.27	716	0.19	1.59
Orozco (13)	RA	2072	0.25	2474	0.22	1.25
Palomino-Morales (15)	RA	257	0.39	410	0.32	1.36
Remmers (9)	RA	3149	0.26	3516	0.22	1.27
Zervou (16)	RA	311	0.28	344	0.17	1.9
Pooled	**RA**	**14310**	**0.29**	**13575**	**0.24**	**1.26**
Kawasaki (17)	SLE	308	0.46	306	0.34	1.71
Kobayashi (10)	SLE	575	0.42	1895	0.31	1.61
Palomino-Morales (15)	SLE	144	0.43	410	0.32	1.62
Remmers (9)	SLE	1039	0.31	1248	0.22	1.55
Taylor (11)	SLE	1998	0.31	2560	0.23	1.56
Pooled	**SLE**	**3464**	**0.35**	**6419**	**0.26**	**1.59**
Martinez (18)	T1DM	311	0.24	716	0.19	1.36
Zervou (19)	T1DM	101	0.28	203	0.17	1.94
Kerrouin (20)	SS	120	0.30	1112	0.22	1.47
Martinez (18)	IBD	674	0.23	716	0.19	1.29
Martinez (18)	MS	426	0.19	716	0.19	1.02
Present	**JIA**	**443**	**0.28**	**641**	**0.24**	**1.24**
Present	**JIA poly**	**124**	**0.32**	**641**	**0.24**	**1.50**

Figure 1. Case-control association results of STAT4 variant rs7574865

RA: rheumatoid arthritis, SLE: systemic lupus erythematosus, T1D: type 1 diabetes, SS: Sjogren's syndrome, IBD: inflammatory bowel disease, MS: Multiple sclerosis. OR: Odds ratio (Allelic). OR (Vertical line) and 95% confidence intervals (horizontal lines) are shown. Red color indicates pooled analyses of RA and SLE studies. Blue color indicates JIA association.

Table 1

Case-control analysis of autoimmunity associated variants and JIA.

Locus	Variant	Alleles	Controls					Cases					OR (95%CI)	P
		1 2	N	1/1	1/2	2/2	MAF	N	1/1	1/2	2/2	MAF		
TNFAIP3	rs10499194	C T	621	0.52	0.39	0.09	0.29	433	0.58	0.37	0.05	0.23	**0.74 (0.61-0.91)**	**0.004**
TNFAIP3	rs6920220	G A	619	0.67	0.29	0.04	0.19	441	0.59	0.37	0.04	0.23	1.30 (1.05-1.61)	**0.015**
RSBN1	rs6679677	C A	642	0.81	0.18	0.01	0.10	444	0.77	0.21	0.02	0.13	1.28 (0.98-1.68)	0.070
C12orf30	rs17696736	A G	631	0.36	0.48	0.16	0.41	443	0.30	0.51	0.20	0.45	**1.20 (1.01-1.43)**	**0.041**
TRAF1/C5	rs3761847	G A	641	0.34	0.49	0.18	0.42	440	0.34	0.47	0.19	0.42	1.02 (0.86-1.21)	0.818
IL2RA	rs2104286	A G	634	0.55	0.39	0.06	0.25	438	0.53	0.41	0.05	0.26	1.04 (0.85-1.27)	0.693
STAT4	rs7574865	G T	641	0.56	0.39	0.04	0.24	443	0.51	0.43	0.07	0.28	**1.24 (1.02-1.51)**	**0.029**

MAF: Minor allele frequency.

Odds ratio for the carriage of allele 2 are shown.

Arthritis Rheum. Author manuscript; available in PMC 2011 May 31.

Table 2

Case-control analysis of autoimmunity associated variants and JIA subtypes

Locus	Variant	Phenotype	N	MAF	OR	95%CI	p
TNFAIP3	rs10499194	Controls	621	0.29			
		All JIA	433	0.23	**0.75**	**0.61-0.91**	**0.004**
		Oligoarticular	198	0.22	**0.71**	**0.55-0.93**	**0.012**
		All poly	131	0.23	0.75	0.55-1.02	0.065
		Polyarticular	98	0.24	0.75	0.53-1.07	0.116
		Polyarticular RF+	33	0.23	0.72	0.40-1.30	0.279
		ERA	37	0.19	0.57	0.32-1.04	0.064
		Systemic	35	0.30	1.05	0.62-1.78	0.844
		Other	32	0.26	0.82	0.46-1.46	0.500
TNFAIP3	rs6920220	Controls	619	0.19			
		All JIA	441	0.23	**1.30**	**1.05-1.61**	**0.017**
		Oligoarticular	199	0.24	**1.39**	**1.05-1.82**	**0.019**
		All poly	133	0.23	1.34	0.97-1.84	0.072
		Polyarticular	99	0.23	1.33	0.93-1.92	0.116
		Polyarticular RF+	34	0.24	1.35	0.76-2.04	0.300
		ERA	40	0.20	1.10	0.63-1.92	0.738
		Systemic	36	0.19	1.06	0.58-1.92	0.841
		Other	33	0.22	1.19	0.65-2.17	0.581
C12orf30	Rs17696736	Controls	631	0.41			
		All JIA	443	0.45	**1.20**	**1.01-1.43**	**0.041**
		Oligoarticular	201	0.47	**1.28**	**1.02-1.60**	**0.033**
		All poly	133	0.44	1.17	0.90-1.53	0.244
		Polyarticular	98	0.44	1.17	0.87-1.59	0.302
		Polyarticular RF+	35	0.44	1.17	0.72-1.90	0.529
		ERA	40	0.39	0.93	0.58-1.48	0.758
		Systemic	36	0.42	1.05	0.65-1.70	0.843
		Other	33	0.47	1.38	0.84-2.27	0.198

Locus	Variant	Phenotype	N	MAF	OR	95%CI	p
STAT4	rs7574865	Controls	641	0.28			
		All JIA	443	0.28	**1.24**	**1.02-1.5**	**0.031**
		Oligoarticular	201	0.26	1.09	0.85-1.42	0.485
		All polyarticular	134	0.32	**1.50**	**1.13-2.00**	**0.005**
		Polyarticular	99	0.31	**1.45**	**1.04-2.00**	**0.026**
		Polyarticular RF+	35	0.34	**1.66**	**1.0-2.76**	**0.050**
		ERA	39	0.29	1.33	0.80-2.20	0.268
		Systemic	36	0.26	1.14	0.66-1.95	0.637
		Other	33	0.28	1.19	0.68-2.08	0.538