IL1RN Variation Influences both Disease Susceptibility and Response to Human Recombinant IL-1RA Therapy in Systemic Juvenile Idiopathic Arthritis

Victoria L. Arthur, BA[1,*], Emily Shuldiner, BA[1,*], Elaine F. Remmers, PhD[2], Anne Hinks, PhD[3], Alexei A.Grom, MD[4,5], Dirk Foell, MD[6], Alberto Martini, MD[7,8], Marco Gattorno, MD[8], Seza Özen, MD[9], Sampath Prahalad, MD[10,11], Andrew S. Zeft, MD[12], John F. Bohnsack, MD[13], Norman T. Ilowite, MD[14], Elizabeth D. Mellins, MD PhD[15], Ricardo Russo, MD[16], Claudio Len, MD[17], Sheila Oliveira, MD PhD[18], Rae S. M. Yeung, MD PhD[19,20,21], Alan M. Rosenberg, MD[22], Lucy R. Wedderburn, MD PhD[23-25], Jordi Anton, MD PhD[26], Johannes-Peter Haas, MD[27], Angela Rösen-Wolff, MD PhD[28], Kirsten Minden MD PhD[29,30], Ann Marie Szymanski, MD[1], INCHARGE Consortium[31], Wendy Thomson, PhD[3,32], Daniel L. Kastner, MD PhD[2], Patricia Woo, MBBS PhD[23] and Michael J. Ombrello, MD[1]

[1] Translational Genetics and Genomics Section, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, US Department of Health & Human Services, Bethesda, MD, USA; [2] Inflammatory Disease Section, National Human Genome Research Institute, National Institutes of Health, US Department of Health & Human Services, Bethesda, MD, USA; [3] Arthritis Research UK Centre for Genetics and Genomics, Centre for Musculoskeletal Research, University of Manchester, Manchester, UK; [4] Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA; [5] Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [6]Department of Pediatric Rheumatology and Immunology,

University Hospital Münster, Münster, Germany; [7]Direzione Scientifica, G. Gaslini Institute, Genoa, Italy; [8] Clinica Pediatrica e Reumatologia, G. Gaslini Institute and University of Genoa, Genoa, Italy; [9]Department of Pediatric Rheumatology, Hacettepe University, Ankara, Turkey; [10]Departments of Pediatrics and Human Genetics, Emory University School of Medicine, Atlanta, GA, USA; [11]Children's Healthcare of Atlanta, Atlanta, GA; [12]Department of Pediatrics, Cleveland Clinic, Cleveland, OH, USA; [13]Department of Pediatrics, University of Utah, Salt Lake City, UT, USA; [14]Department of Pediatrics, Albert Einstein College of Medicine and Children's Hospital at Montefiore, Bronx, NY, USA; [15]Department of Pediatrics, Stanford University, Stanford, CA, USA; [16]Service of Immunology and Rheumatology, Hospital de Pediatria Garrahan, Buenos Aires, Argentina; [17]Department of Pediatrics, Universidade Federal de São Paulo, São Paulo, Brazil; [18]Universidade Federal de Rio de Janeiro, Rio de Janeiro, Brazil; [19]Department of Pediatrics, University of Toronto, Toronto, Canada; [20]Department of Immunology, University of Toronto, Toronto, Canada; [21]Institute of Medical Science, University of Toronto, Toronto, Canada; [22]Department of Pediatrics, University of Saskatchewan, Saskatoon, Canada; [23]University College London Great Ormond Street Hospital Institute of Child Health, University College London, London, UK; [24]Center of Paediatric and Adolescent Rheumatology, University College London, London, UK; [25]NIHR GOSH Biomedical Research Centre, London, UK; [26]Pediatric Rheumatology Unit, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain; [27]German Center for Pediatric and Adolescent Rheumatology, Garmisch-Partenkirchen, Germany; [28]University Hospital Cal Gustav Carus, Dresden, Germany; [29]Charité University Medicine, Berlin, Germany; [30]German Rheumatism Research Centre, Epidemiology

Unit, Berlin, Germany; [31]The full authorship of the International Childhood Arthritis Genetics (INCHARGE) Consortium is provided in the supplementary appendix; [32]National Institute for Health Research Manchester Biomedical Centre, Central Manchester National Health Service Foundation Trust, Manchester Academic Health Centre, University of Manchester, Manchester, UK.

* These authors contributed equally to this work.

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Corresponding Author:

Dr. Michael J. Ombrello, M.D.

Translational Genetics and Genomics Unit

Intramural Research Program

National Institute of Arthritis and Musculoskeletal and Skin Diseases

National Institutes of Health

US Department of Health & Human Services

10 Center Drive, 12N248A

Building 10, MSC1560

Bethesda, Maryland, 20852, USA

+1 (301) 435 4027

Michael.Ombrello@nih.gov

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Abstract

Objective: To determine whether systemic juvenile idiopathic arthritis (sJIA) susceptibility loci identified by candidate gene studies demonstrated association with sJIA in the largest study population assembled to date.

Methods: Single nucleotide polymorphisms (SNPs) from 11 previously reported sJIA risk loci were examined for association in 9 populations, including 770 sJIA cases and 6947 control subjects. The effect of sJIA-associated SNPs on gene expression was evaluated *in silico* in paired whole genome and RNA sequencing data from lymphoblastoid cell lines (LCL) of 373 European 1000 Genomes Project subjects. The relationship between sJIA-associated SNPs and response to anakinra treatment was evaluated in 38 US patients for whom treatment response data were available.

Results: We found no association of the 26 SNPs previously reported as sJIA-associated. Expanded analysis of the regions containing the 26 SNPs revealed only one significant association, the promoter region of *IL1RN* ($p<1E-4$). sJIA-associated SNPs correlated with *IL1RN* expression in LCLs, with an inverse correlation between sJIA risk and *IL1RN* expression. The presence of homozygous *IL1RN* high expression alleles correlated strongly with non-response to anakinra therapy (OR 28.7 [3.2, 255.8]).

Conclusion: *IL1RN* was the only candidate locus associated with sJIA in our study. The implicated SNPs are among the strongest known determinants of *IL1RN* and IL1RA levels, linking low expression with increased sJIA risk. Homozygous high expression alleles predicted non-response to anakinra therapy, nominating them as candidate biomarkers to guide sJIA treatment. This is an important first step towards the personalized treatment of sJIA.

Systemic juvenile idiopathic arthritis (sJIA) is a rare, severe childhood inflammatory disease[1, 2] that develops in the absence of an identifiable cause. sJIA is marked by the presence of chronic arthritis that occurs in the context of profound systemic inflammation, including quotidian fever, lymphadenopathy, hepatosplenomegaly, a salmon pink evanescent skin rash and serositis. It may also be accompanied by life threatening complications, including pericardial effusion, interstitial lung disease, amyloidosis, and macrophage activation syndrome, a highly lethal secondary form of hemophagocytic lymphohistiocytosis. Among children with sJIA, approximately half develop a destructive form of chronic arthritis that persists throughout their lives.

Despite its unifying inflammatory characteristics, sJIA is a heterogeneous condition with three distinct disease courses and variable expression of clinical manifestations and complications[3]. Regardless of the disease course and specific manifestations, the goal of sJIA treatment is to extinguish the systemic inflammation as rapidly as possible, taking advantage of the early therapeutic “window of opportunity” in an effort to avoid the development of persistent arthritis[4]. Achievement of this goal is often complicated by the fact that children with sJIA do not respond uniformly to the currently available therapies[5, 6]. A subset of sJIA responds to treatments targeting interleukin (IL)-1, a subset responds to IL-6 directed therapies, a subset responds to tumor necrosis factor (TNF)-α blockade, and a subset does not respond to any of these treatment strategies. Importantly, there is no objective determinant or biomarker that assists in predicting which therapeutic approach will be successful in individual patients, and thus there are often delays in ameliorating the systemic inflammation.

The pathophysiology of sJIA is poorly understood, as is the basis of its phenotypic heterogeneity. Due to its rare nature, most genetic studies of sJIA have utilized a candidate gene approach to examine small case-control collections. These studies produced a list of over two dozen single nucleotide polymorphisms (SNPs) at 11 distinct susceptibility loci that were reported as sJIA-associated loci. These include the *IL1A/B*[7,8], *GLI2*[7], *IL1RN/PSD4*[7], *IL1R2*[7], *IL10/20*[9,10], *IL6*[11,12], *MVK*[8], *CCR5*[13], *MIF*[14], *SLC26A2*[15], and *TAPBP*[16] loci (Table 1). Importantly, the original evidence supporting these associations was modest, and in many cases, the associations were not observed in studies of independent populations. Despite these facts, these associations are regularly included in discussions of sJIA pathophysiology.

We have recently performed the largest genetic study of sJIA, a multi-national effort that included children with sJIA from 9 countries[17,18]. We identified two *bona fide* sJIA susceptibility loci and 24 additional loci suggestively associated with sJIA, however there was no overlap between the peak sJIA susceptibility loci in our studies and those reported in the earlier candidate gene studies. To evaluate the relationship between sJIA risk and the sJIA susceptibility loci identified by candidate gene studies, we have undertaken a regional association study of the 11 reported candidate susceptibility loci in the International Childhood Arthritis Genetics Consortium (INCHARGE) sJIA case-control collection.

Materials and Methods

Study design and participants

Directly observed and imputed SNP genotype data from the 9 case-control populations of the INCHARGE sJIA collection were evaluated for this study[17,18]. The INCHARGE sJIA collection includes children fulfilling International League of Associations for Rheumatology (ILAR) criteria for sJIA and control subjects from the United States, the United Kingdom, Germany, Turkey, Italy, Brazil, Argentina, Canada and Spain. SNP genotyping of genomic DNA from cases and controls was performed using Human Omni1M arrays and an iScan reader (Illumina). SNP genotypes were stratified by country of origin and, where available, combined with existing SNP genotype datasets, in silico, from geographically-matched healthy control individuals. Each geographically-defined stratum was subjected to rigorous quality control processes to remove samples and SNPs of poor quality using standard metrics. Ancestral outliers were removed from each geographically-defined stratum using a combination of principal components analysis and multidimensional scaling. The degree of matching was assessed using genomic control inflation factors (λ_{GC}), which were < 1.004 for each of the 9 strata. Detailed information about case and control populations included in the INCHARGE collection, along with technical descriptions and visualizations of the quality control processes and their results can be found in the supplementary material of our earlier papers[17,18]. For the present study, genotypes of SNPs residing in 11 candidate loci (Tables S1) were examined in 770 children with sJIA and 6947 control samples from the United States, the United Kingdom, Germany, Turkey, Italy, Brazil, Argentina, Canada and Spain. For candidate loci defined by a single sJIA-associated SNP, the study interval was defined as ± 100 kilobases (Kb) from the position of that SNP. When more

than one SNP association was present within a locus, the study interval was defined as ± 100 Kb from the mean of the positions of the reported sJIA-associated SNPs.

Statistical analysis

Association testing of candidate SNPs was performed under the additive model, adjusted for sex and ancestry-informative principal components, in each of the 9 sJIA case-control collections using SNPTESTv2[19]. Association results were then combined across collections using fixed-effect meta-analysis with GWAMA software[20]. Heterogeneity was evaluated using the i^2 statistic and SNPs exhibiting moderate evidence of heterogeneity ($i^2 > 0.5$) were excluded from our analysis. Association data were visualized using SNP and Variation Suite 8 (SVS8, Golden Helix, Bozeman, Montana) and custom R scripts (R version 3.4.0). Haplotype analysis and examination of LD were performed using Haploview[21]. The SNP set was pruned for pairwise linkage disequilibrium (LD) of $r^2 < 0.5$ by the Estimation-Maximization method with PLINK[22] to determine the number of independent SNPs in the study. The threshold for study-wide significance was defined by a Bonferroni correction for the total number of independent SNPs across all candidate loci.

Gene expression analysis

The effect of sJIA-associated SNPs on gene and/or protein expression was examined using the Haploregv4.1 database[23]. The correlation of sJIA-associated SNPs with gene expression was investigated by an integrated examination of RNA-sequencing (RNA-seq) and whole genome sequencing (WGS) data from 1000 Genomes Project

subjects[24,25]. RNA-seq data from the set of 373 lymphoblastoid cell lines (LCLs) of European 1000 Genomes Project subjects were downloaded from the Geuvadis website (http://www.geuvadis.org/web/geuvadis/RNAseq-project) and WGS data from the corresponding individuals was downloaded from the 1000 Genomes Project website (http://www.internationalgenome.org/data/). RNA-seq data (normalized reads per kilobase per million reads or RPKM) were stratified by sJIA risk allele genotype and the difference in relative expression between genotypes was evaluated using the non-parametric Kruskal-Wallis test. Box plots of relative expression were generated using R.

Therapeutic response analysis

The relationship between sJIA-associated *IL1RN* SNPs and therapeutic response to human recombinant IL-1RA (anakinra) or tocilizumab treatment was examined in sJIA patients from the U.S. stratum for whom therapeutic response data were available. This included 38 anakinra treated subjects and 14 subjects treated with tocilizumab. Treatment response data were extracted from medical records by the treating pediatric rheumatologist, who encoded either “no response” or “any response” for each subject. “No response” was defined as no improvement of either fever (if present) or arthritis. “Any response” was defined as any degree of improvement in either fever or arthritis. Treatment response was then tested for association with sJIA-associated SNPs by logistic regression under the dominant model using SVS8. The threshold of significance for the association test was defined by a Bonferroni correction for the number of independent SNPs tested, as defined by pairwise LD pruning ($r^2 < 0.5$).

Results

Association testing of sJIA candidate SNPs and loci

We first performed association testing of the 26 SNPs for which associations with sJIA had been previously reported (Table 1). After applying Bonferroni correction for 26 SNPs, association meta-analysis of the 9 INCHARGE sJIA study populations revealed no significant associations with sJIA ($p < 0.05 \div 26 = 1.9 \times 10^{-3}$, Table 1, Figure S1). To evaluate whether the 11 candidate loci containing these 26 SNPs harbored sJIA risk SNPs distinct from those previously described, we extended our analysis to test all SNPs within these candidate risk loci for association with sJIA. The candidate regions included a total of 5479 SNPs (Table S2), but LD pruning at a level of $r^2 < 0.5$ determined that only 500 of them were independent. This defined the threshold of study-wide significance ($p < 0.05 \div 500 = 1.0 \times 10^{-4}$). By this standard, association meta-analyses of these 11 loci revealed a single significant association signal within the *IL1RN* locus (Figure 1). The association peak was located 4.3 Kb upstream from *IL1RN*, with 3 SNPs exceeding the significance threshold and the top 7 SNPs in strong LD with one another (Figure 2). In fact, LD mapping and haplotype analysis of the top 25 SNPs within this locus revealed that the top 7 sJIA-associated SNPs were inherited as a part of a common haplotype (Figure 2).

sJIA-associated *IL1RN* variants and gene expression

A query of the HaploRegv4.1 database revealed that many of the top sJIA-associated SNPs were known expression quantitative trait loci (eQTL) for *IL1RN* in whole blood[26]

and LCLs[25] (Table 2). Moreover, a review of the literature found that sJIA-associated SNPs also correlated with IL-1RA protein levels in the largest study of genetic predictors of IL-1RA levels[27]. The SNP that most strongly correlated with IL-1RA in that study, rs4251961, was one of the top sJIA-associated SNPs and was a constituent of the 7 SNP haplotype (Figure 2, Table 2). These observations were corroborated by our direct analyses of LCL RNA-seq data from 1000 Genomes Project subjects[25], which found that the sJIA-associated SNPs were strongly correlated with *IL1RN* expression (Figures 3 and S2). Specifically, alleles that were protective against sJIA correlated with high *IL1RN* expression and those that were risk factors for sJIA correlated with reduced *IL1RN* expression (Figure 3). Importantly, all three of the studies mentioned above parsimoniously demonstrated that sJIA risk alleles of the top 42 sJIA-associated SNPs were correlated with decreased levels of *IL1RN* expression or circulating IL-1RA protein (Figure 3).

sJIA-associated *IL1RN* variants and response to anakinra therapy in sJIA

Given that the response of sJIA to treatment with recombinant human IL-1RA (anakinra) is variable, we hypothesized that individuals with the highest genetically-encoded levels of IL-1RA may fail to respond to anakinra treatment more often than those with lower genetically-encoded levels. To evaluate this possibility, we examined clinical and SNP genotype data in 38 sJIA patients from the U.S. collection that had received anakinra and for whom clinical data were available. Within this group of anakinra treated subjects, there were 9 non-responders and 29 “any responders”. An examination of the top 7 sJIA-associated *IL1RN* SNPs found that for each SNP, homozygosity for the

IL1RN high expression alleles was associated with non-response to anakinra treatment ($p < 0.05$, Table 3). rs555447483 showed the strongest association with anakinra non-response ($p = 7.7 \times 10^{-4}$; OR 28.7 [3.2, 255.8], with homozygous high expression alleles predicting non-response with a sensitivity of 92% and a specificity of 71%.

To determine whether the relationship between these SNPs and sJIA treatment failure were specific to anakinra, we performed an identical examination of the 14 sJIA patients from the U.S. collection who were treated tocilizumab, an anti-IL-6 monoclonal antibody. Within this group, which included 3 tocilizumab non-responders and 11 tocilizumab "any responders," we found no association between sJIA-associated *IL1RN* SNPs and response to tocilizumab treatment (Table S3). Moreover, 8 of the 14 patients treated with tocilizumab were anakinra non-responders who received tocilizumab as second line treatment. Among the 8 anakinra non-responders, 6 were tocilizumab "any responders." Taken together, these facts support the hypothesis that sJIA-associated *IL1RN* SNPs specifically predict non-response of sJIA to anakinra treatment, as opposed to identifying individuals whose sJIA is more broadly refractory to treatment.

Discussion

Through an examination of common genetic variants at 11 previously reported sJIA susceptibility loci in the INCHARGE sJIA collection, this study has yielded three important observations. First, this study has demonstrated that the *IL1RN* locus is a *bona fide* sJIA susceptibility locus. Second, it has revealed that genetically-encoded high expression of *IL1RN* and production of IL-1RA are protective against sJIA (and

conversely that genetically-encoded low expression/production are risk factors for developing sJIA.) Most importantly, it has shown that homozygosity for the high expression alleles of sJIA-associated *IL1RN* SNPs is strongly associated with unresponsiveness to anakinra treatment in sJIA patients.

The original studies describing these 11 candidate loci reported modest associations that were identified in small case-control collections[7-16]. At most of these loci, the associations with sJIA were not observed in subsequent studies of other populations, calling their proposed relationships with sJIA into question. We sought to evaluate these associations more rigorously by using the INCHARGE sJIA collection, which provided greater statistical power than any previous study of these loci while also allowing for internal validation through the examination of 9 independent populations. Using this approach, we found that only one of these candidate loci, *IL1RN,* was associated with sJIA. At this locus, we observed 3 sJIA-associated SNPs that tagged a 7 SNP haplotype in the promoter region of *IL1RN,* as well as a cluster of 39 other SNPs with intermediate evidence of association with sJIA. Importantly, the *IL1RN* association signal identified in the present study did not include any of the SNPs that were previously reported as sJIA-associated (Table 1) or any SNPs that were in strong LD with those SNPs (Figures 1 and S1). This observation suggests that the historical candidate gene studies of sJIA could have been negatively impacted by poor statistical power, as has been the case in other genetically complex diseases, such as schizophrenia[28].

Given that the association signal of the *IL1RN* locus was within the promoter region, we hypothesized that these SNPs may influence sJIA risk by altering gene expression. By examining previously published gene expression studies and integrating our association data with publicly available gene expression datasets, we found that the risk alleles of the top 42 sJIA-associated *IL1RN* SNPs correlated with reduced *IL1RN* expression and circulating IL-1RA levels (Figure 2, Table 2). Furthermore, we observed that the top 7 sJIA-associated SNPs were among the SNPs most strongly associated with *IL1RN* expression levels in whole blood and LCLs, and with circulating levels of IL-1RA protein, in published studies (Table 2)[25-27]. Taken together these observations suggest that the sJIA-associated *IL1RN* SNPs influence sJIA risk through their effect on *IL1RN* expression and production of IL-1RA.

IL-1RA is a well-documented positive acute phase protein[29] and it has been shown to be highly expressed in the blood of children with active sJIA[30-31]. Therefore, one would expect that gene expression studies should find increased expression of *IL1RN* in children with active sJIA compared to healthy subjects or children with quiescent sJIA. There have been several studies that have examined gene expression in sJIA peripheral blood mononuclear cells. In one of these studies, the expression of positive acute phase genes was upregulated in children with sJIA and the authors noted that *IL1RN* was among this cluster[32]. However, three other studies found no relationship between sJIA and *IL1RN* expression[33-35]. There are a couple of potential reasons for these conflicting results. These studies were undertaken in relatively small numbers of sJIA cases, so it is possible that they lacked the statistical power to identify a

relationship between sJIA and *IL1RN* expression. It is also possible that these studies were affected by confounding variables that altered *IL1RN* expression in the sJIA patients, such as the duration of sJIA, the level of sJIA disease activity or the treatment(s) administered for sJIA. By examining the correlation between sJIA-associated *IL1RN* variants and gene expression in healthy individuals, the present study could identify the relationship between *IL1RN* expression and sJIA without the interference of these potential confounders.

Looking beyond disease risk, we also observed that high expression alleles of sJIA-associated *IL1RN* SNPs were strongly associated with non-response to anakinra therapy. The lack of association between these SNPs and non-response to tocilizumab treatment suggests that these SNPs are specifically associated with anakinra non-responsiveness, as opposed to being associated with more global therapeutic recalcitrance. In the context of the bi-phasic hypothesis of sJIA pathophysiology, new onset sJIA is treated with the goal of rapidly inducing remission within the therapeutic window of opportunity[4]. Anakinra is commonly chosen as the first line treatment because its effects can be observed within days of initiation and because its dosing can be rapidly escalated, but it is not effective in all patients[36]. In the subset of sJIA cases that ultimately don't respond to anakinra, their time to remission is extended by the failed therapeutic course of anakinra. The findings of this study can be used to identify the subset of children with sJIA that are unlikely to respond to anakinra and facilitate the selection of an alternative treatment. In doing so, one can avoid the delay associated with a first-line therapeutic failure and reduce the time to remission, as well as prevent

unnecessary exposure to the risks of anakinra treatment. This is the first candidate biomarker that can prospectively guide therapeutic decision making in sJIA.

Despite the strength of our findings, it is important to consider potential limitations of our study. This study evaluated genetic associations in 9 independent sJIA case-control collections. It will be important to examine the *IL1RN* region in larger, independent groups of patients. There were several limitations to the evaluation of genetic predictors of anakinra response. Therapeutic response to anakinra was examined in 38 sJIA patients, which is a relatively small group. The anakinra treated patients were not treated in a standardized fashion, with potential variation in the drug dosing and duration, timing of dose escalation and co-administration of other agents (ie. glucocorticoids). The clinical response data were extracted from medical records in a *post hoc* analysis and clinical response metrics were not standardized. We anticipated that these factors would complicate differentiating incomplete and complete response, but should not influence the identification of non-response. Therefore, we chose to compare non-response to “any response.” Nonetheless, it will be important to evaluate the correlation between *IL1RN* SNPs and response to anakinra in prospective studies of larger numbers of patients treated and monitored in a standardized manner.

By identifying a prospective biomarker capable of guiding the treatment of sJIA, this study brings precision medicine to the rheumatology clinic. Looking forward, it will be important to determine whether these findings are generalizable beyond anakinra and sJIA. For example, can the *IL1RN* SNPs predict therapeutic response with other IL-1

directed therapies, such as monoclonal anti-IL-1β antibodies (canakinumab) or the IL-1 trap (rilonacept), in sJIA. Similarly, these SNPs may predict therapeutic response to anakinra (or other IL-1 directed therapies) in conditions other than sJIA, such as adult-onset Still's disease or monogenic autoinflammatory diseases. Given that recently published trials have found that canakinumab treatment significantly reduces the risk of recurrent cardiovascular events[37], as well as the incidence of and mortality from lung cancer[38], it is even possible that the utility of this prospective biomarker may extend beyond the field of rheumatology.

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Figure Legends

Figure 1. INCHARGE sJIA case-control regional association plots of loci previously implicated by candidate gene studies. Regional association plots for previously reported sJIA candidate susceptibility loci near *IL1A/B* (**A**), *GLI2* (**B**), *IL1RN/PSD4* (**C**), *IL1R2* (**D**), *IL10/20* (**E**), *IL6* (**F**), *MVK* (**G**), *CCR5* (**H**), *MIF* (**I**), *SLC26A2* (**J**), and *TAPBP* (**K**) show minimal significance in INCHARGE case-control dataset, except for a cluster of SNPs in the *IL1RN/PSD4* region **(C)**. None of the top SNPs from previous candidate studies (labeled and denoted by red diamonds) showed even nominal significance with sJIA. Other SNPs in the candidate loci are shown as blue circles. The brown horizontal line demonstrates the study-wide significance threshold.

Figure 2. Variants of the *IL1RN* locus are associated with sJIA in the INCHARGE case-control collection. SNP associations within the *IL1RN* locus are shown, colored by pairwise linkage disequilibrium (LD) with the most strongly associated SNP, rs55663133 **(A)**. The brown horizontal line demonstrates the study-wide significance threshold. In **Panel B**, a forest plot demonstrates the effect size of rs55663133 by meta-analysis and in individual study populations. **Panel C** displays pairwise LD with the peak sJIA-associated SNP, rs55663133 (star) in the U.S. case-control population. The top 7 sJIA associated markers (19-25) form a strong LD block.

Figure 3. Relationship of *IL1RN* expression and IL-1RA protein levels with sJIA-associated SNPs. *IL1RN* expression by RNA sequencing from the study of Lappalainen *et al.*[25] is shown, stratified by genotype, for representative sJIA-associated SNPs **(A and B)**. Dot plots depict all SNPs with reported correlations with *IL1RN* expression **(C and D)** or IL-1RA protein levels **(E)** in the studies by Westra *et al.*[26], Lappalainen *et al.*[25], and Herder *et al.*[27], respectively. SNPs among the top 42 sJIA-associated SNPs are highlighted in green (sJIA protective alleles) and gold (sJIA risk alleles), and the top 7 sJIA-associated SNPs are highlighted in red.

Table 1. Association results of 26 systemic juvenile idiopathic arthritis candidate SNPs in INCHARGE sJIA study collection

			Previous Study		INCHARGE Study			
Previous Study	**SNP**	**Gene**	***P* value**	**OR (95 C.I.)**	***P* value**	**OR (95 C.I.)**	**i^2**	**N / n**
Stock *et. al.*	rs6712572	IL1 Ligand (*CKAP2L*)	0.0045	1.62 (1.16, 2.29)	0.66	1.03 (0.91, 1.15)	0.34	9/7708
	rs2071374	IL1 Ligand (*IL1A*)	0.0060	1.65 (1.15, 2.37)	0.11	1.11 (0.98, 1.25)	0	9/7711
	rs3783516	IL1 Ligand (*IL1A/IL1B*)	0.0053	1.64 (1.15, 2.27)	0.04	1.13(0.80, 1.26)	0.30	9/7711
	rs4848123	IL1 Ligand (*GLI2*)	0.0030	1.70 (1.19, 2.44)	0.24	0.27 (0.12, 2.38)	0.80	2/449
	rs3917368	IL1 Ligand (*IL1B*)	0.0096	1.57 (1.11, 2.22)	0.18	1.08 (0.96, 1.22)	0.43	9/7715
	rs1688075	IL1 Ligand (*IL1RN*)	0.0089	3.04 (1.58, 5.85)	0.64	0.95 (0.77, 1.17)	0.06	8/7603
	rs4849159	IL1 Ligand (*PSD4*)	0.040	1.61 (1.02, 2.54)	0.17	0.89 (0.75, 1.05)	0	8/5755
	rs6760120	IL1 Ligand (*PSD4*)	0.020	1.49 (1.06, 2.21)	0.33	0.92 (0.77, 1.09)	0.19	9/7713
	rs12712122	IL1 Receptor (*IL1R2*)	0.0031	1.71 (1.21, 2.41)	0.04	1.32 (1.03, 1.69)	0	9/7710
	rs4851531	IL1 Receptor (*IL1R2*)	0.0087	1.59 (1.11, 2.28)	0.58	0.97 (0.86, 1.09)	0.13	9/7708
Omoyinmi *et. al.*	rs1400986	IL-10 Family (*IL20*)	0.0004	1.53 (1.21, 1.93)	0.27	1.11 (0.93, 1.32)	0.48	8/7519
	rs4129024	IL-10 Family (*MAPKAPK2*)	0.0027	0.68 (0.53, 0.88)	0.05	0.87 (0.75, 1.00)	0.08	9/7712
Fife *et. al.*	rs1800896	IL-10 Family (*IL10*)	0.031	1.34 (n.p.)	0.02	1.15 (1.02, 1.28)	0	9/7716
	rs1400986	IL-10 Family (*IL20*)	0.028	1.51 (n.p.)	0.27	1.11 (0.93, 1.32)	0.48	8/7519
Fishman *et. al.*	rs1800795	*IL6*	0.03	n.p.	0.34	0.94 (0.84, 1.06)	0.32	9/7710
Hinks *et. al.*	rs2071374	*IL1A*	0.001	1.50 (1.16, 1.92)	0.11	1.11 (0.98, 1.25)	0	9/7711
	rs11836136	*MVK*	0.03	1.34 (1.03, 1.74)	0.58	1.05 (0.89, 1.23)	0	9/7717
Scheibel *et. al.*	rs333	*CCR5*	0.004	n.p.	0.16	0.86 (0.69, 1.06)	0.63	4/7009
De Benedetti *et. al.*	rs755622	*MIF*	0.017	n.p.	0.11	0.88 (0.76, 1.03)	0	8/7513
Lamb *et. al.*	rs1541915	*SLC26A2*	0.0003	2.3 (1.4, 3.7)	0.76	0.98 (0.87, 1.11)	0.19	8/7516
	rs245056	*SLC26A2*	0.00002	2.8 (1.7, 4.6)	0.72	1.03 (0.86, 1.23)	0.26	8/7513
	rs245055	*SLC26A2*	0.004	2.5 (1.2, 5.0)	0.56	0.95 (0.81, 1.12)	0	9/7709
	rs245051	*SLC26A2*	0.0005	2.3 (1.4, 3.7)	0.42	0.95 (0.85, 1.07)	0.44	9/7708
	rs245076	*SLC26A2*	0.0015	2.7 (1.3, 5.6)	0.46	0.94 (0.80, 1.11)	0	9/7715
	rs8073	*SLC26A2*	0.04	2.3 (0.9, 5.6)	0.25	0.91 (0.77, 1.07)	0	9/7714
Bukulmez *et. al.*	rs2071888	*TAPBP*	0.04 (TDT)	n.p.	0.15	1.09 (0.97, 1.22)	0	9/7715

SNP, single nucleotide polymorphism; INCHARGE, International Childhood Arthritis Genetic Consortium; OR, odds ratio, 95 CI, 95% confidence interval; i^2, i^2 test for heterogeneity; N, number of strata included in meta-analysis; n, number of samples included in meta-analysis; n.p., not provided; TDT, transmission disequilibrium testing

Table 2. Association of *IL1RN* SNPs with sJIA risk and their effect on *IL1RN* expression / IL-1RA concentration

					***IL1RN* in LCLs Lappalainen *et al.*[25]**		***IL1RN* in whole blood Westra *et al.*[26]**		**IL-1RA in serum Herder *et al.*[27]**	
SNP	**Risk Allele**	**Meta P**	**Meta OR (95 CI)**	**r^2**	**P-value**	**Effect Size**	**P-value**	**Effect Size**	**P-value**	**Effect Size**
rs55663133	AAT	5.9×10^{-5}	1.3 (1.1, 1.4)	1	1.0×10^{-6}	-0.25				
rs62158854	G	7.2×10^{-5}	1.3 (1.1, 1.4)	1	6.5×10^{-7}	-0.25				
rs62158853	T	8.3×10^{-5}	1.3 (1.1, 1.4)	1	2.6×10^{-7}	-0.26				
rs55709272	C	2.8×10^{-4}	1.2 (1.1, 1.4)	0.87						
rs7580634	T	3.7×10^{-4}	1.2 (1.1, 1.4)	0.91	2.8×10^{-6}	-0.24				
rs4251961	C	4.6×10^{-4}	1.2 (1.1, 1.4)	0.86	1.0×10^{-6}	-0.25	1.6×10^{-11}	-6.74	2.2×10^{-34}	-0.08
rs555447483	A	5.0×10^{-4}	1.2 (1.1, 1.4)	0.89						
rs28648961	A	8.5×10^{-4}	1.2 (1.1, 1.4)	0.75	2.8×10^{-6}	-0.24				
rs111354213	-	9.8×10^{-4}	1.2 (1.1, 1.4)	0.74						
rs6743171	C	1.1×10^{-3}	1.2 (1.1, 1.4)	0.77	3.2×10^{-6}	-0.24	5.8×10^{-8}	-5.42	6.4×10^{-13}	-0.09
rs17207494	C	1.1×10^{-3}	1.2 (1.1, 1.4)	0.75	2.9×10^{-6}	-0.24	3.8×10^{-8}	-5.50	1.3×10^{-11}	-0.08
rs10171849	C	1.1×10^{-3}	1.2 (1.1, 1.4)	0.75	5.5×10^{-6}	-0.23	2.6×10^{-8}	-5.57	1.4×10^{-11}	-0.08
rs4496335	T	1.2×10^{-3}	1.2 (1.1, 1.4)	0.77	3.0×10^{-6}	-0.24	5.2×10^{-8}	-5.44	6.4×10^{-13}	-0.09
rs6730516	T	1.2×10^{-3}	1.2 (1.1, 1.4)	0.77	3.5×10^{-6}	-0.24	6.2×10^{-8}	-5.41	6.4×10^{-13}	-0.09
rs55896126	C	1.2×10^{-3}	1.2 (1.1, 1.4)	0.75	2.7×10^{-6}	-0.24				
rs6734238	G	1.2×10^{-3}	1.2 (1.1, 1.4)	0.73			2.4×10^{-8}	-5.58	1.1×10^{-12}	-0.08
rs13410964	A	1.2×10^{-3}	1.2 (1.1, 1.4)	0.77	2.7×10^{-6}	-0.24	6.4×10^{-8}	-5.41	6.4×10^{-13}	-0.09
rs13424580	A	1.2×10^{-3}	1.2 (1.1, 1.4)	0.75	2.4×10^{-6}	-0.24	5.3×10^{-8}	-5.44	1.4×10^{-11}	-0.08
rs1446510	T	1.2×10^{-3}	1.2 (1.1, 1.4)	0.77	2.8×10^{-6}	-0.24	6.2×10^{-8}	-5.41	6.5×10^{-13}	-0.09
rs10176274	G	1.3×10^{-3}	1.2 (1.1, 1.4)	0.77	2.7×10^{-6}	-0.24	5.8×10^{-8}	-5.42	6.4×10^{-13}	-0.09
rs10188292	T	1.3×10^{-3}	1.2 (1.1, 1.4)	0.77	2.6×10^{-6}	-0.24	5.8×10^{-8}	-5.42	6.4×10^{-13}	-0.09
rs1446509	T	1.3×10^{-3}	1.2 (1.1, 1.4)	0.77	2.0×10^{-6}	-0.24	6.2×10^{-8}	-5.41	6.5×10^{-13}	-0.09
rs62158846	T	1.3×10^{-3}	1.2 (1.1, 1.4)	0.75						
rs6738239	A	1.3×10^{-3}	1.2 (1.1, 1.4)	0.77	4.7×10^{-6}	-0.23	6.1×10^{-8}	-5.42	6.5×10^{-13}	-0.09
rs13382561	G	1.3×10^{-3}	1.2 (1.1, 1.4)	0.75	1.9×10^{-6}	-0.24	3.7×10^{-8}	-5.51	1.4×10^{-11}	-0.08
rs7587033	G	0.001296	1.2 (1.1, 1.4)	0.77	2.9×10^{-6}	-0.24				

rs6750559	A	0.001325	1.2 (1.1, 1.4)	0.77	2.5×10^{-6}	-0.24	6.1×10^{-8}	-5.42	6.4×10^{-13}	-0.09
rs7574427	A	0.001344	1.2 (1.1, 1.4)	0.77	2.1×10^{-6}	-0.24	4.3×10^{-8}	-5.48	1.4×10^{-11}	-0.08
rs6722922	T	0.001374	1.2 (1.1, 1.4)	0.77	2.7×10^{-6}	-0.24	6.1×10^{-8}	-5.42	6.4×10^{-13}	-0.09
rs6741180	A	0.001376	1.2 (1.1, 1.4)	0.77	3.1×10^{-6}	-0.24	6.0×10^{-8}	-5.42	6.4×10^{-13}	-0.09
rs7574159	A	0.001393	1.2 (1.1, 1.4)	0.75	2.1×10^{-6}	-0.24	5.3×10^{-8}	-5.44	1.2×10^{-11}	-0.08
rs13398728	C	0.001434	1.2 (1.1, 1.4)	0.77	2.7×10^{-6}	-0.24	6.0×10^{-8}	-5.42	6.4×10^{-13}	-0.09
rs13409371	A	0.001445	1.2 (1.1, 1.4)	0.79	6.3×10^{-7}	-0.25	5.2×10^{-9}	-5.84	3.8×10^{-12}	-0.08
rs13409360	A	0.001467	1.2 (1.1, 1.4)	0.79	6.5×10^{-7}	-0.25	3.8×10^{-9}	-5.89	7.8×10^{-13}	-0.08
rs12329129	A	0.001468	1.2 (1.1, 1.4)	0.77	2.8×10^{-6}	-0.24	6.1×10^{-8}	-5.42	6.4×10^{-13}	-0.09
rs12328368	G	0.001473	1.2 (1.1, 1.4)	0.77	2.7×10^{-6}	-0.24	6.1×10^{-8}	-5.42	6.4×10^{-13}	-0.09
rs7596350	G	0.001475	1.2 (1.1, 1.4)	0.75	1.7×10^{-6}	-0.24				
rs6746979	A	0.001485	1.2 (1.1, 1.4)	0.75	2.1×10^{-6}	-0.24	5.1×10^{-8}	-5.45	1.4×10^{-11}	-0.08
rs58865280	A	0.001494	1.2 (1.1, 1.4)	0.75						
rs9973741	G	0.001514	1.2 (1.1, 1.4)	0.75	2.1×10^{-6}	-0.24				
rs12328766	G	0.001562	1.2 (1.1, 1.4)	0.77	3.0×10^{-6}	-0.24	6.2×10^{-8}	-5.41	6.4×10^{-13}	-0.09
rs550593914	T	0.001593	1.2 (1.1, 1.4)	0.77						

SNP, single nucleotide polymorphism; Meta P, fixed effect meta-analysis P value; Meta OR, fixed effect meta-analysis odds ratio; 95 CI, 95% confidence interval; r^2, pairwise r^2 with rs55663133 using the Estimation-Maximization method in the U.S. case-control population; LCL, lymphoblastoid cell line. The top 7 sJIA-associated SNPs, which are inherited as an LD block, are shown in bold italics.

Table 3. Association between sJIA-associated quantitative trait loci for *IL1RN* expression (and serum levels of IL-1RA protein) and response to anakinra therapy in 38 patients from the INCHARGE U.S. population.

SNP	Effect allele (high expression)	Homozygote frequency: Non-responder (n=9)	Homozygote frequency: Any responder (n=29)	P value	OR (95CI)
rs55663133	-	0.67	0.22	1.6×10^{-2}	7.0 (1.3, 36.7)
rs62158854	T	0.67	0.22	1.6×10^{-2}	7.0 (1.3, 36.7)
rs62158853	C	0.67	0.24	2.1×10^{-2}	6.3 (1.2, 32)
rs55709272	T	0.67	0.1	9.8×10^{-4}	17.3 (2.8, 108.1)
rs7580634	G	0.67	0.1	9.8×10^{-4}	17.3 (2.8, 108.1)
rs4251961	T	0.78	0.21	1.8×10^{-3}	13.4 (2.2, 82)
rs555447483	-	0.71	0.08	7.7×10^{-4}	28.7 (3.2, 255.8)

SNP, single nucleotide polymorphism; OR, odds ratio; 95CI, 95% confidence interval.

Figures

Figure 1

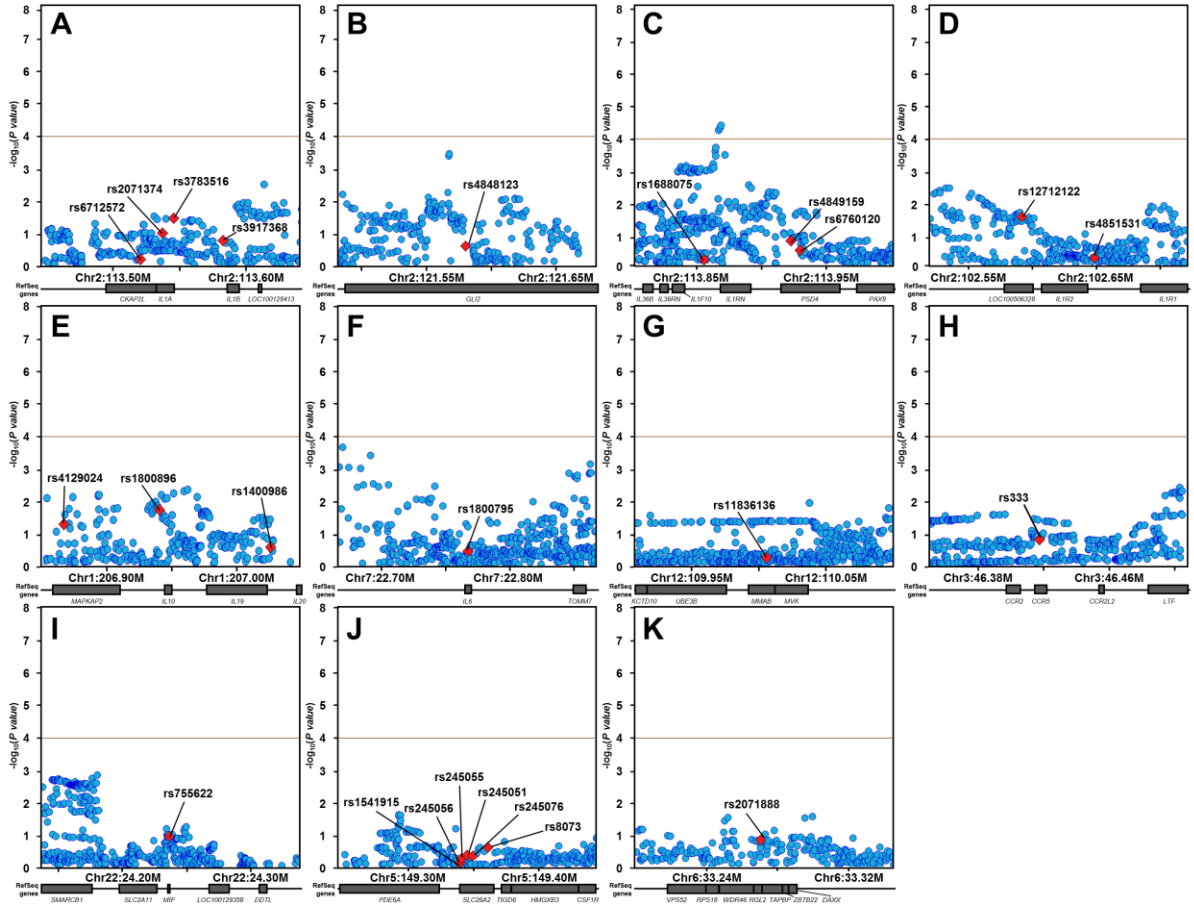


Figure 2

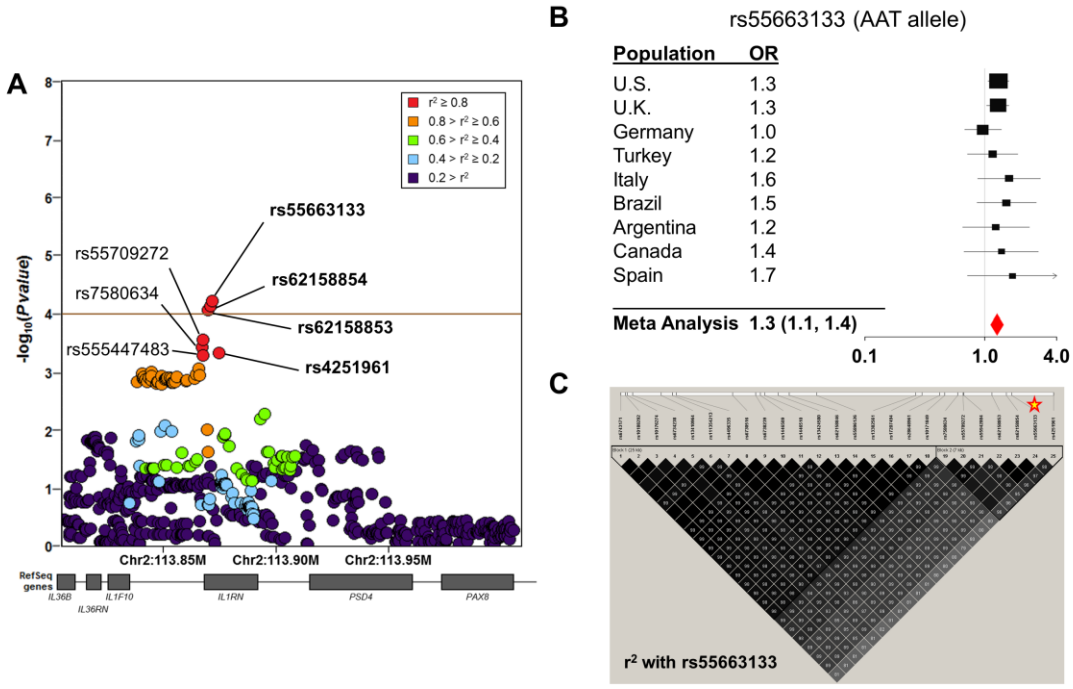
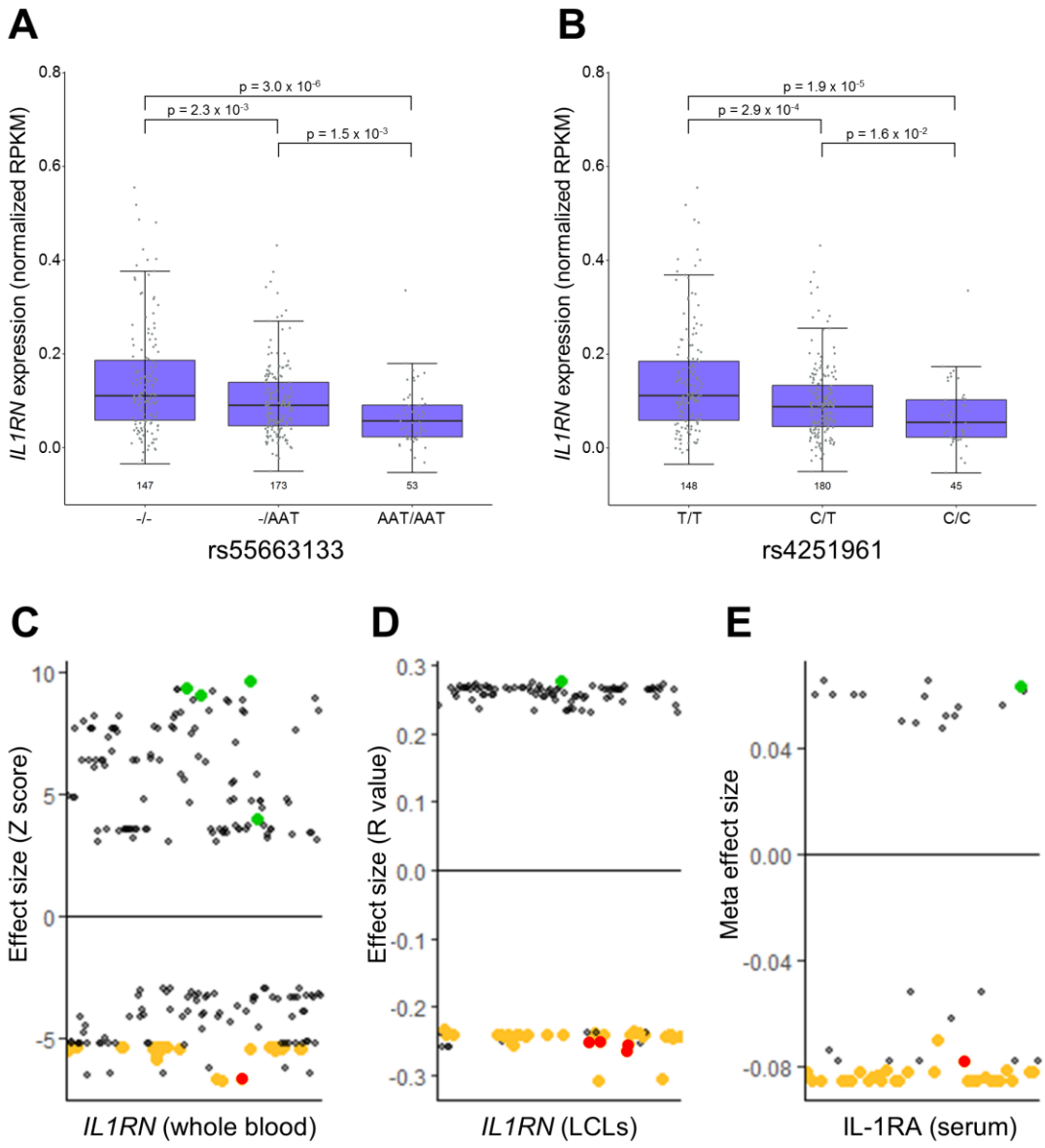

A
B
C
rs55663133 (AAT allele)
Population OR
U.S. 1.3
U.K. 1.3
Germany 1.0
Turkey 1.2
Italy 1.6
Brazil 1.5
Argentina 1.2
Canada 1.4
Spain 1.7
Meta Analysis 1.3 (1.1, 1.4)
0.1
1.0
4.0
r² ≥ 0.8
0.8 > r² ≥ 0.6
0.6 > r² ≥ 0.4
0.4 > r² ≥ 0.2
0.2 > r²
rs55663133
rs55709272
rs62158854
rs7580634
rs62158853
rs555447483
rs4251961
-log10(Pvalue)
Chr2:113.85M
Chr2:113.90M
Chr2:113.95M
RefSeq genes
IL36B
IL1F10
IL36RN
IL1RN
PSD4
PAX8
r² with rs55663133

Figure 3


A
B
C
D
E
IL1RN expression (normalized RPKM)
p = 3.0 x 10-6
p = 2.3 x 10-3
p = 1.5 x 10-3
147
173
53
-/-
-/AAT
AAT/AAT
rs55663133
p = 1.9 x 10-5
p = 2.9 x 10-4
p = 1.6 x 10-2
148
180
45
T/T
C/T
C/C
rs4251961
Effect size (Z score)
IL1RN (whole blood)
Effect size (R value)
IL1RN (LCLs)
Meta effect size
IL-1RA (serum)