*HLA-DRB1*11* and variants of the MHC class II locus are strong risk factors for systemic juvenile idiopathic arthritis

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Systemic juvenile idiopathic arthritis (sJIA) is an often severe, potentially life-threatening childhood inflammatory disease, the pathophysiology of which is poorly understood. To determine whether genetic variation within the MHC locus on chromosome 6 influences sJIA susceptibility, we performed an association study of 982 children with sJIA and 8,010 healthy control subjects from nine countries. Using meta-analysis of directly observed and imputed SNP genotypes and imputed classic HLA types, we identified the MHC locus as a bona fide susceptibility locus with effects on sJIA risk that transcended geographically defined strata. The strongest sJIA-associated SNP, rs151043342 [$P = 2.8 \times 10^{-17}$, odds ratio (OR) 2.6 (2.1, 3.3)], was part of a cluster of 482 sJIA-associated SNPs that spanned a 400-kb region and included the class II HLA region. Conditional analysis controlling for the effect of rs151043342 found that rs12722051 independently influenced sJIA risk [$P = 1.0 \times 10^{-5}$, OR 0.7 (0.6, 0.8)]. Meta-analysis of imputed classic HLA-type associations in six study populations of Western European ancestry revealed that *HLA-DRB1*11* and its defining amino acid residue, glutamate 58, were strongly associated with sJIA [$P = 2.7 \times 10^{-16}$, OR 2.3 (1.9, 2.8)], as was the *HLA-DRB1*11—HLA-DQA1*05—HLA-DQB1*03* haplotype [6.4×10^{-17}, OR 2.3 (1.9, 2.9)]. By examining the MHC locus in the largest collection of sJIA patients assembled to date, this study solidifies the relationship between the class II HLA region and sJIA, implicating adaptive immune molecules in the pathogenesis of sJIA.

systemic juvenile idiopathic arthritis | Still's disease | human leukocyte antigen | autoinflammation

Juvenile idiopathic arthritis (JIA) is a classification term describing children under the age of 16 who develop chronic arthritis (persisting for more than 6 wk) without an identifiable cause (1). Under this classification scheme there are seven subtypes of JIA, each with its own unique set of clinical characteristics and manifestations (1). One of these subtypes is systemic juvenile idiopathic arthritis (formerly known as systemic juvenile rheumatoid arthritis or systemic juvenile chronic arthritis, henceforth referred to as sJIA), a rare chronic, inflammatory disease of childhood whose etiology is poorly understood (2).

Although sJIA is classified among the JIA subtypes, reflecting the importance of arthritis in its definition, its overtly inflammatory phenotype clearly distinguishes sJIA from the other six subtypes (3). Children with sJIA exhibit recurrent episodes of unexplained, quotidian (daily spiking) fever, together with chronic arthritis and other manifestations, including generalized lymphoid hyperplasia, hepatosplenomegaly, serositis, and a characteristic salmon pink, evanescent skin rash (1). During periods of active inflammation, children with sJIA can develop profound elevation of serum acute-phase reactants and ferritin, and at these times, up to one-third of children with sJIA begin to manifest features of macrophage activation syndrome, a severe and life-threatening form of cytokine storm (4, 5). The presentation and clinical course of sJIA varies, with some cases involving predominantly

Significance

To determine whether genetic variation within the MHC locus influences the risk of developing systemic juvenile idiopathic arthritis (sJIA), we examined a dense set of MHC region single nucleotide polymorphisms, classic HLA alleles, and the individual amino acids of HLA molecules in nine independent sJIA case-control populations. Association testing revealed that genetic variants within the MHC class II gene cluster significantly influenced sJIA risk in every study population. The strongest risk factor for sJIA was *HLA-DRB1*11*, which conferred at least a two-fold increase in disease risk in each population studied. These data implicate the interaction of antigen presenting cells with T cells in the pathogenesis of sJIA.

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inflammatory features that tend to respond to anti–IL-1 therapies (6) and others following an arthritis-predominant disease course that may ultimately lead to chronic, destructive, treatment-refractory arthritis (7, 8).

sJIA is a genetically complex trait, meaning that its development depends on a combination of genetic and environmental risk factors (9). Investigations of genetically complex diseases rely upon comparisons of variant allele frequencies in ancestrally similar populations of affected individuals and healthy subjects. Given an estimated incidence of 0.6 new cases of sJIA per 100,000 children per year among populations of European ancestry (10), the assembly of case-control collections has been the primary obstacle to the genetic investigation of sJIA. As a result, most genetic studies of sJIA have examined modestly sized collections. The most intensively studied genetic locus in sJIA has been the cluster of HLA molecules within the MHC locus (11–16). HLA molecules are a critical component of the adaptive immune system, acting to present peptide antigens to antigen receptors on T lymphocytes. Studies of classic HLA locus-types in sJIA have often reported one or more sJIA-associated alleles (11–16), frequently at class II HLA loci, although no association has met the contemporary standard of genome-wide significance. In fact, there has been little agreement and no consensus among these studies, adding uncertainty to the proposed relationship between sJIA and the MHC locus.

To determine whether genetic variation of the MHC locus influences sJIA risk, we assembled the largest sJIA study population to date, including nine populations from North America, South America, and Europe. Using a combination of SNP genotyping and imputation of both SNPs and classic HLA alleles, we performed association testing and meta-analyses to identify sJIA risk factors within the MHC locus.

Results

Dataset Assembly and Quality Control. We performed SNP genotyping in a collection of 1,413 children from nine countries, including 982 children with sJIA and 431 healthy children. SNP genotype data from this collection were combined with SNP genotype data, in silico, from five healthy control populations that included an additional 7,579 control subjects, producing a total study population of 8,992 individuals. After quality-control operations, the final dataset included nine geographically defined, ancestrally matched case-control collections with a total of 770 sJIA patients and 6,947 control subjects (Figs. S1–S3 and Table S1). The majority of subjects excluded from the study (174 of 212 cases, 744 of 1,063 controls) were removed because of ancestral dissimilarities with their strata (Fig. S1 and Table S1*A*). This approach produced nine very well-matched case-control strata, as evidenced by genomic control inflation factors (λ_{GC}) between 1.00 and 1.004 (Fig. S3). Importantly, because some strata included in silico control data with sets of SNPs from different genotyping platforms that only partially intersected with the set of SNPs that we generated in the cases, the number of analyzable SNPs was reduced in these strata. As a result, the number of directly genotyped MHC region SNPs that passed the quality control process in both cases and controls varied by stratum, ranging from 938 to 9,575 SNPs (Table S1*B*). These sets of high-quality SNPs were used as the basis for SNP imputation with the multiancestral 1,000 Genomes reference population, generating datasets of between 27,005 and 34,992 MHC region SNPs with minor allele frequencies (MAF) > 0.05 that were imputed with high quality (Table 1 and Table S1*B*).

Association Testing and Meta-Analysis of MHC Locus SNPs in Nine sJIA Populations. Association testing of the MHC region SNPs was performed in each of the nine case-control populations under the additive and dominant models. Fixed-effect meta-analysis of the associations identified the strongest associations between sJIA and the MHC locus under the dominant model (Fig. 1). Among the 38,441 MHC region SNPs that were genotyped or imputed in at least three populations, we identified 482 sJIA-associated markers that exceeded the stringent threshold of genome-wide significance corrected for the two models tested ($P < 2.5 \times 10^{-8}$) (Fig. 1*A* and Table S2*A*). sJIA-associated SNPs were positioned between *NOTCH4* and *HLA-DQB1* (HLA-DQβ1 chain), with one peak of association in the *BTNL2*/*HLA-DRA* (HLA-DRα chain) region and a second in the *HLA-DRB1* (HLA-DRβ1 chain)/*HLA-DQA1* (HLA-DQα1 chain) region. The top SNP from this analysis, rs151043342, was located 8.1 kb 3′ of *HLA-DRA* [$P = 2.8 \times 10^{-17}$; odds ratio (OR) 2.6 (2.1, 3.3)] (Fig. 1*A*). This SNP conferred risk of sJIA in five of nine study populations, and the OR was suggestive of risk in three other study populations despite having 95% confidence intervals that included one (Fig. 1*B* and Tables S2*A* and S3). The top SNP in the *HLA-DRB1*/*HLA-DQA1* region was rs115124338 [$P = 1.9 \times 10^{-14}$; OR 2.2 (1.8, 2.7)] (Fig. 1*A* and Tables S2*A* and S3), located 6.1-kb upstream of *HLA-DQA1*. To determine whether multiple independent sJIA risk factors existed within the MHC locus, we repeated association testing while adjusting for the effect of the top SNP from the study, rs151043342 (Fig. 1*A*). This process revealed that rs12722051, encoding the Y25F missense variant of HLA-DQα1, independently influenced sJIA [$P_{regressor} = 1.0 \times 10^{-5}$; OR 0.7 (0.6, 0.8)] (Fig. 1 *A* and *C* and Table S3). HLA-DQα1 Y25F is most frequently found in *HLA-DQA1*02*, which demonstrated suggestive evidence of association with sJIA (Table 2).

Fixed-effect meta-analysis identified a high probability of heterogeneity ($I^2 > 0.7$) in 398 of 38,441 MHC region SNPs, including 2 of the 482 sJIA-associated markers (rs79174031 and rs149061153). Upon repeating the association meta-analysis of these 398 SNPs under the random-effects model, we found no association between sJIA and any of these SNPs (Fig. S4).

Imputation, Association Testing, and Meta-Analysis of Classic HLA Types and Amino Acids in sJIA. To further investigate the relationship between sJIA and the MHC locus, we performed SNP-based imputation of classic HLA types and amino acids in six study populations. Importantly, the strata from Turkey, Brazil, and Argentina were not included in this analysis because the reference dataset used for HLA imputation did not contain individuals ancestrally matched to these populations. The accuracy of HLA imputation was evaluated in subsets of the United States and United Kingdom populations for which directly observed HLA-type data were available. The imputed *HLA-DRB1* types were 97.5% and 95% concordant at two-digit resolution and 93.5% and 91.1% concordant at four-digit resolution in the United States and United Kingdom populations, respectively (Table S4). Association testing of two-digit and four-digit HLA types was performed separately in the six study populations. Fixed-effect meta-analysis of the HLA-type associations identified strong associations between sJIA and several class II HLA alleles (Table 2). The strongest association was between sJIA and *HLA-DRB1*11* alleles under the dominant model [$P = 2.7 \times 10^{-16}$; OR 2.3 (1.9, 2.8)] (Fig. 1*D* and Table 2), with both the *HLA-DRB1*11:01* [$P = 7.1 \times 10^{-14}$; OR 2.3 (1.9, 2.9)] and *HLA-DRB1*11:04* [$P = 7.8 \times 10^{-5}$; OR 2.0 (1.4, 2.7)] alleles conferring significant risk of sJIA (Fig. 2 *A* and *B*, Table 2, and Table S3). Association testing conditioned for the effect of *HLA-DRB1*11* on sJIA risk revealed no significant residual associations among HLA locus-types or regional SNPs (Fig. 1*E*).

To examine the molecular basis of sJIA risk within class II HLA molecules, we examined the polymorphic amino acid positions of classic HLA molecules for association with sJIA. Fixed-effect meta-analysis of amino acid associations from six study populations identified 14 sJIA-associated positions whose significance exceeded the genome-wide significance threshold, as well as an additional 20 positions that exceeded a significance threshold Bonferroni-corrected for 580 observed polymorphic amino acid positions ($P < 8.6 \times 10^{-5}$) (Table S2*B*). Among these, the dimorphic position 58 of HLA-DRβ1 had the strongest association with sJIA [$P = 3.1 \times 10^{-16}$; OR 2.3 (1.9, 2.8)] (Figs. 1*D* and 2) with metrics of association that were virtually identical to those observed for *HLA-DRB1*11*. Because *HLA-DRB1*11* is the only imputable HLA-DRβ1 that contains glutamate 58, the effects of these two variables on sJIA risk are indistinguishable. After

Table 1. Membership of nine geographically defined sJIA case-control strata following quality control operations

Stratum	Cases	Controls	MHC-region SNPs*
United States	243	1,718	34,992
United Kingdom	202	4,097	32,105
Germany	115	193	34,458
Turkey	49	94	33,115
Italy	49	59	34,434
Brazil	48	62	31,814
Argentina	33	115	32,870
Canada	17	427	31,988
Spain	14	182	27,005
Total	770	6,947	

*Imputed with info score > 0.8 and MAF > 0.05.

completely adjusting for the effect of position 58 on sJIA risk, association testing identified no significant residual associations among the amino acids, HLA locus-types, or SNPs (Fig. 1*E*).

Analysis of Haplotypes and Linkage Disequilibrium Within the MHC Locus. Given that the sJIA susceptibility locus contained disease-associated SNPs spanning at least eight genes, we hypothesized that sJIA-associated *HLA-DRB1*11* alleles were inherited as a part of a longer haplotype. To test this hypothesis, we first assembled and tested haplotypes of best-guess, imputed classic HLA locus-types in each study population for association with sJIA. Fixed-effect meta-analysis of haplotype associations identified a strong, dominant association between sJIA and the common European haplotype, *HLA-DRB1*11–HLA-DQA1*05–HLA-DQB1*03* ($P = 6.4 \times 10^{-17}$) (Table 2 and Table S3). Moreover, this haplotype was composed of two subhaplotypes, one bearing *HLA-DRB1*11:01* and a second bearing *HLA-DRB1*11:04*, both of which conferred risk of sJIA (Fig. 3 *A* and *B*, Table 2, and Table S3).

To define the boundaries of the *DRB1*11*-containing risk haplotypes, we separately combined the top 100 population-specific sJIA-associated SNPs with the classic HLA locus-types in the United States and United Kingdom populations and repeated the haplotype analyses. In the United States population, a 243-kb sJIA risk haplotype that included the *DRB1*11–DQA1*05–DQB1*03* haplotype and 99 SNPs spanned the region from *HLA-DRA* to *HLA-DQB1* [$P = 8.1 \times 10^{-5}$; OR 1.8 (1.3, 2.4)] (Fig. 3*C*). This risk haplotype was present at a frequency of 14.2% in United States sJIA cases and 8.6% in United States healthy controls. In the United Kingdom population, we identified a 397-kb sJIA risk haplotype composed of 93 SNPs and the *DRB1*11–DQA1*05–DQB1*03* haplotype. This haplotype encompassed the region from *C6orf10* to *HLA-DQB1* and it was present at a frequency of 12.2% in United Kingdom sJIA cases and 4% in United Kingdom healthy controls [$P = 2.6 \times 10^{-10}$; OR 3.4 (2.3, 5.0)] (Fig. 3*C*). Given that the entire United States risk haplotype was contained within the United Kingdom risk haplotype, we examined the intersection of markers from the United States and United Kingdom risk haplotypes to determine the extent of shared identity between their respective sJIA risk haplotypes. Based upon 12 intersecting SNPs and HLA types from the DRβ1, DQα1, and DQβ1 loci, the United States risk haplotype was identical to the homologous segment of the United Kingdom risk haplotype (Table S3). To validate this finding, we expanded the analysis to include the top 400 population-specific sJIA-associated SNPs from the United States and United Kingdom populations, revealing an intersection of 110 SNPs. Examination of the 110 intersecting SNPs and the HLA types revealed that an identical, 243-kb haplotype was present in both populations. This haplotype, which included

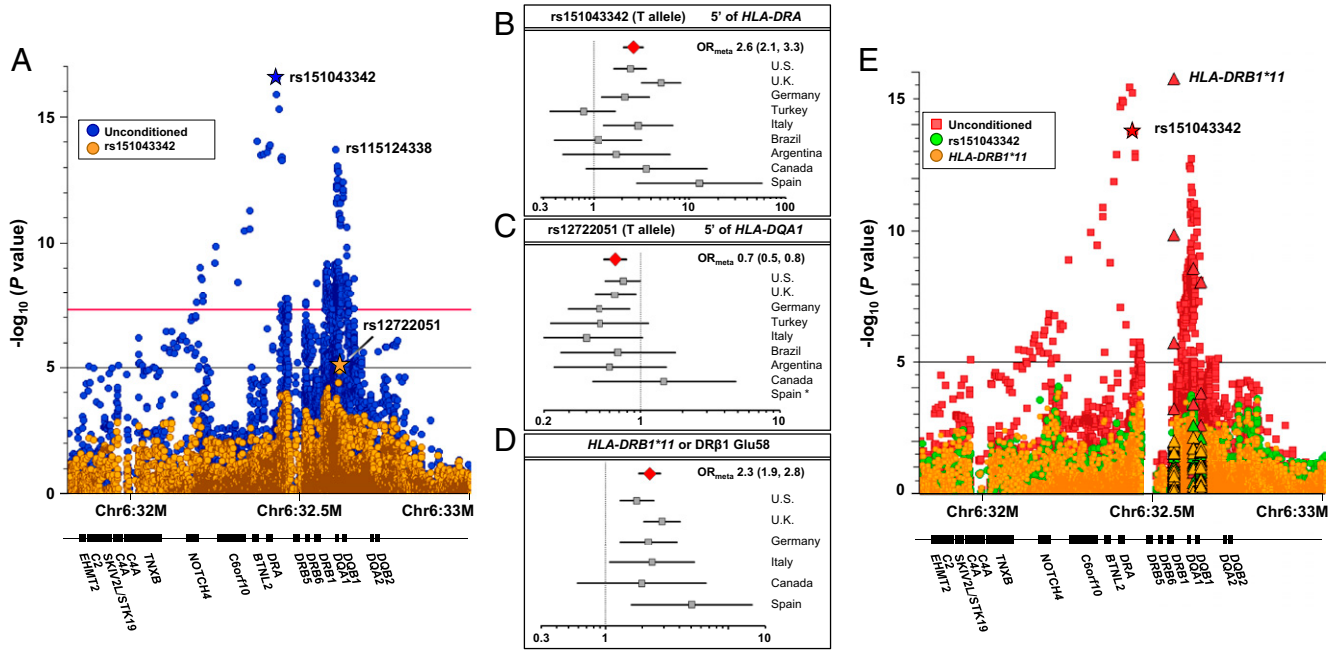

Fig. 1. The MHC is an sJIA susceptibility locus. (*A*) Dominant-model association results from fixed-effect meta-analysis of MHC-region SNPs in nine independent sJIA case-control populations are depicted by blue circles. The results of association testing conditioned on the effect of the top sJIA-associated MHC-region SNP (rs151043342, blue star) are shown as orange circles, and the top SNP from the conditional analysis (rs12722051) is marked by an orange star. (*B–D*) Forest plots depict the magnitude of the effects of rs151043342 (*B*), rs12722051 (*C*), and *HLA-DRB1*11* (*D*) on sJIA risk in the individual study populations and by meta-analysis. (*E*) Association results from fixed-effect meta-analysis of best-guess MHC-region SNPs (squares) and classic HLA types (triangles) from six Western European study populations are depicted by red shapes. Association results conditioned on the effect of the top SNP from *A* (rs151043342, red star) are shown as green shapes. Association results conditioned on the effect of the study's top risk factor (*HLA-DRB1*11*, red triangle) are shown as orange shapes. *rs12722051 was not imputed with high quality in this population.

Table 2. sJIA-associated classical HLA alleles and haplotypes identified by meta-analysis of six independent populations

Allele or haplotype	P_{meta}	OR (95CI)	I^2
*HLA-DRB1*11*	2.7×10^{-16}	2.3 (1.9, 2.8)	0.17
*HLA-DRB1*11:01*	7.1×10^{-14}	2.3 (1.9, 2.9)	0.00
*HLA-DQA1*05:01*	2.6×10^{-9}	1.7 (1.4, 2.0)	0.00
*HLA-DQA1*05*	2.6×10^{-9}	1.7 (1.4, 2.0)	0.00
*HLA-DQB1*03:01*	9.2×10^{-9}	1.7 (1.4, 2.0)	0.11
*HLA-DRB1*11:04*	7.8×10^{-5}	2.0 (1.4, 2.7)	0.00
*HLA-DQB1*03*	1.5×10^{-4}	1.4 (1.2, 1.7)	0.40
*HLA-DQA1*02*	5.4×10^{-4}	0.7 (0.6, 0.8)	
*DRB1*11–DQA1*05–DQB1*03*	6.4×10^{-17}	2.3 (1.9, 2.9)	0.32
*DRB1*11:01–DQA1*05:01–DQB1*03:01*	3.1×10^{-11}	2.2 (1.7, 2.8)	0.00
*DRB1*11:04–DQA1*05:01–DQB1*03:01*	1.5×10^{-6}	2.3 (1.6, 3.1)	0.22

After correcting for 175 imputed classic HLA types, significance was defined as $P < 2.9 \times 10^{-4}$. P_{meta}, P value from fixed-effect meta-analysis of dominant model associations; OR, odds ratio under the dominant model; 95CI, 95% confidence interval; I^2, I^2 test of heterogeneity.

HLA-DRA, the entire *HLA-DRB* gene cluster, *HLA-DQA1*, and *HLA-DQB1*, was associated with sJIA risk in both the United States and United Kingdom populations (Fig. 3*C* and Table S3).

Discussion

By using a stratified study design to interrogate a combination of directly observed and imputed SNP genotypes and imputed classic HLA alleles in nine independent sJIA case-control populations, this study provides the most extensive investigation of the MHC locus in sJIA to date. The data clearly demonstrate that genetic variants of class II HLA genes influence sJIA susceptibility in multiple populations of European ancestry. This work identifies *HLA-DRB1*11* alleles as the strongest single risk factor for sJIA with a pooled OR of 2.3, and further shows that the *DRB1*11-DQA1*05-DQB1*03* haplotype is associated with sJIA in each of the six populations examined. Haplotype analysis of SNP and HLA data in the two largest case-control subpopulations (United States and United Kingdom) revealed a conserved, 243-kb sJIA-associated class II HLA haplotype in both populations, raising the possibility that additional constituents of the haplotype may influence sJIA risk, in concert with *HLA-DRB1*11*. By demonstrating that class II HLA molecules influence sJIA susceptibility, this study implicates adaptive immunity in the pathophysiology of sJIA.

We and others have long sought to determine whether HLA genes are involved in the pathogenesis of sJIA. This relationship has been examined in children from a variety of ancestral backgrounds. Many alleles of HLA-DRβ1, including *HLA-DRB1*11* (16), have been reported to affect sJIA risk in studies of single populations (11–16). However no association has reached the level of genome-wide significance and no single *HLA-DRB1* allele has been found to influence sJIA risk across studies or populations (9, 17), leading some to conclude that class II HLA molecules are not risk factors for sJIA (18).

In designing the present study, we sought to overcome the factors that have limited earlier HLA studies of sJIA. The sample sizes of the HLA studies cited above were modest (35–108 cases) producing underpowered studies. We addressed this by establishing an international consortium and examining the largest sJIA study collection ever assembled. The case-control populations in the earlier studies were assembled without the benefit of ancestry-informative genetic markers. As a result, they were susceptible to population stratification, which occurs when ancestry-specific genetic differences between the case and control groups are errantly classified as disease-associated genetic differences. We addressed population stratification by performing clustering analyses of genome-wide SNP data to strictly define the membership of each stratum, and again by adjusting the association analyses for ancestry informative principal components (PCs). Additionally, serologic and PCR-based HLA-typing assays that were used in the earlier studies of sJIA are very costly, making direct HLA-typing cost prohibitive in our study. Therefore, we used a highly accurate imputation method to determine the classical HLA types in a population of over 7,000 subjects.

Each subject included in this study was evaluated by a senior pediatric rheumatologist expert in the diagnosis and treatment of sJIA, and every case fulfilled the International League of Associations for Rheumatology criteria for sJIA. This study includes patient collections that were assembled for independent genetic investigations at more than 15 separate pediatric rheumatology centers, many before the availability of genome-wide association analysis or the creation of the International Childhood Arthritis Genetics (INCHARGE) Consortium. As a result, the selection and timing of clinical data points varied among the populations. Additionally, the inability to recontact subjects within the consent documents at some centers precluded additional data collection from those subjects. Nonetheless, we have assembled clinical information from approximately half of the subjects in the study population (Table S5). Among this population subset, the proportion of subjects with the monophasic, polycyclic, and persistent disease courses, macrophage activation syndrome and persistent arthritis were each consistent with previous reports (4, 9). Although subanalyses stratified by these phenotypes would be of great interest, they were not performed in the current study because subdivision of the study population would have greatly reduced the statistical power to detect associations.

The implication of *HLA-DRB1*11* in sJIA susceptibility reinforces the uniqueness of sJIA among the JIA subtypes on a genetic level, clearly distinguishing sJIA from the two class I HLA-associated JIA subtypes, enthesitis-related JIA and psoriatic JIA. It also differentiates sJIA from seropositive polyarticular JIA, which is strongly associated with shared epitope-encoding alleles of *HLA-DRB1*, but not with *HLA-DRB1*11* (19). Surprisingly, *HLA-DRB1*11* alleles and *DRB1*11-DQA1*05-DQB1*03*

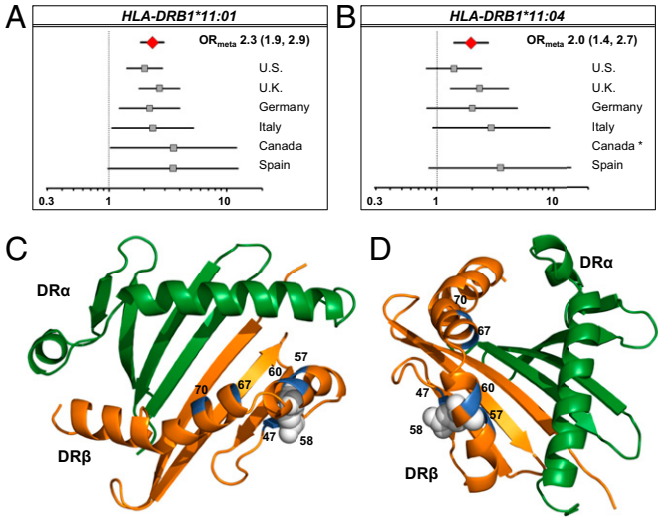

Fig. 2. Multiple *HLA-DRB1*11* alleles are associated with sJIA. Forest plots depict the odds ratios of the sJIA-associated *HLA-DRB1*11* family members, *HLA-DRB1*11:01* (*A*) and *HLA-DRB1*11:04* (*B*). Ribbon models of an HLA-DR molecule (*C* and *D*) demonstrate the two defining features of *HLA-DRB1*11* molecules: a glutamate residue at position 58 (white) on the exterior surface of the molecule with its side-chain pointing away from the peptide-binding groove, and the combination of peptide-binding groove residues that is unique to *HLA-DRB1*11* (shown in blue). The models were created from PDB ID code 3LQZ. *Allele frequency < 0.01%.

haplotypes also influence the risk of developing oligoarticular JIA (oJIA) and seronegative polyarticular JIA (snpJIA), which are phenotypically very similar to one another but which are very different from sJIA (14, 16). Unlike sJIA, where only the *HLA-DRB1*11* allele influences disease susceptibility, *HLA-DRB1* shows allelic heterogeneity in oJIA/snpJIA with numerous *HLA-DRB1* alleles affecting disease risk. Additionally, unlike oJIA and snpJIA, sJIA demonstrated no association with either the *HLA-DP* gene cluster or with the class I HLA locus, even after controlling for the effect of *HLA-DRB1*11*.

Class II HLA molecules present peptide antigens on the surfaces of antigen-presenting cells (APCs) for recognition by T-cell receptors (TCR) on $CD4^+$ T cells (20). It is possible that *HLA-DRB1*11* molecules participate in sJIA pathogenesis through an antigen-dependent mechanism, thereby implicating $CD4^+$ T lymphocytes and adaptive immunity in the pathogenesis of sJIA. Although the evidence for autoimmunity in sJIA is scant, there are some data that support a role for T lymphocytes in its pathophysiology, particularly in the subset of sJIA with an arthritis-predominant course. Alterations in the pattern of T-cell–secreted cytokines with a mixed Th1/Th2 pattern have been observed in sJIA patients (21). It has also been observed that children with sJIA have an increased proportion of the proinflammatory Th1 and Th17 cells, relative to age-matched healthy subjects (22). Additionally, two studies of abatacept (CTLA4-Ig), which prevents T-cell activation by inhibiting costimulation through CD80 and CD86, found it to be an effective treatment for children with chronic, articular sJIA (7) and recalcitrant sJIA with systemic features (8).

Class II HLA molecules also have a role in innate immunity and the regulation of APCs. Engagement of class II HLA on the surface of APCs by TCR and non-TCR ligands activates signaling pathways in APCs that regulate their function and survival (23–25). For example, ligation of surface-expressed class II HLA molecules on APCs by superantigens activates the myeloid differentiation primary response gene 88 signaling pathway (26), inducing expression of proinflammatory cytokines (27). Moreover, intracellular class II HLA molecules are important regulators of Toll-like receptor signaling in both monocytes and dendritic cells (24). Given that sJIA is a disease marked by systemic inflammation with enhanced production of proinflammatory cytokines (3, 17), it is attractive to hypothesize that *HLA-DRB1*11* molecules contribute to sJIA pathogenesis through dysregulation of innate immunity and promotion of proinflammatory cytokine production by APCs.

*HLA-DRB1*11* alleles are defined by glutamate at position 58 (Fig. 2 *C* and *D*), a residue whose side-chain is directed away from the peptide-binding groove. The exterior positioning of residue 58 may indicate a disease-relevant effect that is independent of antigen presentation, such as a superantigen-like reaction. However, it is not clear from our analyses whether the association of *HLA-DRB1*11* alleles with sJIA is driven by glutamate 58 or by the combination of highly polymorphic peptide-binding groove amino acids that uniquely define *HLA-DRB1*11* (Fig. 2 *C* and *D*). Ultimately, functional studies are necessary to elucidate the mechanisms through which *HLA-DRB1*11* participates in the pathophysiology of sJIA.

In summary, this study provides strong evidence for a role for *HLA-DRB1*11* as a major risk factor for sJIA. Our data demonstrate that alleles of this family confer a large effect (OR 2.3) on sJIA risk across study populations, reinforcing their potential importance in disease pathogenesis. Further attention should be focused on determining the specific mechanism through which *HLA-DRB1*11* alleles influence sJIA risk, whether through effects on T cells, APCs, or both, to allow for the rational design of therapeutics for sJIA.

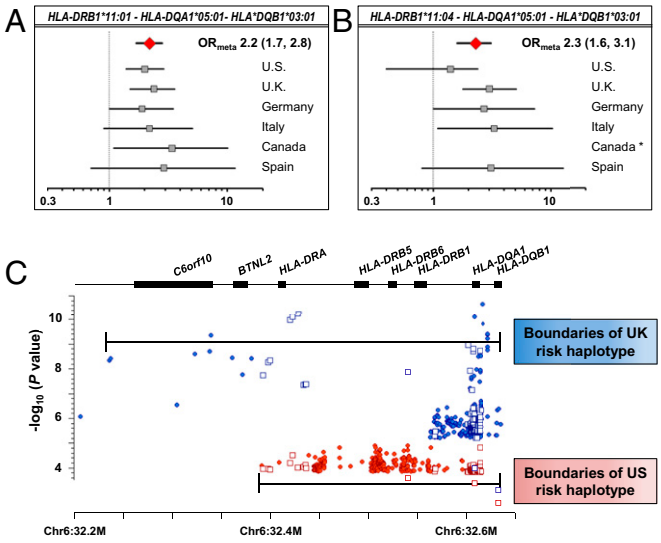

Fig. 3. Extended, *HLA-DRB1*11*-containing haplotypes are associated with sJIA. Forest plots depict the magnitude of the effects of the *HLA-DRB1*11:01* (*A*) and *HLA-DRB1*11:04* (*B*) containing forms of the *HLA-DRB1*11-HLA-DQA1*05-HLA-DQB1*03* haplotype on sJIA risk. To define haplotypic boundaries (*C*), we independently analyzed haplotypes of HLA types with the top 400 population-specific, sJIA-associated SNPs in the United Kingdom (blue circles) and the United States (red circles) populations. This revealed a 397-kb sJIA-associated haplotype in the United Kingdom population [$P = 2.6 \times 10^{-10}$; OR 3.4 (2.3, 5.0)] and a 243-kb disease-associated haplotype in the United States population [$P = 1.8 \times 10^{-5}$; OR 1.8 (1.3, 2.4)] that was fully contained within the United Kingdom haplotype. Comparison of the alleles of the 113 markers in common between the two haplotypes (open boxes) revealed that the United States and United Kingdom haplotypes were identical.

Methods

Patient Samples. Blood samples were obtained from children who were diagnosed with sJIA by pediatric rheumatologists at collaborating centers in nine countries (*SI Methods*). Blood samples from geographically matched healthy control subjects were obtained, and where available existing SNP genotype data from geographically matched healthy control individuals were used, in silico. The INCHARGE project was approved by an institutional review board (IRB) at the University of Manchester; subjects were enrolled in accordance with all local ethics regulations, with informed parental consent, and with approval of local IRBs at each contributing center (*SI Methods*).

SNP Genotyping, Imputation, Association Testing, and Meta-Analysis. We performed SNP genotyping of genomic DNA from children with sJIA and healthy children using Human Omni1M beadchips and an iScan reader (Illumina) according to the manufacturer's specifications. Samples were stratified by country of origin and rigorous quality control operations were performed separately in each case and control group (*SI Methods*). Geographically matched case and control populations were then assembled into nine case-control strata, each composed of the SNP intersection between the respective case and control groups. MHC locus SNPs (chromosome 6: 29 M–33 M; human genome build 19) were extracted from the conditioned, high-quality SNP data of each case-control stratum and were used as the basis for SNP imputation (*SI Methods*). Imputed SNP data were filtered to include common SNPs (MAF > 0.05) that were imputed with high quality (info > 0.8). Probabilistic genotypic data were subjected to frequentist association testing adjusted for ancestry informative PCs and meta-analyses were performed.

Imputation, Association Testing, and Meta-Analysis of Classic MHC Alleles and Amino Acid Residues. Imputation of HLA types and their corresponding amino acid polymorphisms at the eight classic HLA loci was performed in the United States, United Kingdom, German, Italian, Canadian, and Spanish case-control collections using MHC region SNP genotypes, SNP2HLA software, and a specially designed reference panel (*SI Methods*). Probabilistic HLA data were tested for association with sJIA using logistic regression adjusted for ancestry informative PCs and association results were meta-analyzed. Haplotype analyses were performed with Haploview and SVS7.

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