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Hierarchy of risk of childhood onset rheumatoid arthritis conferred by HLA-DRB1 alleles encoding the shared epitope

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Abstract

Objective—Associations between shared epitope (SE)-encoding *HLA-DRB1* alleles and rheumatoid arthritis (RA) are well established but only a limited number of studies have investigated these alleles in childhood onset RA (CORA), defined as rheumatoid factor (RF) and/or anti-citrullinated protein antibody (ACPA) positive juvenile idiopathic arthritis. We sought to investigate the largest cohort of CORA for association with SE alleles, and to determine whether there was a hierarchy of risk based on the amino acid sequence of the SE.

Methods—High resolution *HLA-DRB1* genotypes were obtained for 204 children with CORA and 373 healthy controls. Odds ratios (OR) and 95% CI were calculated for different SE-encoding *HLA-DRB1* alleles. We also calculated genotypic OR for combinations of SE alleles classified into S2, S3P or L alleles, based on amino acids in positions 70-74 of the DRβ1 chain as proposed by Tezenas Du Montcel et al (2005).

Results—We confirmed associations between *HLA-DRB1* SE alleles and CORA (76% of cases versus 46% of controls; OR=3.81 (95%CI 2.4-6.0), $p < 1 \times 10^{-7}$). We also found associations between individual SE alleles (*HLA-DRB1**0101, *0401, *0404, *0405, *0408 and *1001) and CORA. Genotype-specific risk estimates suggested a hierarchy of risk, with the highest risk among those heterozygous for S2/S3P (OR=22.3 (9.9-50.5) $p < 0.0001$).

Conclusions—We confirm the association between SE-encoding *HLA-DRB1* alleles and susceptibility to CORA. The excess risk conferred by individuals who carry the combination of

S2/S3P risk alleles suggests that children with DR β 1 chains containing KRAA and Q/RRRAA are especially prone to RA.

Keywords

juvenile idiopathic arthritis; rheumatoid arthritis; shared epitope; association

Rheumatoid arthritis (RA), which is estimated to affect ~1% of the adult population, is the most common form of inflammatory arthritis in the world. A substantial majority of individuals with RA have rheumatoid factor (RF) and/or anti-citrullinated protein antibodies (ACPA), and the presence of these antibodies has been associated with a worse prognosis. Some children with juvenile idiopathic arthritis (JIA) have a disease that phenotypically resembles RA, characterized by chronic inflammatory arthritis and the presence of the characteristic biomarkers seen in RA including RF and ACPA. Such JIA patients presumably represent a subset of RA patients with childhood onset of RA (CORA), but it is unclear if CORA shares the same risk factors associated with adult-onset RA, which has its peak age of onset in the fifth decade, or if patients with CORA have additional risk factors that, once identified, will offer important insight into the etiopathogenesis of RA.

Both genetic and environmental factors have been associated with the risk of developing RA in adults. A recent meta-analysis of genome-wide associations studies of RA brought the total number of loci with confirmed genetic association to 31(1). The strongest association observed to date has been with a subset of alleles encoding the class II Human leukocyte antigens (HLA). RA associated HLA-DRB1 alleles encode DR β 1 chains with conserved amino acid sequence in positions 70-74. The molecular structure encoding the susceptibility residues is termed the shared epitope (SE)(2). While associations between SE and risk of RA are well established, some alleles (e.g. *HLA-DRB1*0401*) appear to confer a greater degree of risk than others (*HLA-DRB1*0101*)(3). Tezenas Du Montcel et al proposed a classification based on the amino acid sequence at positions 70-74, and suggested that some classes of SE alleles conferred greater susceptibility than others (4, 5).

While SE alleles have been evaluated in childhood cases of RA, often referred to in the literature as “seropositive juvenile rheumatoid arthritis” or “polyarticular RF+ juvenile idiopathic arthritis”, most prior cohorts were small, used low resolution HLA typing, and thus have been limited to evaluation of the presence or absence of SE (6-11). Our objectives were to investigate the association between SE-encoding *HLA-DRB1* alleles and CORA, and to explore a possible hierarchy of risk conferred by SE alleles based on the amino-acid sequence in position 70-74, in a large cohort of CORA patients. We also sought to compare the frequencies of SE genotypes in children with CORA versus published frequencies in adults with RA.

Subjects and methods

Subjects

Cases were 204 children (149 non-Hispanic White (NHW), 21 African American, 25 Hispanic children, and 9 other) with JIA who had one or more positive tests for RF and/or ACPA. The mean age of onset of RA was 10.6 years. Of the 204 cases, 176 (86%) were female. All children were RF positive except for 3 children who had two negative tests each for RF and had positive tests for anti CCP antibodies (> 40 units). Of 98 children tested for ACPA, 72% were positive. The 21 NHW children who were ACPA negative were slightly younger than the 47 who were positive for ACPA (8.6 years vs. 10.9 years, *p* 0.042 by T-test). There was no difference in the gender distribution between ACPA positive vs. negative cases. Cases had been enrolled in genetic studies of JIA at various US pediatric

rheumatology centers, and also included some children who were enrolled as part of the Trial of Early Aggressive Therapy in Juvenile Idiopathic Arthritis (TREAT) study (clinical trials identifier NCT00443430). Controls were 373 healthy NHW individuals from Utah and Cincinnati (52% female; mean age of 20.3 at the time of enrollment). All subjects were enrolled in studies approved by the respective institutional review boards.

HLA-DRB1 genotyping and classification: DNA was extracted from peripheral blood mononuclear cells using established protocols. High resolution sequence based *HLA-DRB1* genotypes were determined on all cases and controls. The following *HLA-DRB1* alleles were considered to be SE alleles as previously described: HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *1001, *1303, *1402, *1406 (12). SE alleles were designated as proposed by the Tezenas du Montcel classification as follows: **S2**: (KRAA at amino acid positions 71-74) *HLA-DRB1**0401, and *1303; **S3P**: (Q/R-RRAA at amino acid positions 70-74) *HLA-DRB1**0101, *0102, *0404, *0405, *0408, *1001, *1402, *1406. All other alleles were designated as low-risk (L) alleles.

Statistical analyses

The frequencies of SE-encoding *HLA-DRB1* alleles were compared between NHW cases with CORA and controls using Chi-square or Fisher's exact tests as appropriate. Odds Ratio (OR) and 95% confidence intervals were calculated. Because we did not ascertain controls for children with African American or Hispanic ancestry, SE allele frequencies among African American and Hispanic subjects were compared against published allele frequencies of 2405 African American and 1999 Hispanic subjects from the National Marrow Donor Program (NMDP) Registry using Chi-square tests (13). Logistic regression was performed using NHW cases and controls to calculate the genotypic OR of various combinations of SE alleles classified by the Tezenas du Montcel system, with L/L as the reference group, using SAS 9.1.3 (SAS Institute, Cary, NC, USA).

Results

SE and HLA-DRB1 allele frequencies in NHW cases with CORA and controls

The frequency of SE-encoding *HLA-DRB1* alleles was 55% (163 of 298 alleles) among the 149 NHW cases with CORA versus 26% (192 of 746 alleles) among the 373 NHW controls (OR=3.48 (2.6-4.7); $p < 1 \times 10^{-7}$; bottom row of Table 1). Of the 149 NHW cases, 76% carried 1 or 2 SE alleles compared to 46% of controls (OR=3.81 (2.4-6.0), $p < 1 \times 10^{-7}$). A significantly greater proportion of cases carried 2 copies of SE alleles compared to controls (33% vs. 5.4%; OR=8.6, (4.7-15.8) $p < 1 \times 10^{-7}$). There were no differences in allelic or genotypic frequencies of SE between ACPA-positive and ACPA-negative cases.

When we examined the individual SE-encoding *HLA-DRB1* alleles among cases with CORA and controls, we confirmed associations between *HLA-DRB1**0101, *0401, *0404, *0405, *0408, and *1001 and CORA (Table 1). The other SE alleles were infrequent in our cohorts and were not significantly different between cases and controls. Significantly more cases than controls were heterozygous for *HLA-DRB1* *0401/0404 (10 of 149 cases versus 1 of 373 controls; OR=28.8 (3.5-564); $p < 0.0001$).

Evaluation of a possible hierarchy of SE in CORA and control cohorts

The frequency of *HLA-DRB1* SE alleles encoding DR β 1 chains containing Q/R-RRAA at positions 70-74 (S3P) was 33% (97 of 298 alleles) among the 149 NHW cases with CORA versus 16% (117 of 746 alleles) among the 373 NHW controls (OR=3.4 (2.4-4.8); $p < 1 \times 10^{-7}$). Similarly, the frequency of SE alleles encoding DR β 1 chains containing KRAA

at positions 71-74 (S2) was 22% (66 of 298 alleles) among the cases with CORA versus 10% (75 of 746 alleles) among the controls (OR=3.6 (2.4-5.4); $p < 1 \times 10^{-7}$).

Genotype-specific risk estimates with L/L as reference group suggested a hierarchy of risk (Table 2). Subjects who had a single copy of S2 (S2/L genotype) had an OR of 2.2 and those with a single copy of S3P (S3P/L genotype) had an OR of 2.6 compared to the reference group (L/L). Similarly, subjects with 2 copies of S2 had an OR of 7.7, while those with 2 copies of S3P had an OR of 7.2. The highest risk was observed among those who had an S2 and an S3P allele, (OR=22.3 (9.9-50.5), $p < 0.0001$). The effect of the number of risk S2 or S3P risk alleles was very significant for each additional risk allele, (OR=2.6 (1.8-3.7); $p = 3 \times 10^{-7}$), but there was a greater risk for S3P/S2 individuals beyond that expected based on the number of risk alleles alone (OR=3.4 (1.4-8.3); $p = 0.007$). Because the female to male ratio was higher among our cases than controls, we also performed the logistic regression including gender as a covariate. The OR estimates were slightly higher and demonstrated a similar trend (Table 2, columns 6 and 7).

SE and HLA-DRB1 allele frequencies in African American and Hispanic cases with CORA

Of 21 African American children with CORA, 10 (48%) carried 1 or 2 SE alleles (Table 3). The frequency of *HLA-DRB1* alleles encoding SE was 26% (11 of 42 alleles), compared to 16% among 2405 African American individuals in the National Marrow Donor Program Registry (OR=1.84 (0.84-3.82); $p = 0.08$) (13). Of 25 Hispanic children with CORA, 19 (76%) carried 1 or 2 SE alleles. The frequency of *HLA-DRB1* alleles encoding the SE was 48% (24 of 50 alleles), compared to 24% among 1999 Hispanic individuals in the National Marrow Donor Program Registry (OR=2.88 (1.59-5.21); $p < 0.0001$).

Discussion

Associations between SE-encoding *HLA-DRB1* alleles and RA have been well established. Previously, studies of small cohorts of children with “seropositive JRA” reported associations with *HLA-DR4* (6-11). Many of these studies, dating back 20 years or more, used lower resolution HLA typing, and were limited to evaluating the presence or absence of SE (Table 4). In the work reported here, we not only confirmed the association between SE alleles and susceptibility to childhood onset of RA using the largest cohort of CORA patients yet assembled, but also demonstrated associations between individual *HLA-DRB1* alleles and CORA for the first time.

In a study limited to 15 children with RA, Vehe et al observed that 11 (73%) children carried an SE-encoding *HLA-DRB1* allele on both haplotypes, and speculated that gene dosage may contribute to the early disease onset that defines CORA (11). In contrast, Barron et al, reported that none of their 13 cases with CORA had 2 copies of the SE (6). Addressing this discrepancy in our larger dataset, we found that the proportion of children with CORA who have two copies of SE was significantly higher than in controls (33% versus 6%), a finding similar to what has been observed in well powered studies of adults with RA. Specifically, 28% of 1320 British adults with RA were found to have 2 SE alleles compared to 7% of controls (14). Likewise in a separate study, 33% of 1275 adults with ACPA-positive RA carried 2 SE alleles, compared to 8% among 2405 controls (15). The similarity between our findings in CORA and adult onset RA suggests that gene dosage at the *HLA-DRB1* locus does not influence the age of onset of RA.

Investigating the allelic combinations present in our population, we found that heterozygosity for *HLA-DRB1**0401/0404 confers a significantly excessive risk of CORA, consistent with findings previously reported in adults with severe RA (16). Furthermore, the frequency of heterozygosity for S2/S3P alleles was higher in our cohort of CORA compared

to that reported for the British cohort of RA reported by Morgan et al. (23.5 % in CORA versus 15.8% in RA were heterozygous for S2/S3P alleles; OR 1.64 (1.07-2.51), $p < 0.02$). Also the genotypic ORs for SEs suggests that heterozygosity for S2/S3P confers a greater risk of CORA compared to RA (OR for S2/S3P versus L/L = 22.3 in CORA compared to 7.4 in RA). The differences between CORA and adult RA may reflect sampling variation from a relatively smaller number of pediatric cases. However, the excess risk conferred by individuals who carry the combination of S2/S3P alleles even beyond that expected based on the presence of two risk alleles suggests that children with HLA-DR β 1 chains containing both KRAA and Q/R-RRAA are especially prone to CORA.

Our study does have some limitations, notably the relatively small number of children who were of African American or Hispanic descent in the cohort. Despite the small sample size, there was an association between CORA and SE in Hispanic American children. There was also a trend towards association between SE and CORA in African American children, likely reflecting the small sample size. It should be noted that the frequency of SE-encoding *HLA-DRB1* alleles among African American children with CORA is comparable to African American adults with early RA reported by Hughes et al (26.2% in CORA versus 25.2% in RA) (17). Our control samples were limited to two sources, (Utah and Ohio), whereas the case samples came from a greater number of institutions, with ~ 70% of cases coming from Utah and Ohio. We do not believe this affected our results, given that the controls were broadly representative of the NHW population of the United States. This is further supported by almost identical frequencies of HLA alleles among controls used in our study and >7000 NHW controls from the NMDP Registry (13). Another limitation was the lack of extensive phenotype information such as erosions or nodules, hence limiting our analyses to association between SE and susceptibility but not severity of disease. Finally, it would have been ideal to perform a meta-analysis using data from published studies. However, the published data spanned several years and lacked appropriate data. Control frequencies were not always available and the SE-encoding alleles included varied in these studies. These factors precluded a meta-analysis.

Important genetic discoveries have resulted from investigations of individuals with an earlier age-of-onset of several complex genetic traits, including inflammatory bowel disease and obesity (18, 19). We believe CORA is also a genetically enriched early-onset cohort that is a promising target for identification of additional genetic factors predisposing to RA. Furthermore, studying children minimizes the impact of environmental influences such as smoking, also known to confer susceptibility to RA (20). Our results suggest that whereas SE-encoding *HLA-DRB1* alleles confer a significant risk for developing CORA, *HLA-DRB1* gene dosage does not appear to be a factor influencing the development of RA in childhood compared to adulthood. However, our results show that compared to adults with RA, children with CORA appear to have a greater frequency of S2/S3P alleles, suggesting that heterozygosity for *HLA-DRB1* alleles encoding SE plays a role in early onset of disease. Investigating the role played by HLA and non-HLA loci, as well as rare variants in larger, well-characterized cohorts of children with CORA will likely yield further insights into the etiopathogenesis of RA.

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immune reactions to autoantigens modified by citrullination. *Arthritis Rheum.* 2006; 54:38–46.
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Table 1

SE-encoding HLA-DRB1 allele frequencies in cases and controls

DRB1 Allele	TDM class	European control Frequency in NMDP N = 7870	NHW Controls N = 373 allele count (allele frequency)	NHW Cases N = 149 allele count (allele frequency)	OR (95%)	P
0101	S3P	0.091	71 (0.095)	44 (0.148)	1.65 (1.08-2.51)	0.014
0102	S3P	0.017	5 (0.007)	2 (0.007)	1 (0.13-5.82)	ns
0401	S2	0.091	64 (0.086)	65 (0.218)	2.97 (2.01-4.40)	<1×10⁻⁷
0404	S3P	0.036	28 (0.038)	27 (0.091)	2.55 (1.43-4.56)	0.0005
0405	S3P	0.004	6 (0.008)	9 (0.030)	3.84 (1.24-12.24)	0.007
0408	S3P	0.002	1 (0.001)	8 (0.027)	20.55 (2.61-440.0)	0.00006
1001	S3P	0.008	5 (0.007)	7 (0.023)	3.56 (1.01-13.02)	0.046
1303	S2	0.010	11 (0.015)	1 (0.003)	0.22 (0.01-1.68)	ns
1402	S3P	0.000	1 (0.001)	0 (0.000)	-	ns
1406	S3P	0.000	0 (0.000)	0 (0.000)	-	-
Total		0.260	192 (0.257)	163 (0.547)	3.48 (2.6-4.66)	<1×10⁻⁷

SE: Shared epitope;

NHW: Non-Hispanic White;

In the system described by Tezenas du Montcel (TDM) et al (4), HLA DRB1 alleles encoding SE were grouped according to amino acids sequence at 70-74 (S2 = QKRAA or DKRAA; S3P=QRRAA or RRRAA). All other alleles were designated as low risk (L) alleles.

NMDP: National Marrow Donor Program

OR = odds ratio; 95% CI: 95% confidence interval.

Table 2

Genotype specific risk estimates of CORA in NHW subjects

Genotype	Number (%) of controls (N=373)	Number (%) of patients (N=149)	Comparison to L/L genotype		Comparison to L/L genotype including gender as covariate	
			OR (95%CI)	P	OR (95%CI)	P
L/L	201 (53.9)	35 (23.5)	1.0 (referent)		1.0 (referent)	
S3P/L	92 (24.7)	42 (28.2)	2.6 (1.6-4.4)	<0.0001	2.9 (1.7-5.0)	<0.0001
S2/L	60 (16.1)	23 (15.4)	2.2 (1.2-4.0)	0.01	2.7 (1.4-5.0)	0.003
S3P/S3P	8 (2.1)	10 (6.7)	7.2 (2.6-19.4)	<0.0001	9.9 (3.2-30.3)	<0.0001
S2/S2	3 (0.8)	4 (2.7)	7.7 (1.6-35.7)	0.01	11.8 (2.1-66.6)	0.005
S2/S3P	9 (2.4)	35 (23.5)	22.3 (9.9-50.5)	<0.0001	26.6 (10.9-65.0)	<0.0001

HLA-DRB1 alleles were grouped according to the Tezenas du Montcel classification system (4). Two susceptibility alleles S2 (KRAA at amino acids 71-74) and S3P (Q/RRRAA at amino acid positions 70-74) were evaluated in comparison with all other alleles which were denoted as low-risk (L). The risk for CORA was calculated for each genotype compared with the L/L group. Results are shown with and without including gender as a covariate.

OR = odds ratio; 95% CI: 95% confidence interval.

Table 3
SE-encoding HLA-DRB1 allele frequencies in African American and Hispanic cases with CORA

DRB1 Allele	TDM class	AA control Frequency in NMDP N = 2405	AA Cases N = 21 (allele frequency)	Hispanic control frequency in NMDP N = 1999	Hispanic Cases N = 25 (allele frequency)
0101	S3P	0.026	0 (0.000)	0.039	5 (0.100)
0102	S3P	0.040	1 (0.024)	0.033	1 (0.020)
0401	S2	0.023	3 (0.071)	0.015	0 (0.000)
0404	S3P	0.007	0 (0.000)	0.055	6 (0.120)
0405	S3P	0.010	4 (0.095)	0.019	3 (0.060)
0408	S3P	0.001	0 (0.000)	0.004	0 (0.000)
1001	S3P	0.019	1 (0.024)	0.015	4 (0.080)
1303	S2	0.037	2 (0.048)	0.015	1 (0.020)
1402	S3P	0.001	0 (0.000)	0.024	1 (0.020)
1406	S3P	0.000	0 (0.000)	0.026	3 (0.030)
Total		0.162	11 (0.262) *	0.243	24 (0.480) **

SE: Shared epitope;

AA: African American

TDM: Tezenas du Montcel et al classification

NMDP: National Marrow Donor Program

* OR=1.84 (0.84-3.82); p=0.08;

** OR=2.88 (1.59-5.21); p <0.0001.

Table 4

Prior investigations of SE in NHW children with CORA.

Authors (Reference)	Year	Controls with SE (%)	Cases with SE		
			N	% with at least 1 SE	% with 2 SE
Clemens et al (7)	1983	27	52	60	NR
Forre et al (8)	1983	27	17	53	NR
Nepom et al (9)	1984	32	22	77	55
Vehe et al (10)	1990	NR	15	80	73
Barron et al (6)	1992	29	13	53	0
Thomson et al (10)	2002	36	37	65	NR
Current study	2011	46	149	76	33

SE: shared epitope

NR: Not reported