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Increased risk of rheumatoid arthritis among mothers with children who carry *DRB1* risk-associated alleles

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Abstract

Objective.—To investigate whether a child's genotype affects a mother's risk of rheumatoid arthritis (RA) beyond the risk associated with her genotype and to test whether exposure to fetal alleles inherited from the father increase risk of RA among mothers without risk alleles.

Methods.—A case-control study was conducted among 1,165 mothers (170 cases/995 controls) and their respective 1,482 children. We tested the association between having any child with alleles encoding amino acids (AA) associated with RA including the "shared epitope" (SE) and DERAA AA sequences at position 70-74; AA valine, lysine, alanine at positions 11, 71, 74 of HLA-DRB1; aspartic acid at position 9 of HLA-B; and phenylalanine at position 9 of DPB1. We used logistic regression models to estimate odds ratios (OR) and 95% confidence intervals (CI) for each group of alleles, adjusting for maternal genotype and number of live births.

Results.—We found increased risk of RA among mothers who had any child with the SE (OR 3.0; 95% CI, 2.0-4.6); DERAA (OR 1.7; 95% CI, 1.1-2.6); or valine (OR 2.3; 95% CI, 1.6-3.5), lysine (OR 2.3; 95% CI, 1.5-3.4) and alanine (OR 2.8; 95% CI, 1.2-6.4) at DRB1 positions 11, 71 and 74 respectively. Among non-carrier mothers, increased risk of RA was associated with having children who carried DERAA (OR 1.7; 95% CI, 1.0-2.7) and alleles encoding lysine at DRB1 position 71 (OR 2.3; 95% CI, 1.5-4.8).

Conclusion.—Findings support the hypothesis that a child's genotype can contribute independently to risk of RA among mothers.

Keywords

Rheumatoid arthritis; epidemiology; autoimmune disease

INTRODUCTION

Rheumatoid arthritis (RA) affects two to three times more women than men [1, 2] for reasons not entirely understood [3]. Various aspects of pregnancy have been investigated due to the observed overall sex dimorphism and findings indicating this time period is relevant to disease risk. Among disease-free women, an increased risk of RA onset takes place during the post-partum period [4, 5]. Further, improvement of symptoms during pregnancy among cases has been described [6]. Paternal antigens from the fetus are hypothesized to influence risk of developing RA. During pregnancy, mothers are exposed to fetal material shed from the placenta as well as fetal cells [7]. Fetal cells may persist in small quantities for up to

decades after delivery [8]. It is possible that fetal antigens could contribute to the development of RA among some women.

The strongest genetic risk factors for RA are variants of the *HLA-DRB1* gene. The "shared epitope" (SE) alleles encoding the QKRRA and QRRRA amino acid sequences at positions 70-74 explain much of the genetic predisposition to RA [9, 10]. At the same positions, the sequence DERAA has been associated with reduced risk of RA [11]. More recently, a large study demonstrated that the association between the major histocompatibility complex (MHC) and RA is best explained by five amino acids; three in *HLA-DRB1* and one each in *HLA-B* and *HLA-DPB1,* of which two are the same as those of the shared epitope [12]. Of the 16 resulting *DRB1* haplotypes, valine/lysine/alanine at positions 11/71/74 is the most strongly associated with RA and it corresponds to the *DRB1**04:01 allele [12], a well-documented association [13]. Clinically, the SE is associated with anti-citrullinated peptide antibody-positive (ACPA) RA and antibody titers [14], disease severity [15] and mortality [16]. The SE alleles have been shown to be more predictive of ACPA presence than of RA [17]. Exposure to SE alleles through non-host genetics during pregnancy is potentially relevant to RA etiology. Two studies found that among SE-negative mothers, SE-positive microchimerism is more frequently found among cases than controls [18, 19]. For this reason, we would expect to find that among SE-negative women, RA cases have more SE-positive children compared to controls. Exposure to DERAA through non-inherited maternal antigens (NIMA) is associated with decreased risk of RA [20]. By analogy, it is possible that having children positive for DERAA reduces risk of RA for mothers. The objective of this study was to investigate whether a child's genotype affects a mother's risk of disease beyond the risk associated with her own genotype, and to test whether exposure to risk alleles of the fetus inherited from the father is associated with risk of RA among mothers who do not carry the risk alleles.

METHODS

Study population.

We conducted a case control study of 1,165 mothers and 1,482 children using data from the University of California San Francisco (UCSF) Mother-Child Immunogenetic Study (MCIS) and research studies conducted at the Inova Translational Medicine Institute (ITMI), Inova Health System, Falls Church, Virginia. White females of European ancestry with at least one living child were eligible to participate. Cases were identified from patients enrolled in genetic studies of autoimmunity at UCSF between 1997 and 2010. All RA cases met the 1987 revised criteria of the American College of Rheumatology (ACR) [21] and had at least one live birth prior to diagnosis. Only children born prior to diagnosis were included in this study. Control mothers had no prior history of autoimmune disease and had at least one live born child. Controls were recruited from various sources including blood donors at the Blood Centers of the Pacific and the Institute for Transfusion Medicine in Pittsburgh, PA and from families who enrolled in studies at the Inova Women's Hospital, Inova Fairfax Medical Center, Falls Church, Virginia. Only participants with genotype data for both mother and at least one child were included in this study. All participants provided written informed consent. The study protocol is in accordance with the Declaration of Helsinki and was

approved by the UCSF and UC Berkeley Institutional Review Boards (IRB). The Western IRB and the Inova Health System IRB approved ITMI studies.

Clinical and questionnaire data.

For cases, we obtained the date of diagnosis and clinical characteristics from medical records. The MCIS collected data from case and control mothers on reproductive history and potential confounders through a self-administered questionnaire. For ITMI control mothers, reproductive history, mother's and child's date of birth were obtained from electronic medical records (EMR). Seropositive RA was defined as rheumatoid factor (RF) and/or cyclic citrullinated peptide (CCP) antibody test-positive. A combination of RF and anti-CCP was used to define seropositive RA due to the lack of available anti-CCP data for some patients; patients recruited at earlier dates primarily had RF status available documenting their diagnosis.

HLA allele imputation.

We used SNP2HLA [22] to impute HLA alleles using post-QA/QC genotype and whole genome sequencing data. In order to minimize confounding by ancestry, we selected participants of European ancestry for inclusion in this study. Using ancestry informative markers for Northern and Southern Europeans [23], we adjusted for ancestry proportions estimated using STRUCTURE (version 2.3.4) [24]. A detailed description of genotyping, QA/QC steps and imputation methods can be found in the supplementary materials.

Statistical analyses.

We classified mothers and children as carriers (yes/no) of RA-associated alleles. We included SE alleles and alleles containing the five amino acids associated with increased risk of RA, as well as alleles containing the amino acid sequence DERAA. Figure 1 includes the alleles in each group as well as the overlap in allele groups. We created a binary variable for each mother to indicate whether she had any children positive for any of the risk-associated alleles *prior to* diagnosis.

We used logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals (CIs) to investigate three questions. First, we tested the independent association between allele groups and RA among mothers in a multivariable model including all the risk allele groups, without inclusion of children's genotype. Second, we tested the association between mother's RA case status and having any children who carried one or two alleles from each allele group. We modeled exposure to a child's genotype using two approaches. One model considered the additional risk associated with having any allele-positive children by including maternal carrier status of the same allele group. The third model specifically addressed whether the allele inherited from the father was associated with increased risk of RA among allele-negative mothers. We used directed acyclic graphs to identify our sufficient adjustment set of variables that met the definition of a confounding variable. Maternal genetic ancestry was considered in all models but it was not included in final models since it did not affect our estimates. The number of live births and maternal carrier status of risk alleles was included in models for the second and third questions.

We tested the robustness of our estimates through a number of sensitivity analyses. In models testing the independent effect of a child's genotype on maternal risk of RA, we adjusted for maternal genotype by including a categorical variable for *DRB1* haplotype carrier status instead of individual amino acid positions to evaluate if the method of defining maternal genotype impacted model estimates. The *DRB1* haplotype is based on amino acids at positions 11, 71 and 74 as defined in previous studies [12, 25]. Haplotype frequencies can be found in Supplementary Table 2. Since our exposure of interest pertained to children born prior to diagnosis, we created a variable for the number of live births prior to RA diagnosis and compared the effect on our estimates for models 2 and 3. We repeated our analyses in the seropositive subgroup and same number of controls. Analyses were conducted using Stata 13 (StataCorp, College Station, Texas) and R [26].

RESULTS

Cases and controls differed in age at study enrollment and number of live births. (Table 1). The average number of live births before diagnosis in cases was similar to the average number of births among controls (2.0±0.9 vs. 1.9±1.0, p=0.09) and slightly higher at the time of interview. Clinical characteristics for cases are presented in Table 1. The average age at diagnosis was 40 years of age and 69% were seropositive, per medical records. Approximately one-third had evidence of rheumatoid nodules and 55% had evidence of radiographic changes. Frequencies of additional ACR criteria in cases are in Supplementary Table 1.

Maternal risk alleles and maternal RA

We evaluated the association between known risk alleles and case control status for mothers (Table 2). As expected, RA cases were more likely to have 1 or 2 SE alleles compared to controls, 82% and 39% respectively. Associations with *DRB1* risk allele groups were in the expected direction. The SE alleles and lysine encoding alleles at position 71 were independently associated with risk of RA. Results were very similar when seropositive cases were compared to controls (data not shown).

Children with risk alleles and maternal RA

Next, we evaluated whether having at least one child positive for any of the risk alleles of each classification (SE, DERAA, five amino acids) was associated with risk of RA in the mother (Table 3). Final models were adjusted for number of live births and maternal genotype (SE, DERAA, five amino acids). We found a three-fold increased risk of RA for mothers who had any child positive for the SE, independent of maternal genotype (OR 3.0; 95% CI, 2.0-4.6). A two-fold increase in risk for maternal RA was also present for other *HLA-DRB1* alleles encoding valine at amino acid position 11, lysine at position 71 and alanine at position 74. Having a child with alleles encoding the reduced risk sequence DERAA was associated with increased risk of RA (OR 1.7; 95% CI, 1.1-2.6). Although among mothers, carrying alleles coding for phenylalanine at position 9 of *DPB1* was not associated with RA, having children with one or more alleles was associated with a four-fold increase in risk of maternal RA (OR 4.0; 95% CI, 1.2-13.4). The effect size for *DPB1* alleles was stronger in the seropositive group (OR 8.5; 95% CI, 1.1-63.5) than among seronegative

cases (OR 1.9; 95% CI, 0.4-8.2). No other striking differences were found by serostatus (data not shown). Estimates adjusted for maternal genotype defined by presence or absence of the highest risk amino acid at each of the 5 positions (Table 3) did not differ from estimates adjusted for *DRB1* haplotype and *HLA-B* and *DPB1* amino acids (Supplementary Table 3).

We repeated our analyses among mothers who did not carry any of the alleles for each of the groups investigated (Table 4). The association between having children who carried DERAA alleles and those for amino acid position 71 and RA was also observed in allele-negative mothers. However, association between children who carried SE alleles or from the other allele groups and RA was not observed. The association between children who carried 1 or 2 SE alleles among SE-negative mothers and RA was attenuated to OR 1.7 from the three-fold increase in the previous model that included all mothers (Table 3). Among allele-positive mothers, positive associations were present between having children positive for the SE; AA 11, 71, 74 of *DRB1*; and AA position 9 of *DPB1* and maternal RA. The association between having children with 1 or 2 DERAA alleles and RA was not statistically significant among DERAA-positive mothers (Table 5).

At the individual allele level, *DRB1**04:01* is strongly associated with risk of RA. *DRB1**04:01* encodes valine, lysine and alanine at positions 11, 71 and 74, respectively and it is the amino acid combination most strongly associated with risk of RA [12]. In our study, the association between **04:01* and RA among mothers was OR 2.9 (95% CI, 1.5-5.6), adjusting for the shared epitope, DERAA and 5 amino acids. Having any **04:01* allele-positive children and adjusting for number of live births and mother's genotype resulted in an OR of 2.3 (95% CI, 1.5-3.5). Among allele-negative mothers, the association between **04:01* allele-positive children and RA was similar (OR 2.2; 95% CI, 1.1-4.3).

DISCUSSION

To our knowledge, this is the first report to investigate the association between a child's genotype and maternal RA. We found increased risk of RA among women with children who carried one or two alleles encoding amino acids or amino acid sequences of DRB1 and DPB1 molecules associated with RA, after adjusting for maternal genotype and number of live births. Thus, a child's genotype is independently associated with maternal RA possibly through exposure to risk-associated HLA alleles. The additive effects of a child's genotype may in part explain why women are more likely to develop RA compared to men. Female cases are less likely to carry SE alleles compared to male cases [27, 28], possibly implicating non-host genetic factors in RA pathogenesis.

An increase in risk of RA was also associated with having children who carried DERAA-encoding alleles among mothers who did not carry DERAA alleles. Our findings are in contrast to what we might expect given that the sequence is associated with reduced risk of RA [11]. The alleles that encode DERAA, also encode alanine at position 74 of the DRB1 molecule (Figure 1), which is associated with increased risk of RA. It is possible that the association is not due to DERAA but only to alanine at position 74 and other amino acids at different positions. However, our estimates are adjusted for maternal genotype at these other

amino acid positions. Another possible explanation is that the observed association is due to one of the DERAA alleles. We excluded one allele at a time and all estimates were within the 95% confidence interval for the reported DERAA ORs. Therefore, the observed association is not due to a single allele. Increased risk associated with exposure during pregnancy is consistent with a mechanism mediated by DRB1-derived epitopes. T-cell cross-reactivity with the DERAA sequence of microbial as well as of self-origin has been identified in RA patients [29]. Likewise, it is possible that exposure to fetal DERAA during pregnancy affects maternal risk of RA through molecular mimicry. Fetal antigens could contribute to the process of epitope spreading prior to disease onset [30]. More work is needed to understand the biological mechanisms underlying the association between *DRB1* alleles and risk of RA in general.

In our study, the SE alleles were strongly associated with RA among mothers. The observed SE frequency among cases (82%) and controls (39%) was similar to the range (69%−80% and 42%−45%) reported in previous studies [14, 31-33]. The estimate for maternal SE status and RA was attenuated once we took into account having any SE+ children (OR 3.1; 95% CI, 2.0-4.7). These findings support the hypothesis that a child's genotype can independently contribute to risk of maternal RA.

SE+ women are more likely to have children who carry at least one SE allele. This is evident in both cases and controls. Since more RA cases carry SE alleles than controls, RA cases are more likely to have SE+ children than controls (88% vs. 62% respectively). This translates to an increase in exposure through non-host genetics of 6% among RA cases compared to 23% among controls. Results from our logistic regression models suggest that the increase in risk associated with having SE+ children (and other alleles associated with risk) is not entirely due to the difference in maternal genotype since it is accounted for in our models. Our results support previous work that demonstrated a dose effect of SE alleles [14, 31]. RA is a complex disease likely caused by a combination of genetic and environmental factors. Exposure to children's SE alleles, regardless of their origin, could serve as one of many environmental "hits" contributing to RA pathogenesis.

Among mothers who did not carry the alleles, we observed an association between RA and having children who carried DERAA, lysine at position 71 and *DRB1*04:01*. The small number of allele-negative cases for various groups including the SE, led to a lack in precision for some estimates. We found an 11% difference in the frequency of SE+ children that may help explain the excess of SE+ microchimerism among cases compared to controls previously reported [18, 19]. One limitation of our study is that we did not have measures of microchimerism to test whether maternal and/or fetal genotype combinations influence its presence and quantity.

In a previous study of systemic lupus erythematosus (SLE) patients, an increase in risk of maternal SLE associated with children who carried *DRB1*04:01* was observed [34]. *DRB1*04:01* does not have a strong association with SLE but it does share sequence similarities with the Epstein-Barr Virus (EBV) [35]; EBV is a risk factor for SLE [36]. Studies in RA have demonstrated that ACPAs react with EBV viral sequences and may

contribute to disease-associated antibody formation [37]. It is possible that EBV-*DRB1* 04:01* molecular mimicry may trigger or contribute to autoimmunity.

Our mother-child study had many strengths. RA cases and controls were clinically well characterized; comprehensive reproductive histories were obtained for each participant, genetic data were collected and HLA genotypes were derived for classical loci using established computational methods. We performed QA/QC measures that increase confidence in our findings. We took into account potential confounding variables in our analyses. Similar results were obtained when adjusting for the number of live births before diagnosis for cases rather than their total live births reported at the time of interview.

Despite the large overall number of mother-child pairs, we had limited sample sizes for some of the allele groups we tested. We did not correct p-values for multiple comparisons due to the lack of independence between allele groups (Figure 1). Among SE-negative mothers, we had only 30% power to detect an association of the observed magnitude or greater. ITMI controls were younger at the time of study enrollment and had fewer births. However, comparing the number of children born prior to diagnosis, RA cases and controls did not differ and inclusion of any version of number of live births in our models did not affect our results. Potential misclassification due to younger age of controls would bias results towards the null; RA is a relatively rare disease and population rates would be expected to apply to our control group. Results by serostatus are limited by the lack of complete anti-CCP data and future studies are needed to confirm and extend these findings. Our study was conducted among women who have given birth to a child and therefore apply to a subset of cases who became pregnant before RA diagnosis.

In conclusion, exposure to a child's genotype during pregnancy may contribute to risk of RA among mothers. Non-host genetic exposure may be relevant to consider in understanding RA pathology. Functional studies are needed to characterize the biological pathways that can explain our observations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

[1]. Crowson CS, Matteson EL, Myasoedova E, Michet CJ, Ernste FC, Warrington KJ et al. The lifetime risk of adult-onset rheumatoid arthritis and other inflammatory autoimmune rheumatic diseases. Arthritis and rheumatism, 2011;63:633–9. [PubMed: 21360492]

[2]. Cooper GS, Stroehla BC. The epidemiology of autoimmune diseases. Autoimmunity reviews, 2003;2:119–25. [PubMed: 12848952]

[3]. McCombe PA, Greer JM, Mackay IR. Sexual dimorphism in autoimmune disease. Current molecular medicine, 2009;9:1058–79. [PubMed: 19747114]

[4]. Ostensen M, Villiger PM, Forger F. Interaction of pregnancy and autoimmune rheumatic disease. Autoimmunity reviews, 2012;11:A437–46. [PubMed: 22154710]

[5]. Wallenius M, Skomsvoll JF, Irgens LM, Salvesen KA, Koldingsnes W, Mikkelsen K et al. Postpartum onset of rheumatoid arthritis and other chronic arthritides: results from a patient register linked to a medical birth registry. Annals of the rheumatic diseases, 2010;69:332–6. [PubMed: 19717397]

[6]. de Man YA, Dolhain RJ, van de Geijn FE, Willemsen SP, Hazes JM. Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. Arthritis and rheumatism, 2008;59:1241–8. [PubMed: 18759316]

[7]. Clifton VL, Stark MJ, Osei-Kumah A, Hodyl NA. Review: The feto-placental unit, pregnancy pathology and impact on long term maternal health. Placenta, 2012;33 Suppl:S37–41. [PubMed: 22118870]

[8]. Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proc Natl Acad Sci U S A, 1996;93:705–8. [PubMed: 8570620]

[9]. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis and rheumatism, 1987;30:1205–13. [PubMed: 2446635]

[10]. Jawaheer D, Li W, Graham RR, Chen W, Damle A, Xiao X et al. Dissecting the genetic complexity of the association between human leukocyte antigens and rheumatoid arthritis. Am J Hum Genet, 2002;71:585–94. [PubMed: 12181776]

[11]. Feitsma AL, van der Helm-van Mil AH, Huizinga TW, de Vries RR, Toes RE. Protection against rheumatoid arthritis by HLA: nature and nurture. Annals of the rheumatic diseases, 2008;67 Suppl 3:iii61–3. [PubMed: 19022816]

[12]. Raychaudhuri S, Sandor C, Stahl EA, Freudenberg J, Lee HS, Jia X et al. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. Nat Genet, 2012;44:291–6. [PubMed: 22286218]

[13]. Mackie SL, Taylor JC, Martin SG, Wordsworth P, Steer S, Wilson AG et al. A spectrum of susceptibility to rheumatoid arthritis within HLA-DRB1: stratification by autoantibody status in a large UK population. Genes and immunity, 2012;13:120–8. [PubMed: 21881596]

[14]. Huizinga TW, Amos CI, van der Helm-van Mil AH, Chen W, van Gaalen FA, Jawaheer D et al. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. Arthritis and rheumatism, 2005;52:3433–8. [PubMed: 16255021]

[15]. Wagner U, Kaltenhauser S, Pierer M, Seidel W, Troltzsch M, Hantzschel H et al. Prospective analysis of the impact of HLA-DR and -DQ on joint destruction in recent-onset rheumatoid arthritis. Rheumatology (Oxford, England), 2003;42:553–62.

[16]. Farragher TM, Goodson NJ, Naseem H, Silman AJ, Thomson W, Symmons D et al. Association of the HLA-DRB1 gene with premature death, particularly from cardiovascular disease, in patients with rheumatoid arthritis and inflammatory polyarthritis. Arthritis and rheumatism, 2008;58:359–69. [PubMed: 18240242]

[17]. van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Huizinga TW, Toes RE, de Vries RR. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. Arthritis and rheumatism, 2006;54:1117–21. [PubMed: 16572446]

[18]. Rak JM, Maestroni L, Balandraud N, Guis S, Boudinet H, Guzian MC et al. Transfer of the shared epitope through microchimerism in women with rheumatoid arthritis. Arthritis and rheumatism, 2009;60:73–80. [PubMed: 19117368]

[19]. Yan Z, Aydelotte T, Gadi VK, Guthrie KA, Nelson JL. Acquisition of the rheumatoid arthritis HLA shared epitope through microchimerism. Arthritis and rheumatism, 2011;63:640–4. [PubMed: 21360493]

[20]. Feitsma AL, Worthington J, van der Helm-van Mil AH, Plant D, Thomson W, Ursum J et al. Protective effect of noninherited maternal HLA-DR antigens on rheumatoid arthritis development. Proc Natl Acad Sci U S A, 2007;104:19966–70. [PubMed: 18077428]

[21]. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis and rheumatism, 1988;31:315–24. [PubMed: 3358796]

[22]. Jia X, Han B, Onengut-Gumuscu S, Chen WM, Concannon PJ, Rich SS et al. Imputing amino acid polymorphisms in human leukocyte antigens. PLoS One, 2013;8:e64683. [PubMed: 23762245]

[23]. Barcellos LF, May SL, Ramsay PP, Quach HL, Lane JA, Nititham J et al. High-density SNP screening of the major histocompatibility complex in systemic lupus erythematosus demonstrates strong evidence for independent susceptibility regions. PLoS genetics, 2009;5:e1000696. [PubMed: 19851445]

[24]. Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics, 2003;164:1567–87. [PubMed: 12930761]

[25]. Viatte S, Plant D, Han B, Fu B, Yarwood A, Thomson W et al. Association of HLA-DRB1 haplotypes with rheumatoid arthritis severity, mortality, and treatment response. Jama, 2015;313:1645–56. [PubMed: 25919528]

[26]. Team RC. R: A Language and Environment for Statistical Computing. 2015.

[27]. Jawaheer D, Lum RF, Gregersen PK, Criswell LA. Influence of male sex on disease phenotype in familial rheumatoid arthritis. Arthritis and rheumatism, 2006;54:3087–94. [PubMed: 17009227]

[28]. del Rincon I, Battafarano DF, Arroyo RA, Murphy FT, Escalante A. Heterogeneity between men and women in the influence of the HLA-DRB1 shared epitope on the clinical expression of rheumatoid arthritis. Arthritis and rheumatism, 2002;46:1480–8. [PubMed: 12115177]

[29]. van Heemst J, Jansen DT, Polydorides S, Moustakas AK, Bax M, Feitsma AL et al. Crossreactivity to vinculin and microbes provides a molecular basis for HLA-based protection against rheumatoid arthritis. Nature communications, 2015;6:6681.

[30]. van der Woude D, Rantapaa-Dahlqvist S, Ioan-Facsinay A, Onnekink C, Schwarte CM, Verpoort KN et al. Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis. Annals of the rheumatic diseases, 2010;69:1554–61. [PubMed: 20448290]

[31]. Balandraud N, Picard C, Reviron D, Landais C, Toussirot E, Lambert N et al. HLA-DRB1 genotypes and the risk of developing anti citrullinated protein antibody (ACPA) positive rheumatoid arthritis. PLoS One, 2013;8:e64108. [PubMed: 23737967]

[32]. Fries JF, Wolfe F, Apple R, Erlich H, Bugawan T, Holmes T et al. HLA-DRB1 genotype associations in 793 white patients from a rheumatoid arthritis inception cohort: frequency, severity, and treatment bias. Arthritis and rheumatism, 2002;46:2320–9. [PubMed: 12355479]

[33]. Kuller LH, Mackey RH, Walitt BT, Deane KD, Holers VM, Robinson WH et al. Rheumatoid arthritis in the Women's Health Initiative: methods and baseline evaluation. Am J Epidemiol, 2014;179:917–26. [PubMed: 24569640]

[34]. Cruz GI, Shao X, Quach H, Ho KA, Sterba K, Noble JA et al. A Child's HLA-DRB1 genotype increases maternal risk of systemic lupus erythematosus. Journal of autoimmunity, 2016.

[35]. Roudier J, Petersen J, Rhodes GH, Luka J, Carson DA. Susceptibility to rheumatoid arthritis maps to a T-cell epitope shared by the HLA-Dw4 DR beta-1 chain and the Epstein-Barr virus glycoprotein gp110. Proc Natl Acad Sci U S A, 1989;86:5104–8. [PubMed: 2472638]

[36]. Ascherio A, Munger KL. EBV and Autoimmunity. Curr Top Microbiol Immunol, 2015;390:365–85. [PubMed: 26424654]

[37]. Pratesi F, Tommasi C, Anzilotti C, Chimenti D, Migliorini P. Deiminated Epstein-Barr virus nuclear antigen 1 is a target of anti-citrullinated protein antibodies in rheumatoid arthritis. Arthritis and rheumatism, 2006;54:733–41. [PubMed: 16508937]

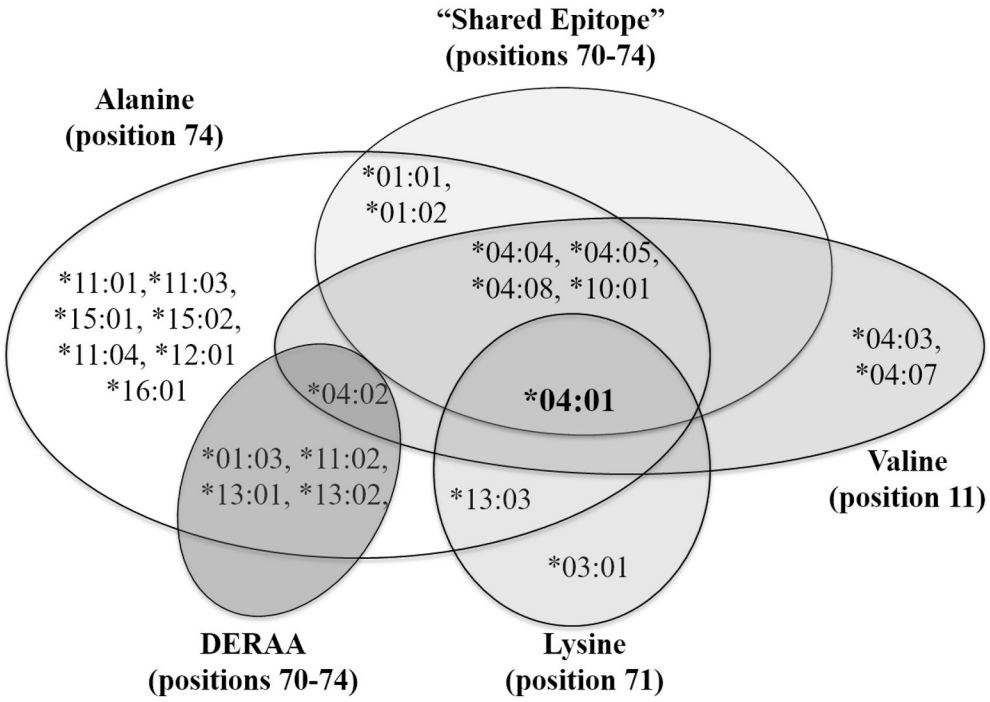

Figure 1.

HLA-DRB1 alleles that encode amino acids associated with increased risk of rheumatoid arthritis at positions 11, 71 and 74 overlap with the alleles with the "Shared Epitope" and the reduced risk amino acid sequence DERAA at positions 70-74.

Table 1.

UCSF Mother-Child Immunogenetic Study (MCIS) participant characteristics

Characteristics	RA Cases Mean ± SD or N (%)	Controls Mean ± SD or N (%)
Mothers, n	170	995
Age at study enrollment	57.0 ± 9.7	38.0 ± 10.5
Number of live births	2.2 ± 1.0	1.9 ± 1.0
Years between 1st childbirth and RA diagnosis	14.4 ± 11.2	--
Age at diagnosis, per medical records	40.1 ± 11.6	--
Seropositive, per medical record[1]	117 (68.8)	--

[1]Cases with a positive cyclic citrullinated peptide (CCP) and/or rheumatoid factor (RF) test.

Table 2.

Multivariable analysis of risk alleles defined by amino acid position and the association with rheumatoid arthritis among mothers

Risk allele group (Mothers)	Alleles	Cases N=170	Controls N=995	RA (Mother) OR (95% CI)[1]	*p*-value
	HLA-DRB1	n (%)	n (%)		
Shared Epitope +	**04:01, *04:04, *04:05, *04:08, *01:01, *10:01, *01:02*	139 (82)	390 (39)	5.0 (2.8-8.9)	<0.001
DERAA +	**04:02, *01:03, *11:02, *13:01, *13:02*	18 (11)	216 (22)	0.7 (0.4-1.2)	0.22
Valine + (AA position 11)	**04:01, *04:08, *04:05, *04:04, *10:01, *04:03, *04:07, *04:02*	107 (63)	281 (28)	1.2 (0.7-2.0)	0.50
Lysine + (AA position 71)	**04:01, *13:03, *03:01*	112 (66)	412 (41)	1.7 (1.1-2.6)	0.03
Alanine + (AA position 74)	**04:01, *04:08, *04:05, *04:04, *10:01, *01:02, *01:01, *16:01, *04:02, *13:03, *15:01, *15:02, *11:01, *11:04, *12:01, *01:03, *11:02, *11:03, *13:01, *13:02*	162 (95)	844 (85)	1.4 (0.6-3.2)	0.46
	HLA-B				
Aspartic acid + (AA position 9)	**08*	48 (28)	227 (23)	1.3 (0.8-2.0)	0.22
	HLA-DPB1				
Phenylalanine + (AA position 9)	**02:01, *02:02, *04:01, *04:02, *05:01, *16:01, *19:01, *23:01*	160 (94)	934 (94)	1.0 (0.5-2.1)	1.0

[1]OR = odds ratio; 95% CI = 95% confidence interval for the association between carrying one or two alleles (+) of each risk allele group compared to none and RA among mothers, mutually adjusted. AA = amino acid.

Table 3.

Case-control association between children carrying 1 or 2 risk alleles and mothers' rheumatoid arthritis status adjusted for mother's carrier status of risk alleles

Risk allele group	Proportion of mothers with children carrying alleles		RA (Mother) OR (95% CI)[1]	*p*-value
	Cases N=170	Controls N=995		
HLA-DRB1	n (%)	n (%)		
Shared Epitope +	133 (78)	391 (39)	3.0 (2.0-4.6)	<0.001
DERAA +	47 (28)	220 (22)	1.7 (1.1-2.6)	0.02
Valine + (AA position 11)	101 (59)	270 (27)	2.3 (1.6-3.5)	<0.001
Lysine + (AA position 71)	115 (68)	389 (39)	2.3 (1.5-3.4)	<0.001
Alanine + (AA position 74)	163 (96)	863 (87)	2.8 (1.2-6.4)	0.01
HLA-B				
Aspartic acid + (AA position 9)	52 (31)	225 (23)	1.3 (0.8-2.0)	0.34
HLA-DPB1				
Phenylalanine + (AA position 9)	164 (98)	932 (94)	4.0 (1.2-13.4)	0.02

[1]OR = odds ratio; 95% CI = 95% confidence interval for the association between having at least one child (born prior to diagnosis for cases) with one or two alleles (+) of each risk allele group compared to none and RA among mothers. Estimates adjusted for mother's carrier status of the *DRB1* "shared epitope", DERAA, AA position 11, 71, and 74, *HLA-B* AA position 9, *HLA-DPB1* AA position 9 and number of live births. AA = amino acid.

Table 4.

Case-control association between children carrying 1 or 2 risk alleles and allele-negative mothers' rheumatoid arthritis status

Risk allele group	*N* Proportion of allele-negative mothers with allele-positive children		RA (Mother) OR (95% CI)[1]	p-value
	Cases	Controls		
HLA-DRB1				
Shared Epitope +	11/31	148/605	1.7 (0.8-3.7)	0.18
DERAA +	35/152	97/779	1.7 (1.0-2.7)	0.03
Valine + (AA position 11)	16/63	114/714	1.7 (0.9-3.3)	0.11
Lysine + (AA position 71)	26/58	127/583	2.7 (1.5-4.8)	0.001
Alanine + (AA position 74)	6/8	100/151	1.4 (0.3-7.4)	0.70
HLA-B				
Aspartic acid + (AA position 9)	17/122	85/768	1.2 (0.7-2.2)	0.58
HLA-DPB1				
Phenylalanine + (AA position 9)	8/10	49/61	0.4 (0.04-3.7)	0.43

[1]OR = odds ratio; 95% CI = 95% confidence interval for the association between having at least one child (born prior to diagnosis for cases) with one or two alleles (+) of each risk allele group compared to none and RA among mothers. Estimates adjusted for number of live births and mother's carrier status at all other risk allele groups. AA = amino acid.

Table 5.

Case-control association between children carrying 1 or 2 risk alleles and allele-positive mothers' rheumatoid arthritis status

Risk allele group	*N* Proportion of allele-positive mothers with allele-positive children		RA (Mother) OR (95% CI)[1]	p-value
	Cases	Controls		
HLA-DRB1				
Shared Epitope +	122/139	242/389	4.2 (2.4-7.3)	<0.001
DERAA +	12/18	123/216	1.7 (0.6-4.9)	0.32
Valine + (AA position 11)	85/107	156/281	3.1 (1.8-5.3)	<0.001
Lysine + (AA position 71)	89/112	262/412	2.0 (1.2-3.4)	0.01
Alanine + (AA position 74)	157/162	763/844	3.3 (1.3-8.7)	0.01
HLA-B				
Aspartic acid + (AA position 9)	35/48	140/227	1.4 (0.7-3.1)	0.35
HLA-DPB1				
Phenylalanine + (AA position 9)	156/157	883/934	10.9 (1.5-81.2)	0.02

[1]OR = odds ratio; 95% CI = 95% confidence interval for the association between having at least one child (born prior to diagnosis for cases) with one or two alleles (+) of each risk allele group compared to none and RA among mothers. Estimates adjusted for number of live births and carrier status at all other risk allele groups. AA = amino acid.