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Fetal-Maternal HLA Relationships and Autoimmune Disease

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Fetal-Maternal HLA Relationships and Autoimmune Disease

By

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Abstract

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University of California, Berkeley

Professor Lisa F. Barcellos, Chair

Autoimmune disease disproportionately affects more women than men. Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are two prototypic female-predominant autoimmune diseases. Despite different clinical manifestations, similarities exist. Both are characterized by a dysregulated immune response to self-tissues. For both diseases, the strongest genetic risk factors are linked to human leukocyte antigen (HLA) alleles. Pregnancy affects their course and may also contribute to risk. This dissertation investigates mother-child HLA relationships and risk of developing RA or SLE.

Chapter 1 provides background on RA and SLE and a literature review outlining the rationale for the dissertation. The chapter states the hypotheses of the project and the specific aims addressed in each chapter.

Chapter 2 addresses the question of mother-child histocompatibility and risk of RA or SLE. The study tests the hypothesis that having any children who are compatible from the perspective of the mother increases the risk of developing RA or SLE. Results show that having any histocompatible children at *HLA-B* and *DPB1* increase risk of RA in the mother. Increased structural similarity as quantified by a sequence similarity matching score at *DPB1* was associated with decreased risk of SLE. Mother-child compatibility at minor histocompatibility antigen ZAPHIR was likewise associated with decreased risk of SLE but compatibility at LB-WNK1 increased risk for mothers who were also HLA-compatible at *HLA-A* with their children. This is the first study investigating the relationship between minor histocompatibility antigen compatibility and risk of SLE. This study supports the hypothesis that mother-child HLA relationships are associated with risk of RA and SLE.

Chapter 3 addresses the question of whether having children who carry HLA alleles associated with increased risk of SLE contributes to its development among mothers. The alleles investigated include *DRB1*03:01, *15:01, *08:01.* In addition, the study tests the hypothesis that molecular mimicry by means of exposure to *DRB1*04:01* that codes for a

similar amino acid sequence to the Epstein-Barr virus, a known SLE risk factor, increases risk among mothers. There was no association between SLE risk-associated alleles. However, increased risk of SLE was associated with having at least one child who carried the *DRB1*04:01* allele among women negative for the allele but with at least one of the predisposing alleles. This study supports the hypothesis that a child's alleles inherited from the father influence a mother's subsequent risk of SLE.

Chapter 4 investigates the association between having children who carry alleles encoding amino acids (AA) associated with RA including the "shared epitope", the sequence DERAA at AA positions 70-74, AA valine, lysine, alanine at positions 11, 71, 74 of the HLA-DRB1 molecule, aspartic acid at position 9 of HLA-B and phenylalanine at position 8 of DPB1. The study finds increased risk of RA among mothers who had any child with SE, DERAA, valine, lysine and alanine encoding alleles, independent of maternal carrier status. Among non-carrier mothers, increased risk of RA was associated with having children who carried DERAA.

Chapter 5 summarizes the key findings and results from each study and the conclusions of this work. Future directions are also addressed.

Dedication

With much gratitude and love I dedicate this dissertation to Dave, Audrey and Owen. Your love and support made it all possible.

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Chapter 1. Introduction

Autoimmune disorders, collectively, affect approximately 15-20 million people in the United States [1-6] and cause significant morbidity, as well as mortality. In general, these conditions are characterized by a humoral and/or cell mediated immune response to self-antigens, which may be *systemic* or *organ* specific. For a large number of autoimmune disorders, women are disproportionately affected. Characteristic features specific to females including hormonal status and events related to pregnancy have been implicated. There is strong evidence for the involvement of genetic and environmental influences in both disease susceptibility and clinical expression; however, the underlying etiology of autoimmunity and causes of gender disparity is unknown.

The physiologic state of pregnancy involves complex fetal-maternal interactions and requires immunological adaptations for its success [7]. For these reasons, better understanding of the effects of exposures associated with this unique experience and time period is especially warranted in diseases with marked sexual dimorphism characterized by aberrant immune responses. The goal of this dissertation is to investigate pregnancy-related exposures, specifically mother-child HLA relationships, and their association with two female predominant autoimmune disorders: rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

This project investigates the potential role of fetal-maternal histocompatibility and other HLA relationships in maternal RA and SLE. Female predominance, onset during childbearing years, and similarities of some autoimmune conditions to graft-versus-host disease (GVHD) have led to the hypothesis that natural processes during pregnancy such as the bi-directional cellular exchange between mother and fetus could be involved in the etiology of some autoimmune conditions. The presence of cells or tissues originating from a different individual is referred to as microchimerism. Studies in scleroderma, rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) have found higher number of fetal cells, or MC, in the maternal circulation years after birth compared to healthy controls [8]. Fetal cells could potentially elicit a graft-versus-host or a host-versus-graft effect triggering autoimmune conditions. However, the overall reduction of risk of RA and SLE associated with parity and the amelioration of RA symptoms during pregnancy, suggest that exposure to fetal DNA or fetal antigens could also be beneficial. The role of microchimerism in disease etiology remains unknown. Human Leukocyte Antigen (HLA) molecules play a key role in immunologic function and are important in immune recognition of self and non-self. Histocompatibility relationships are hypothesized to play a role in the effect and contribute to the persistence of fetal cells.

Background and Literature Review

RA is the most common systemic AD, with a worldwide prevalence approaching 1% [9, 10], affecting an estimated 1.29 million Americans [11]. It is a chronic

inflammatory disease exhibiting clinical heterogeneity and with the potential to cause substantial disability, primarily as a result of the erosive and deforming process in joints experienced by some but not all patients [12, 13]. Two major subtypes are characterized by the presence (seropositive) or absence (seronegative) of autoantibodies, prognostic of disease severity. Rheumatoid factor-positive (RF+) patients experience increased disability, joint damage and mortality compared to RF- patients [14, 15]. Approximately 70% of RA patients are positive for anti-cyclic citrullinated peptide (*anti*-CCP) autoantibodies (ACPA), which are associated with increased joint destruction [16]. Clinical heterogeneity possibly reflects differences in underlying disease etiology [17-19]. RA is associated with increased mortality, particularly among those who develop extra-articular manifestations [20, 21]. Women are more commonly affected than men, 75% of patients are female [22]. Onset usually occurs in middle age [22]. Potential risk factors include subfertility, pregnancy complications, smoking, obesity and recent infections [11, 23].

SLE is a multisystem disease with diverse clinical manifestations. Lupus nephritis (LN) is the most serious SLE complication and becomes clinically evident in up to half of patients [24, 25]. LN is a marker of severe disease, a strong predictor of adverse outcomes, and a leading cause of damage associated with SLE [26-28]. SLE has one of the most pronounced sexual imbalances among autoimmune disorders [29]. The gender difference is most striking in the childbearing years, with a female to male ratio of up to 12:1 [5, 30, 31]. Prevalence estimates range from 14.6 to 78.5 cases per 100,000 persons in the United States [32]. SLE prevalence, incidence, and mortality are highest in non-European populations [33-36]. Although survival has improved over the past five decades, SLE patients are 3-5 times more likely to die compared to the general population [4].

Genetic component and role for HLA in susceptibility to RA and SLE

Associations between various autoimmune disorders with particular Human Leukocyte Antigen (HLA) alleles are well established. The genetic contribution to RA susceptibility is estimated to be 50%-60%, while the contribution of the HLA is estimated to be 11-37% [11, 37, 38]. Several full genome screens of RA cases [38] underscore the importance of the Major Histocompatibility Complex (MHC) for RA susceptibility [39]. The strongest genetic risk factor for developing RA is a set of alleles referred to as the "Shared Epitope" (SE) [40, 41]. The SE alleles encode a specific sequence of amino acids (QRRAA, RRRAA or QKRAA) at positions 70-74 of the third hypervariable region of the DRB1 molecule, encoded by the *HLA-DRB1* gene. The amino acid sequence DERAA, at the same location, has been associated with reduced risk of RA and to less erosive disease [42]. The HLA SE association is mostly found in ACPA+ cases [42] and may not apply to all populations [43, 44]. In addition, there is substantial evidence that supports the existence of other susceptibility genes within the MHC beyond *HLA-DRB1* [45, 46]. Recent work explained most of the MHC association with ACPA+ RA by the presence of five amino acids: three in *HLA-DRB1* and one each in *HLA-B* and *HLA-DPB1* [46].

Similar to RA, the etiology of SLE remains unknown. A substantial genetic contribution to the etiology of SLE is supported by various lines of evidence such as a high sibling risk ratio (λ_S =8-29), familial aggregation, higher concordance in monozygotic twins (24-69%) compared to dizygotic twins (2-5%) and high heritability (66%)[47]. SLE Genome Wide Association Studies (GWAS) have also identified the HLA region as the strongest contributor to genetic risk [47]. A high-density interrogation of the MHC [48] found the strongest associations with *HLA-DRB1*03:01*, **14:01* and **15:01*. The association with *HLA-DRB1*03:01* has been confirmed in a recent meta-analysis [49]. A fine-mapping study [50] investigating the role of amino acid variants in the HLA has identified positions 11, 13 and 26 of *DRB1* as the most significant for risk of SLE. Furthermore, a trans-ancestral mapping study [51] of the MHC in SLE of European and Filipino ancestries identified susceptibility loci at *HLA-DPB1* and *HLA-G*, the latter known for its involvement in fetal-maternal tolerance.

Relationship between pregnancy, RA and SLE

Among women with RA, pregnancy affects disease activity. Improvement or remission of symptoms occurs in about 70% of pregnancies and is typically followed by a "post-partum flare" 3-6 months after parturition [52, 53]. Disease activity has been correlated with serum levels of fetal DNA [54] and fetal-maternal HLA discordance [55-57]. HLA discordance (or incompatibility) refers to the mismatch between the paternal allele carried by the fetus and the non-inherited maternal allele (NIMA) at a given HLA locus. As for risk of developing RA, some studies have reported a *lifetime* reduction in risk of RA associated with parity (compared to nulliparity or lower parity), although results are inconsistent [58]. Guthrie et al. [59] only found reduced risk of RA associated with parity among women younger than 45 (RR=0.52; 95% CI: 0.30-0.90) and among women with more recent births. A transient increase in risk of RA has been reported in the first 12 to 24 months after birth followed by a reduction in risk [60]. A recent study [61] noted increased risk associated with parity among ACPA- cases (OR=2.1; 95% CI: 1.4-3.2) with a stronger association among women who gave birth on the same year as onset of symptoms (OR=2.6; 95% CI: 1.4-4.8). Furthermore, among shared epitope-positive cases, parity reduced risk of RA relative to nulliparous women (OR=0.42; 95% CI: 0.22-0.79) but not among shred epitope-negative cases (OR=0.79; 95% CI: 0.38-1.64) [62]. Increased risk of RA is also associated with complications of pregnancy including preeclampsia [63], extremely low birth weight [64] and vaginal bleeding during pregnancy [65]. The association between parity and risk of RA may depend many factors including age, time since pregnancy, disease subtype and pregnancy characteristics.

In SLE patients, pregnancy is associated with increased disease activity and complications involving both mother and fetus. Flares are more common among pregnant than non-pregnant SLE patients [66] and are the most common complication experienced by LN patients [67]. Most flares occur during the 2nd trimester and postpartum period [66, 68]. SLE patients are at increased risk of preeclampsia, pulmonary hypertension and suffer more adverse pregnancy outcomes including spontaneous miscarriage, intrauterine growth restriction, preterm delivery and stillbirth [67, 69, 70].

Parity is associated with diminished risk of SLE, although the evidence is inconsistent [58, 71, 72]. A Danish population registry-based study found a 26% decrease in risk of SLE among women with one or more births relative to nulliparous women (RR=0.74; 95% CI, 0.64-0.86) [71]. Increasing number of children further reduced risk among women with two compared to one child (RR=0.68; 95% CI, 0.58-0.79) [71]. When time since birth was examined, reduced risk was evident only within 10 years after the most recent birth [71]. Women with a history of idiopathic pregnancy loss have a three-fold risk of developing SLE compared to women with no such history (2 vs. none RR=3.50; 95% CI, 2.38-4.96) [71]. The lack of consistency suggests that the association between parity and risk of developing autoimmune disorders is confounded by a 3rd factor; possibly, a yet-to-be described immunological mechanism.

Pregnancy and microchimerism

During pregnancy, fetal cells enter the maternal circulation and vice versa and the presence of these genetically distinct cells from another individual is called microchimerism [8, 73]. The causes and effects of this naturally occurring exchange are under current investigation, as it is potentially biologically relevant to health and diseases including pregnancy complications, autoimmune disorders and cancer [8]. Fetal cells can be detected in the mother's system as early as 5 weeks of gestation [74] and can persist for decades after pregnancy [75, 76]. Maternal cells in progeny can also persist into adult life [77]. Cells of fetal and maternal origin can engraft and differentiate into various organs in both presence and absence of disease [8]. The exchange of cells during pregnancy can originate from current or previous pregnancies, including spontaneous and induced abortions [78-80] and twins [81], including a "lost twin" [82]. There are also non-pregnancy sources of microchimerism such as transplantation and blood transfusion [83]. Increased levels of microchimerism have been associated with pregnancy complications including preeclampsia [84], fetal growth restriction and pre-term labor [85], and fetal chromosomal abnormalities [86-88].

As each pregnancy presents an opportunity for a new "graft", parity is possibly an important factor. In one study [89], higher parity was not found to be associated with increased prevalence of fetal microchimerism and was inversely related to the presence of maternal microchimerism. The number of detectable fetal cells appears to increase with gestational age and clear soon after parturition in the majority of normal pregnancies [73, 90]. A study [89] that included repeated measure of microchimerism, found intra-individual fluctuation in the presence or absence of fetal DNA not entirely explained by pregnancy. Another study [91] compared the number of fetal DNA copies in SLE patients and controls and found increased number of cells in women with the longest time since their last birth but only among SLE patients. Two other studies [92, 93] found a positive correlation between longer time since the last pregnancy and presence of microchimerism, suggesting that establishment of microchimeric cell lines may take time. A subset of fetal cells transferred to the mother exhibit stem cell-like properties with multi-lineage capacity, as demonstrated by their presence in a wide range of tissues [94]. **It is possible that MC elicits immunological responses analogous to those of a**

foreign graft [8]. Strong evidence suggests that this non-host exposure, in particular, to HLA antigens, is biologically relevant and may play a role in the pathogenesis of autoimmune disorders.

Prevalence of microchimerism in RA and SLE

Presence of microchimerism has been identified in RA [80, 95-99] and SLE [91-93, 95, 100-103] cases. Most studies have found that **RA and SLE patients are more likely to harbor microchimeric cells compared to controls**, with a few exceptions [80, 93, 103, 104]. Presence of microchimerism in RA cases ranges from 18% to 47% [80, 98] and in SLE from 46% to 68% [91, 92] of cases, excluding one study that did not detect any [104]. Higher prevalence of fetal microchimerism has been reported in post-mortem studies of SLE patients where 100% of affected organs of cases harbored cells with a Y chromosome, used as a marker of microchimerism, compared to none in non-affected tissue or controls [101]. Microchimerism has been found in kidneys from lupus nephritis patients twice as often as in healthy controls [102]. In a study of 29 female RA patients, 60% were positive for microchimerism and 18% of sampled rheumatoid nodules also contained HLA-specific sequences of a fetal or maternal source [98]. Similarly, microchimerism was present in about 40% of both synovial tissues and skin fibroblast samples from RA cases [99]. Kekow et al. [95] found no differences in age of onset or markers of disease severity (e.g. *anti*-CCP or *anti*-dsDNA) in either RA or SLE according to presence of microchimerism.

The function of microchimerism in affected tissues is not known but three hypotheses for their role in autoimmunity include both harmful effects (graft-versus-host and host-versus-graft) and beneficial effects such as their involvement in tissue repair [105]. It is possible all three relationships are possible depending on the characteristics of the cell acquired, such as its histocompatibility in relation to the host. Due to the potential relationship between fetal-maternal histocompatibility, microchimerism and autoimmunity, it is necessary to gain better understanding how these factors relate to each other in the hope of understanding the causes of female predominance in many autoimmune conditions.

Fetal-maternal HLA histocompatibility and autoimmunity

One of the factors that determines acceptance of a foreign graft in the transplantation setting is the histocompatibility, or HLA matching, between the donor and the recipient. Minor histocompatibility antigen matching has also been identified as an important factor. By analogy, it is possible that MC persistence might depend on similar factors to those that determine transplantation success. In the context of pregnancy, fetal-maternal histocompatibility can be defined from the perspective of the mother or the fetus. For example, compatibility from the mother's perspective occurs when the non-maternal allele in the offspring is not different from either of the mother's. Both beneficial and harmful effects of MC have been proposed [8]. **An emerging hypothesis**

is that the effect of MC might depend on the histocompatibility with the host. For example, HLA disparity between mother and fetus has been associated with improvement of symptoms during the course of pregnancy in RA [55-57] and inflammatory bowel disease [106]. HLA incompatibility might also be associated with delayed onset of RA post-pregnancy [57]. In a study of 57 pregnancies of 41 women with RA, improvement of symptoms was associated with fetal-maternal disparity from the perspective of the mother in both HLA-DR and HLA-DQ antigens (OR=9.7; 95% CI: 2.2-43.9); 76% of pregnancies discordant for both remitted or improved compared to 25% of those that did not [55]. Relatively few studies have considered fetal-maternal histocompatibility in relation to risk of a small number of autoimmune conditions [107-112]. Findings on the effect of fetal-maternal histocompatibility and autoimmune disorders have been inconclusive. Studies in scleroderma [107, 108] have reported that increased HLA Class II compatibility is more frequent in cases compared to controls. Study design factors cannot be ruled out as a cause of inconsistency of results. Well-characterized studies of fetal-maternal relationships at more HLA loci are needed to better understand how these relationships influence disease risk.

The overall hypothesis of this project is that the nature of the fetal-maternal histocompatibility relationship determines the effect of exposure to fetal cells on risk of disease. Specifically, the following hypotheses will be tested:

1. Fetal-maternal HLA relationships of RA and SLE cases are more likely to have attributes similar to those that are predictive of a successful foreign graft compared to controls.

2. Mothers who are exposed to pregnancies with children carrying known HLA disease-risk alleles are at increased risk of RA or SLE, independent of their own carrier status.

Chapter 2 investigates the association between risk of RA or SLE and exposure to children who are compatible at classical HLA loci (*A, B, C, DPB1, DQA1* and *DRB1)* and at single nucleotide polymorphisms for HLA-restricted minor histocompatibility antigens (SLC19A1, LB-WNK1, HA-3, ZAPHIR, HEATR1 and C19orf48). The study also investigates whether degree of similarity in amino acid sequence at classical HLA loci is associated with risk of RA or. Chapter 3 addresses the question of whether having children who carry *DRB1*03:01, *15:01, *08:01* contribute to the development of SLE among mothers. In addition, the study tests the hypothesis that molecular mimicry by means of exposure to *DRB1*04:01* that shares amino acid sequence similarities to the Epstein-Barr virus increases risk of SLE among mothers. Chapter 4 investigates the relationship between having children who carry alleles encoding amino acids (AA)

associated with RA including the "shared epitope", the sequence DERAA at AA positions 70-74, AA valine, lysine, alanine at positions 11, 71, 74 of the HLA-DRB1 molecule, aspartic acid at position 9 of HLA-B and phenylalanine at position 8 of DPB1. Chapter 5 takes the findings from the studies and discusses their significance in context of the overarching role of fetal-maternal HLA relationships in female-predominant autoimmune disorders and future directions for research.

Chapter 2. Mother-child histocompatibility and risk of rheumatoid arthritis and systemic lupus erythematosus among mothers

Abstract

Objective. The objective of this study is to test the hypothesis that having histocompatible children increases the risk of RA and SLE, possibly by contributing to the persistence of fetal cells acquired during pregnancy.

Methods. We conducted a case control study using data from the UC San Francisco Mother Child Immunogenetic Study and studies at the Inova Translational Medicine Institute. We imputed HLA alleles and minor histocompatibility antigens (mHags). We created a variable of exposure to histocompatible children. We estimated an average sequence similarity matching (SSM) score for each mother based on discordant mother-child alleles. We used logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals.

Results. A total of 161 RA, 156 SLE and 973 control mothers were analyzed. Increased risk of RA was associated with having any child compatible at *HLA-B* (OR 1.8; 1.2-2.9) and *DPB1* (OR 1.5; 1.1-2.2). Reduced risk of SLE was associated with SSM quartile (most compatible vs. least) at *DPB1* (OR 0.6; 0.3-1.0). Compatibility at ZAPHIR was associated with reduced risk of SLE among mothers carrying the HLA-restriction allele *B*07:02* (n =262; OR 0.4; 0.2-0.8).

Conclusions. Our findings support the hypothesis that mother-child histocompatibility is associated with risk of RA and SLE.

Introduction

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are two prototypic autoimmune conditions. Despite differing clinical manifestations, a common feature is the production of B- and T-cell responses directed against self-antigens. Both diseases have strong associations with human leukocyte antigen (HLA) alleles [39] and predominately affect women of reproductive age [22, 113]. In RA, the female to male ratio is 3:1 and 9:1 in SLE [22]. The gender disparity has prompted investigation into various female-specific exposures as potential etiologic factors including pregnancy-related factors [113].

The effect of pregnancy on RA and SLE activity is well documented and supports the hypothesis that immunologic changes during pregnancy are implicated in RA and SLE disease processes. The improvement of RA symptoms with pregnancy has been noted for decades [114]. A recent prospective study on RA disease activity during pregnancy reported that symptoms improved among 48% of patients and 39% experienced a flare post-partum [115]. In SLE, relative to non-pregnant patients, pregnant patients have higher disease activity scores [68] and experience more flares [66]. Risk of RA increases during the first year or two after birth [61, 116, 117]. In contrast to RA, a recent birth is associated with a reduction in risk of developing SLE lasting up to 10 years [71]. Better understanding of the underlying mechanisms responsible for these observations could lead to improved treatment or possibly prevention among susceptible individuals.

One factor that has been investigated is the role of microchimerism as a contributor to female autoimmunity [8]. Fetal microchimerism refers to the presence of a small subset of fetal cells commonly acquired by the mother during pregnancy. Presence of persistent fetal microchimerism is more common in RA and SLE patients compared to controls [95]. The function and relevance of fetal microchimerism is yet unknown and it may have beneficial as well as harmful effects [8, 105]. One hypothesis is that the effect of microchimerism may be mediated by HLA relationships between mother and fetus [118]. HLA disparity has been associated with improvement of RA [55-57] and inflammatory bowel disease [106] symptoms during the course of pregnancy and with delayed onset of RA post-pregnancy [57]. Given this limited but suggestive evidence, a more comprehensive investigation into the association between mother-child histocompatibility and risk of female-predominant autoimmune diseases is warranted.

Interest in microchimerism and autoimmunity was raised by the similarity between graft-versus-host disease (GVHD) after blood transfusion and bone marrow transplantation and clinical manifestations of scleroderma [119, 120]. HLA matching is a major determinant of GVHD [121] as is matching on minor histocompatibility antigens (mHags) among HLA-matched donor-recipients [122]. Minor histocompatibility antigens are polymorphic peptides encoded outside of the HLA that are presented in an HLA-restricted manner [123]. Unlike the HLA, little is known about whether mHags also contribute to autoimmunity.

Together, these observations and various lines of evidence led us to hypothesize that exposure to compatible offspring during pregnancy is associated with increased risk

of RA and SLE. The objective of this study is to investigate the association between RA and SLE and mother-child histocompatibility at classical HLA loci and HLA-restricted minor histocompatibility antigens in a cohort of mother-child pairs.

Patients and methods

We conducted a case control study of 3,746 individuals including 1,614 mothers, 2,132 children. Data were collected as part of 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 female RA and SLE cases with at least one child born prior to diagnosis enrolled in genetic studies of autoimmunity at UCSF were invited to participate. Cases were ascertained by the most current American College of Rheumatology (ACR) criteria available at the time of enrollment. RA cases met the 1987 revised ACR criteria [124] and SLE cases met the 1997 revised ACR criteria [125, 126]. Control mothers had no prior history of autoimmune disease and had at least one 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. In addition, children and the children's fathers (spouses) when possible were invited to participate. 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 and mother's history of RA and SLE were obtained from electronic medical records (EMR).

Genotyping. Cases were genotyped for a panel of 2,360 SNPs in the MHC region using the combined MHC Exon-Centric and Mapping panels from Illumina. Full genome profiling of MCIS controls and children was conducted using the Illumina 660K SNP array or the Illumina ImmunoChip. QA/QC criteria were applied to all genotype data resulting in the exclusion of SNPs that were genotyped in <80% of samples and/or had a minor allele frequency (MAF) <1%. Whole genome sequencing (WGS) data was available for ITMI control families. We excluded SNPs that were genotyped in <90% of samples and/or had an MAF <1%. ITMI WGS methods have been previously described [127]. We verified familial relationships.

HLA allele imputation. We used SNP2HLA [128] to impute HLA alleles using post-QA/QC genotype and whole genome sequencing data. After imputation, we excluded variants with a low measure of imputation accuracy (r^2 <0.3). As an additional QC

measure, we compared imputation results at the allelic level to typed two-field *DRB1* data available for a subset of MCIS participants (n=2,136) using the method described by Raychaudhuri et al. [46]. The imputation procedure correctly called 93.1% of alleles. Using a chi-square test and a significance threshold of α=0.05, we compared allele frequencies by platform among controls. We did not find statistically significant differences by platform for the *DRB1* alleles investigated (data not shown). The process of imputation and QA/QC steps resulted in different sample sizes available at each locus at the two-field resolution. We took a complete case approach in order to facilitate the interpretation of results. To maximize sample size as well as HLA data, we report on 6 loci (*HLA-A, B, C, DPB1, DQA1, DQB1,* and *DRB1*). We compared histocompatibility variables between included and excluded participants using Fisher's exact test. We found that more RA cases had at least one histocompatible child at *DQA1* (p=0.01) among cases with complete data compared to those without. We did not find any other statistically significant differences histocompatibility variable frequencies between the two groups at any HLA locus among cases or controls.

Minor histocompatibility antigens. We identified single nucleotide polymorphisms (SNPs) correlated with minor histocompatibility antigens (mHags) from a previously published report [129]. We obtained ImmunoChip data for MCIS SLE cases available from other studies of autoimmunity at UCSF. ImmunoChip genotype data were available for SLE cases and their children for mHags SLC19A1 (rs1051266), LB-WNK1 (rs12828016), and ZAPHIR (rs2074071). In order to investigate as many mHags as possible, we imputed mHag SNPs using IMPUTE2 and the 1000 genomes (Phase 3) reference panel [130]. After QA/QC, we had three additional SNPs for mothers and at least one child born prior to diagnosis: HA-3 (rs2061821), HEATR1 (rs2275687), C19orf48 (rs3745526). Imputation info score for the three SNPs was $\geq$ 0.96. We compared the results of our imputed rs2061821 with genotyped data from Illumina's 550K platform available for a subset of SLE cases and found 100% concordance. For ITMI controls, whole genome sequencing data were available for all 6 SNPs.

Classification of mother-child histocompatibility profiles. All children were classified into one of two histocompatibility categories at each classical HLA locus (*HLA-A, B, C, DPB1, DQA1, DQB1,* and *DRB1*) and SNPs for mHags SLC19A1, LB-WNK1, ZAPHIR, HA-3, HEATR1 and C19orf48. For case mothers, only children born before diagnosis were included. A child was classified as histocompatible from the mother's perspective if his or her paternally inherited allele was indistinguishable from either of the mother's alleles. Based on this classification, a binary variable for having any histocompatible children was generated for each mother at each HLA locus and mHag SNP.

Histocompatibility defined by sequence similarity matching (SSM) score. In hematopoietic stem cell transplantation (HSCT) an exact match may be difficult to find. The risk of GVHD increases with the number of mismatched alleles [131]. In the case of a mismatch, it may be possible to reduce the risk of GVHD by estimating the allogeneic potential of the mismatch by selecting the least structurally different allele [132].

HistoCheck is a bioinformatics web tool that estimates a sequence similarity matching score (SSM) based on amino acid sequence similarity [132]. This quantitative approach can be informative in estimating degree of similarity between the non-inherited maternal allele and the paternal allele carried by the child. We estimated an SSM score for mother-child pairs who were not HLA-identical at each locus. Where mother and child did not differ at either allele, we assigned a score of zero. For each mother, we created an average SSM score at individual HLA loci. We categorized average SSM score into four groups according to values in the control group. Smaller values indicate increased similarity between mothers and children and are intended to represent greater degree of histocompatibility.

Statistical analysis

Analyses for questions pertaining to mother-child histocompatibility at classical HLA loci were conducted in a sample of 161 RA, 156 SLE and 973 control mothers and their respective children. We included mothers who had complete data at 6 HLA loci (*A, B, C, DPB1, DQA1, DRB1*). A total of 206 SLE cases and 965 controls and 1,387 children were available for mHag-related analyses. Only cases with at least one child with data born prior to diagnosis were included. We used logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between mother's RA and SLE status and any histocompatibility at each HLA locus or mHag SNP and quartiles of average SSM score. We analyzed mHag compatibility in an HLA-restricted manner, meaning that only mothers who carried the HLA allele associated with the mHag were included. In addition, we tested whether case mothers were more likely to have any HLA-compatible/mHag compatible children compared to controls using Fisher's exact test.

We used directed acyclic graphs (DAGs) to identify potential confounders and proceeded to include number of live births in our analyses. To correct for multiple testing, we applied a Bonferroni correction to classical HLA analyses [133]. We tested for the presence of a linear trend in our analyses of SSM score quartiles at each locus and autoimmune disease. Statistical analyses were conducted using Stata 13 (StataCorp, College Station, Texas) and R [134].

Results

RA and SLE cases were older at the time of interview or EMR extraction compared to controls (Table 1). On average, RA cases were diagnosed at age 40 and SLE cases at age 42. Cases had a greater number of live births compared to the average number among controls.

Classical HLA mother-child compatibility

Over the 6 individual HLA loci tested in RA and SLE separately, *HLA-B* and *DPB1* were associated with increased risk of RA when mothers had one or more children who was histocompatible from the mother's perspective (Table 2). The association between *HLA-B* and RA was OR 1.8; 95% CI, 1.2-2.9 and between *DPB1* and RA OR 1.5; 95% CI, 1.1-2.2. Among SLE cases, there was no evidence of an association between

having any histocompatible children at any of the classical HLA loci tested and risk of disease (Table 3). A minority of cases and controls had children who were histocompatible at more than 3 out of 6 loci (13% of RA cases, 12% of SLE and 6% of controls). Compatibility at all 6 loci was rare regardless of disease status (1% to 3%).

Results for our approach using SSM score to categorize mother-child compatibility at each locus are found in Tables 4 and 5. The SSM score range varied across HLA loci. Supplementary Table 1 describes the range, mean and median at each locus by disease status. *DQA1* had the smallest range in score and *HLA-A* and *B* had the greatest. The difference by disease status was minimal. However, the multivariable model results (Table 5) show that relative to the highest quartile representing the least average similarity, women in the lowest quartile at *DPB1* were less likely to have been diagnosed with SLE (OR 0.6; 95% CI, 0.3-1.0, *p* for linear trend= 0.01). We did not find any significant association between SSM score quartiles and RA (Table 4). SSM score was correlated with our allele-based definition of histocompatibility where average scores were significantly lower in the histocompatible group, as expected (data not shown).

Minor histocompatibility antigen mother-child compatibility

Table 6 presents our results for the association between mHag compatibility versus incompatibility and SLE among mothers. The number of mothers who carried the HLA restriction allele for each mHag determined the sample size for each model. We found reduced risk of SLE associated with having one or more children who were compatible for ZAPHIR, restricted for *HLA-B*07:02*, regardless of mother-child histocompatibility status; OR 0.4; 95% CI, 0.2-0.8. Taking HLA compatibility into account, SLE cases were less likely to have HLA-compatible/mHag compatible children (yes/no) compared to controls (0% vs. 9% respectively, Fisher's exact *p*-value = 0.05). However, HLA-compatibility combined with mHag compatibility at LB-WNK1 was more likely among cases than control mothers (23% vs. 13% respectively, Fisher's exact *p*-value = 0.02). We did not find any other association between compatibility and SLE at other mHags investigated.

Discussion

Our study addressed the hypothesis that mother-child histocompatibility increases risk of RA and SLE possibly by facilitating the persistence of fetal cells. We found that RA cases were more likely to have a child histocompatible at *HLA-B* and at *DPB1* compared to controls. Once we corrected for multiple comparisons, the association between RA and *DPB1* was borderline statistically significant. However, *DPB1* was also associated with SLE in the analysis using SSM score quartiles, providing evidence that histocompatibility at this locus may be relevant. SLE cases were *less* likely to be in the lowest quartile of SSM, representing increased similarity relative to the top quartile with the least similarity (OR 0.6; 95% CI, 0.3-1.0). Our findings in regards to histocompatibility are consistent in the context of the contrasting pattern of the effect of pregnancy on RA and SLE [60]. The associations between RA and SLE at *DPB1* do not

convincingly support the hypothesis that compatibility is likely to function as a common mechanism in autoimmunity such as by facilitating the persistence of fetal cells.

HLA Class II incompatibility has been reported to be associated with remission or improvement of RA symptoms during pregnancy and possibly with risk of developing RA in the post-partum period [55, 57]. One study found increased risk of RA in the first year after birth was associated with incompatibility at *DQA1* (OR 3.86; 95% CI, 1.03-14.52) and *DQB1* (OR 4.23, 95% CI, 1.12-15.9) [57]. However, the study was based on a small sample of 16 women who developed RA during this period. Compatibility at HLA Class II has been associated with increased risk of scleroderma [107]. Among controls, the frequency of compatibility in our study at *DRB1* (17%) and *DQA1* (35%) was similar to the one reported in the scleroderma study (16% and 34% respectively) [107]. Estimates for cases differ according to disease, possibly due to relative differences in pathophysiology. Our findings are in agreement with a previous study that failed to find evidence of an association between mother-child histocompatibility at *DRB1* and SLE [110].

Compatibility at the minor histocompatibility antigen ZAPHIR was associated with decreased risk of SLE among mothers who carried the presenting HLA allele *B*07:02* (OR 0.4; 95% CI, 0.2-0.8). Furthermore, we found a similar association once we restricted our analysis to the group of mothers and children who were both histocompatible at *HLA-B* and ZAPHIR (p=0.05). Among this doubly compatible group, compatibility at *HLA-A* and LB-WNK1 was more common among SLE cases compared to controls (p=0.02). Minor histocompatibility antigen disparity is immunogenic as evidence from their involvement in GVHD among HLA-matched transplant recipients and in the potentially curative graft-versus-leukemia effect (GVL) [135]. The use of mismatched mHags as a way to trigger GVL is evidence that mHags can be manipulated for therapeutic applications [136]. As more mHags are discovered, their relevance and function is likely to depend on the context of the interaction.

To our knowledge, this is the first report to investigate the association between mother-child minor histocompatibility antigen compatibility and SLE. CD8+ T-cells specific for minor histocompatibility antigens of fetal origin have been found in mothers up to 22 years after delivery [137] as have minor histocompatibility antigen-specific regulatory T-cells (T-regs) [138]. Exposure to fetal minor histocompatibility antigens is likely to come from microchimerism or from particles that are shed from the placenta and enter the maternal circulation [139]. Minor histocompatibility antigens are expressed in placental material [140]. Placental vesicles of various sizes carry fetal nucleic aids, proteins and lipids and are hypothesized to have immunomodulatory effects [141, 142]. It is possible antigens from fetal sources mediate the effect of pregnancy on RA and SLE. The shedding of placental material could overload the dysfunctional clearance mechanism in SLE patients, leading to exacerbation of symptoms [143]. Functional studies of maternal immune cell responses to fetal minor histocompatibility antigens are needed to understand their biological effect in the context of autoimmunity.

To our knowledge, this is the most comprehensive report published on mother-child histocompatibility and risk of RA and SLE. We conducted a case-control study in a well-characterized family-based study of autoimmunity. The MCIS is a unique resource

with genotype data for case and control families, detailed questionnaire and medical record abstracted clinical data. Our exposure definition of histocompatibility at both classical HLA loci and minor histocompatibility antigens was based on biology.

Our study also has limitations. Inclusion of controls from pregnancy cohorts at ITMI resulted in a significant difference in the age at interview or EMR data extraction. It is possible that the younger age of controls could bias our results by including future cases. RA and SLE are both relatively rare in the general population and we expect similar rates apply to this cohort. Moreover, inclusion of future cases is likely to bias our results towards the null. Differences in data collection (self-administered questionnaire vs. EMR) could result in information bias. For our study, the only variable included in analyses was the number of live births. Studies have shown a high level of concordance in the number of live births reported by self-report and medical records (kappa=1.0) [144, 145], therefore minimizing concern that the method of collection contributed to the results. The lower number of live births among controls compared to cases could reduce the probability of exposure. However, all models are adjusted for the number of live births. The probability that our results could be due to selection bias introduced by our complete case analysis approach is low. We did not find differences in the proportion of exposure to histocompatible offspring at *HLA-B* nor at *HLA-DPB1* by case control status and inclusion status.

In conclusion, we found evidence for mother-child HLA-mediated effects in RA and SLE. A comprehensive evaluation of immunogenic peptides, such as minor histocompatibility antigens, are needed as they may help explain pregnancy-related effects and may be promising therapeutic targets.

TABLES AND FIGURES

Table 1. Characteristics of mothers by rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and control status

Characteristics	RA	SLE	Controls
N[1]	161	156	973
Age at interview, mean ± SD	56.7 ± 9.7	55.9 ± 10.9	37.5 ± 10.2
Number of live births, mean ± SD	2.2 ± 1.0	2.5 ± 1.3	1.9 ± 0.9
Age at diagnosis, mean ± SD	39.6 ± 12.2	42.3 ± 10.7	--

[1]Participants included in HLA-compatibility analyses.

Table 2. Association between one or more children histocompatible from the mother's perspective among rheumatoid arthritis (RA) cases compared to controls[1]

Any histocompatible children	RA n (%)	Controls n (%)	OR (95% CI)[2]	Bonferroni corrected p-value
HLA Class I				
A				
None	125 (77)	727 (75)	Reference	
1 or more	38 (23)	247 (25)	0.9 (0.6-1.3)	1.0
B				
None	133 (82)	871 (89)	Reference	
1 or more	30 (18)	103 (11)	1.8 (1.2-2.9)	0.05
C				
None	125 (77)	800 (82)	Reference	
1 or more	38 (23)	174 (18)	1.3 (0.9-2.0)	0.90
HLA Class II				
DPB1				
None	81 (50)	593 (61)	Reference	
1 or more	82 (50)	381 (39)	1.5 (1.1-2.2)	0.06
DQA1				
None	96 (59)	631 (65)	Reference	
1 or more	67 (41)	343 (35)	1.2 (0.9-1.7)	1.0
DRB1				
None	128 (79)	810 (83)	Reference	
1 or more	35 (21)	164 (17)	1.2 (0.8-1.8)	1.0

[1]Analyses are for each HLA locus and are adjusted for number of live births. [2]OR= odds ratio, 95% CI = confidence interval.

Table 3. Association between one or more children histocompatible from the mother's perspective among systemic lupus erythematosus (SLE) cases compared to controls[1]

Any histocompatible children	SLE n (%)	Controls n (%)	OR (95% CI)[2]	Bonferroni corrected *p*-value
HLA Class I				
A				
None	108 (69)	727 (75)	Reference	
1 or more	48 (31)	247 (25)	1.3 (0.9-1.9)	1.0
B				
None	134 (86)	871 (89)	Reference	
1 or more	22 (14)	103 (11)	1.3 (0.8-2.1)	1.0
C				
None	121 (78)	800 (82)	Reference	
1 or more	35 (22)	174 (18)	1.2 (0.8-1.9)	1.0
HLA Class II				
DPB1				
None	91 (58)	593 (61)	Reference	
1 or more	65 (42)	381 (39)	1.1 (0.8-1.6)	1.0
DQA1				
None	94 (60)	631 (65)	Reference	
1 or more	62 (40)	343 (35)	1.1 (0.8-1.6)	1.0
DRB1				
None	120 (77)	810 (83)	Reference	
1 or more	36 (23)	164 (17)	1.3 (0.9-2.0)	1.0

[1]Analyses are for each HLA locus and are adjusted for number of live births. [2]OR= odds ratio, 95% CI = confidence interval.

Table 4. Mean sequence similarity matching (SSM) score and risk of rheumatoid arthritis (RA) among mothers

Histocompatibility per SSM Score (Quartile)	RA n (%)	Controls n (%)	OR (95% CI)[1]	*p* for linear trend
HLA Class I				
A				
Least (4th)	36 (22)	251 (26)	Reference	
Most (1st)	40 (25)	243 (25)	1.2 (0.7-1.9)	0.39
B				
Least (4th)	42 (25)	248 (26)	Reference	
Most (1st)	41 (25)	244 (25)	1.0 (0.6-1.5)	0.97
C				
Least (4th)	43 (27)	243 (25)	Reference	
Most (1st)	38 (24)	237 (24)	0.9 (0.6-1.4)	0.53
HLA Class II				
DPB1				
Least (4th)	34 (21)	253 (26)	Reference	
Most (1st)	38 (24)	255 (26)	1.1 (0.7-1.8)	0.83
DQA1				
Least (4th)	27 (17)	261 (27)	Reference	
Most (1st)	24 (15)	226 (23)	1.0 (0.6-1.9)	0.66
DRB1				
Least (4th)	39 (24)	252 (26)	Reference	
Most (1st)	33 (21)	236 (24)	0.9 (0.5-1.5)	0.51

[1]OR= odds ratio, 95% CI = confidence interval. Adjusted for number of live births. The SSM score estimates degree of similarity between two alleles. A lower score indicates increased similarity relative to a higher score, therefore the 4th quartile of scores is intended to represent the least histocompatible group and the 1st quartile the most.

Table 5. Mother-child similarity score - Mean sequence similarity matching (SSM) score and risk of systemic lupus erythematosus (SLE) among mothers

Histocompatibility per SSM Score (Quartile)	SLE n (%)	Controls n (%)	OR (95% CI)[1]	*p* for linear trend
HLA Class I				
A				
Least (4th)	39 (25)	251 (26)	Reference	
Most (1st)	39 (25)	243 (25)	1.1 (0.7-1.8)	0.59
B				
Least (4th)	34 (22)	248 (26)	Reference	
Most (1st)	46 (29)	244 (25)	1.3 (0.8-2.1)	0.52
C				
Least (4th)	43 (28)	243 (25)	Reference	
Most (1st)	35 (22)	237 (24)	0.8 (0.5-1.3)	0.29
HLA Class II				
DPB1				
Least (4th)	43 (28)	253 (26)	Reference	
Most (1st)	24 (15)	255 (26)	0.6 (0.3-1.0)	0.01
DQA1				
Least (4th)	36 (23)	261 (27)	Reference	
Most (1st)	27 (17)	226 (23)	0.9 (0.5-1.5)	0.67
DRB1				
Least (4th)	30 (19)	252 (26)	Reference	
Most (1st)	36 (23)	236 (24)	1.2 (0.7-2.1)	0.65

[1]OR= odds ratio, 95% CI = confidence interval. Adjusted for number of live births. The SSM score estimates degree of similarity between two alleles. A lower score indicates increased similarity relative to a higher score, therefore the 4th quartile of scores is intended to represent the least histocompatible group and the 1st quartile the most.

Table 6. Mother-child compatibility at minor histocompatibility antigens (mHag) and risk of systemic lupus erythematosus (SLE) among mothers with the corresponding HLA restriction

Any mHag-matched children	**HLA restriction allele**	**SLE n (%)**	**Controls n (%)**	**OR (95% CI)**[1]	***p***
SLC19A1					
None	*DRB1*15:01*	24 (52)	111 (53)	Reference	
1 or more		22 (48)	98 (47)	0.9 (0.5-1.7)	0.76
LB-WNK1					
None	*A*02:01*	45 (47)	252 (57)	Reference	
1 or more		50 (53)	188 (43)	1.5 (0.9-2.3)	0.11
HA-3					
None	*A*01:01*	32 (48)	152 (52)	Reference	
1 or more		34 (52)	140 (48)	1.2 (0.7-2.1)	0.50
ZAPHIR					
None	*B*07:02*	29 (74)	117 (52)	Reference	
1 or more		10 (26)	106 (48)	0.4 (0.2-0.8)	0.01
HEATR1					
None	*B*08:01*	31 (54)	127 (64)	Reference	
1 or more		26 (46)	73 (37)	1.5 (0.8-2.7)	0.22
C19orf48					
None	*A*02:01*	44 (46)	245 (56)	Reference	
1 or more		51 (54)	195 (44)	1.4 (0.9-2.3)	0.13

[1]OR= odds ratio, 95% CI = confidence interval. Adjusted for number of live births.

Table 7. Association between having any children who are mHag and HLA compatible and risk of systemic lupus erythematosus (SLE) among mothers [1]

Any HLA-matched/ mHag-matched children	**HLA restriction allele**	**SLE n (%)**	**Controls n (%)**	***p***
SLC19A1	*DRB1*15:01*			
None		44 (96)	189 (90)	
1 or more		2 (4)	20 (10)	0.39
LB-WNK1	*A*02:01*			
None		73 (77)	384 (87)	
1 or more		22 (23)	56 (13)	0.02
HA-3	*A*01:01*			
None		57 (86)	251 (86)	
1 or more		9 (14)	41 (14)	1.0
ZAPHIR	*B*07:02*			
None		39 (100)	202 (91)	
1 or more		0 (0)	21 (9)	0.05
HEATR1	*B*08:01*			
None		51 (89)	189 (95)	
1 or more		6 (11)	11 (5)	0.22
C19orf48	*A*02:01*			
None		75 (79)	366 (83)	
1 or more		20 (21)	74 (17)	0.37

[1] Histocompatible from the mother's perspective at the restriction HLA locus and at the minor histocompatibility antigen. [2] Fisher's exact test *p*-value

Supplementary Table 1. Distribution of mother-child average Sequence Similarity Matching (SSM) Score by disease status

HLA locus	RA (n=161)	SLE (n=156)	Controls (n=973)
A			
Mean ± SD	21.3 ± 9.3	21.3 ± 9.0	20.8 ± 10.5
Median	22.6	22.4	23.2
Range	0-36.6	0-35.8	0-37.9
B			
Mean ± SD	21.2 ± 7.9	21.2 ± 7.2	21.5 ± 8.2
Median	21.8	21.9	22.2
Range	0-37.2	0-35.4	0-37.2
C			
Mean ± SD	14.2 ± 6.3	14.5 ± 6.2	13.9 ± 6.5
Median	15.7	16.6	15.4
Range	0-23.9	0-23.5	0-23.9
DPB1			
Mean ± SD	5.1 ± 4.3	5.9 ± 4.0	5.2 ± 4.4
Median	4.3	6.2	3.6
Range	0-12.9	0-12.9	0-13.2
DQA1			
Mean ± SD	3.6 ± 2.3	3.4 ± 2.4	3.5 ± 2.6
Median	3.4	3.4	3.1
Range	0-6.7	0-6.7	0-6.7
DRB1			
Mean ± SD	13.9 ± 5.8	13.3 ± 5.5	13.5± 6.2
Median	13.6	13.4	13.2
Range	0-26.4	0-26.4	0-26.4

Chapter 3. A Child's *HLA-DRB1* Genotype Increases Maternal Risk of Systemic Lupus Erythematosus

Abstract

Objective. Systemic lupus erythematosus (SLE) disproportionately affects women of reproductive age. During pregnancy, women are exposed to various sources of fetal material possibly constituting a significant immunologic exposure relevant to the development of SLE. The objective of this study was to investigate whether having any children who carry *DRB1* alleles associated with SLE increase the risk of maternal SLE.

Methods. This case-control study is based on the University of California, San Francisco Mother-Child Immunogenetic Study and from studies at the Inova Translational Medicine Institute. Analyses were conducted using data for 1,304 mothers (219 cases/1,085 controls) and their respective 1,664 children. We selected alleles based on their known association with risk of SLE (*DRB1*03:01*, **15:01*, or **08:01)* or Epstein-Barr virus (EBV) glycoproteins (**04:01*) due to the established EBV association with SLE risk. We used logistic regression models to estimate odds ratios (OR) and 95% confidence intervals (CI) for each allele of interest, taking into account maternal genotype and number of live births.

Results. We found an increase in risk of maternal SLE associated with exposure to children who inherited *DRB1*04:01* from their father (OR 1.9; 95% CI, 1.1-3.2), among **04:01* allele-negative mothers. Increased risk was only present among mothers who were positive for one or more SLE risk-associated alleles (**03:01, *15:01* and/or **08:01*). We did not find increased risk of maternal SLE associated with any other tested allele.

Conclusions. These findings support the hypothesis that a child's alleles inherited from the father influence a mother's subsequent risk of SLE.

Keywords: systemic lupus erythematosus; genetic epidemiology; human leukocyte antigen genes; pregnancy

Introduction

Systemic lupus erythematosus (SLE) has one of the most striking sex ratios ranging from 4-13 females per every male affected and a peak in incidence during the reproductive years [146]. Fetal microchimerism has been previously proposed to contribute to female-predominant autoimmune diseases [8, 118]. Microchimerism, or the presence of a small number of cells from one individual present in another, is a natural consequence of bi-directional cellular trafficking that takes place between mother and fetus. Previous studies have found a higher prevalence of fetal microchimerism in SLE cases compared to controls [91, 95].

These observations support the hypothesis that fetal material such as fetal cells, fetal cell free DNA or the contents of placental vesicles, may constitute a significant immunologic exposure to the mother that contributes to an increased incidence of SLE among women. Fetal material enters the maternal circulation during normal pregnancy and has immunomodulatory effects [142]. Fetal DNA, lipids and proteins are released into the maternal circulation by the syncytiotrophoblast of the placenta [141]. The contents of placental exosomes are believed to be an important source of fetal-maternal communication [147]. To date, little is known about how the exposure to fetal material shapes the long-term health of the mother.

It has previously been reported that SLE autoantibodies precede diagnosis, diversify and accumulate [148, 149]. Exposure to fetal material through the maternal circulation may contribute to the development of autoantibodies. We hypothesize that risk of maternal SLE is increased depending on the characteristics of the fetal material the mother is exposed to during pregnancy. Using a genetic approach, we investigated whether having any children who carry SLE-associated HLA alleles *DRB1*03:01*, **15:01*, or **08:01* [48, 150, 151] increases the risk of maternal SLE. It is also possible that fetal material may act through molecular mimicry of certain infections [152]. Therefore, we also investigated the relationship between having any children who carry *DRB1*04:01* and maternal SLE due to the association between Epstein-Barr virus (EBV) and SLE [153] and the amino acid sequence similarity between EBV glycoproteins and *DRB1*04:01* [154].

Materials and methods

Study population. We conducted a case control study of 1,304 mothers and their children using data from the UC 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. All SLE cases met the 1997 revised criteria of the American College of Rheumatology [125, 126] and had at least one live birth prior to diagnosis. 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). ITMI studies were approved by the Western IRB and the Inova Health System IRB.

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).

HLA allele imputation. We used SNP2HLA [128] 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 [48], we adjusted for ancestry proportions estimated using STRUCTURE (version 2.3.4) [155]. A detailed description of genotyping, QA/QC steps and imputation methods are found in the Supplementary material section.

Statistical analyses. We used logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between mother's SLE status and a child's *DRB1* genotype. For each eligible mother, we included data from all children (n=1,664; 386 from cases/1,278 from controls) who participated in the study. We created a binary variable for each *DRB1* allele of interest *(*03:01, *15:01, *08:01* and **04:01*) to indicate whether any child carried one or two alleles vs. none and constructed a model for each allele. We addressed three main questions: a) is the *DRB1* allele associated with SLE; b) is a child's *DRB1* allele associated with maternal SLE; and c) is a child's *DRB1* allele associated with maternal SLE among mothers who were not carriers of the same allele. Using directed acyclic graphs we identified 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 was included in models for the second and third questions. In order to isolate the independent effect of a child's genotype and maternal SLE, we adjusted for maternal carrier status of each tested allele for the second set of models and excluded mothers with the allele in the third set. To correct for multiple testing, we applied a Bonferroni correction to each of the three questions investigated [133]. Statistical analyses were conducted using Stata 13 (StataCorp, College Station, Texas) and R [134].

Interaction and sensitivity analyses. We investigated potential mother-child genotype interaction between a child's **04:01* carrier status and maternal *DRB1* risk-allele carrier

status of at least one allele of **03:01*, **15:01*, or **08:01*. We conducted the analysis among **04:01* allele-negative mothers. In addition, we performed sensitivity analyses to rule out alternative explanations for our findings. Due to the case control difference in the number of participating children, we conducted a sensitivity analysis where we only included mothers with one participating child. We also adjusted our models to account for the difference in the number of live births between cases and controls using total number of children included in the study instead of total number of live births, as this may affect the probability of exposure. We reclassified our exposure of having allele-positive children to only include children born pre-diagnosis rather than including all children.

Since the results of mother-child allele combinations could be a surrogate for a genetic effect of allele combinations, we tested statistically significant combinations in a larger dataset of SLE cases and controls (n=12,935). Data for the analysis was obtained from a previously published study that includes our 219 cases [151]. Furthermore, to investigate whether the time of exposure to the pregnancy was within a plausible time period for the development of autoantibodies [149], we conducted an analysis excluding cases diagnosed more than 10 years after their last pregnancy.

Results

Controls were younger at study enrollment and had fewer births (1.9 vs. 2.3) due to the inclusion of ITMI pregnancy cohorts (Table 1). Genotype data were available for some but not all children. The average number of children included in the study was slightly greater for SLE cases than controls (1.8 vs. 1.2). The average age at SLE diagnosis among cases was 38.6 ± 12.6 and 18% had a lupus nephritis diagnosis. Antibody test results were available for 91% to 97% of cases, depending on the antibody. Antibodies to double stranded DNA (anti-dsDNA) and Ro (anti-Ro) were present in 40% and 30% of all cases, respectively. Frequencies for other antibodies are in Table 1. The frequency of each of the 1997 ACR revised criteria for SLE [125, 126] is available in Supplementary Table 1.

DRB1 and maternal SLE. For comparison to published reports, we first investigated the association between presence of each *DRB1* allele and SLE by comparing mothers in a traditional case control manner. Our results were consistent with previously reported associations between **03:01*, **15:01*, and **04:01* and SLE (Table 2). The association between **08:01* and SLE was in the expected direction but estimates lacked precision.

Child's DRB1 genotype and maternal SLE. We investigated the association between having any children who carried one or two copies of each *DRB1* allele and maternal SLE in separate models (Table 3). We found a two-fold increase in risk of maternal SLE among women who had an **04:01* allele-positive child compared to controls (OR 2.1; 95% CI, 1.4-3.1). Models were adjusted for maternal **04:01* status and number of live births. The association was stronger when comparing cases with lupus nephritis to controls (OR 2.7; 95% CI, 1.2-6.1). We did not find an association between any other *DRB1* allele investigated and maternal SLE. Our study had more than 80% power to

detect a 20% increase in risk associated with having any children positive for the **03:01* allele and maternal SLE, but power was lower for detecting the observed level of increase in risk associated with **15:01* (67%) and **08:01* (32%).

To rule out the possibility that the effect was limited to children who inherited the allele from their mother, we conducted the analysis excluding mothers who carried each allele (Table 4). Among **04:01* allele-negative mothers (177 cases / 920 controls), having any **04:01* allele-positive children was associated with maternal SLE (OR 1.9; 95% CI, 1.1-3.2); an attenuated but significant result compared to allele-positive mothers (n= 42 cases /165 controls; OR 2.4; 95% CI, 1.1-4.9). The increase in risk is present regardless of whether the child inherited the allele from his or her father or mother.

In order to determine whether the effect of a child's genotype on maternal disease was modified by the mother's own genetic susceptibility, we stratified on whether mothers carried one or more *DRB1*03:01, *15:01,* or **08:01* alleles. We found that the association between **04:01* allele-positive children and maternal SLE was restricted to mothers who carry one or more risk-associated *DRB1* alleles (Table 5; *p-interaction* = 0.09). Our study was not adequately powered (n=134, 27% power) to test if having any children positive for one or more alleles of **03:01*, **15:01* and/or **08:01* and negative for **04:01* among mothers who were **04:01*-positive and negative for the other three alleles increased the risk of maternal SLE. We found a slightly stronger association between having **04:01* allele-positive children and maternal SLE among mothers diagnosed within 10 years of a child's birth compared to controls (n= 978; OR 2.7; 95% CI, 1.3-5.7).

Our estimates remained consistent in analyses where we only included mothers with one participating child (n=455, OR 2.6; 95% CI, 1.1-6.0), or when we adjusted for number of participating children rather than number of live births. As it was possible that our results could have been affected by our method of classifying the genotype of children born at any time independent of date of diagnosis, we reclassified exposure by limiting to those born prior to diagnosis; results were similar (n=585, OR=2.1; 95% CI, 1.1-4.1). Due to the difference in age at interview of ITMI and MCIS mothers, we compared the frequency of having any **04:01*-allele positive children between the two control groups. We did not find a statistically significant difference (p=0.18). The age at interview for MCIS control mothers did not differ compared to case mothers (55 vs. 54 years, respectively; p=0.14).

Furthermore, among mothers we did not find strong evidence of additional risk associated with carrying *DRB1*04:01* in combination with any one of the *DRB1* risk-associated alleles **03:01, *15:01*, and/or **08:01* relative to mothers who were **03:01, *15:01* and/or **08:01* positive and **04:01*-negative in this study (OR 1.7; 95% CI, 1.0-3.0, p=0.05) or in our larger dataset (OR 0.9; 95% CI, 0.8-1.0, p=0.15).

Discussion

To our knowledge, this is the first report of an association between a child's genotype and maternal SLE risk. We found that having children who are *DRB1*04:01* allele-positive is associated with a two-fold increase in risk of SLE among mothers who

carry at least one *DRB1* risk allele *(*03:01, *15:01*, and/or **08:01*). The association is present among women who are positive as well as negative for the allele. Among allele-negative mothers this means that **04:01* was inherited from the father, suggesting that *DRB1* alleles may also increase risk of disease through non-genetic effects. We did not find evidence of increased risk of SLE associated with carrying **04:01* in combination with **03:01*, **15:01*, or **08:01*. The increase in risk of maternal SLE associated with **04:01* is only present when exposure occurs through the allele of the child. These findings support the hypothesis that exposure to fetal material during pregnancy may contribute to the development of SLE. There are several plausible explanations for our findings based on current understanding of the biology of pregnancy as well as the natural history of SLE.

One possibility is that fetal material that enters the maternal circulation during pregnancy mediates the production of autoantibodies that contribute to the development of SLE. During pregnancy, mothers can make antibodies against placental material and specifically against the paternal HLA of the fetus [156-159]. Antibodies to fetal paternally inherited HLA molecules have been detected in nearly a third of pregnant women [159]. Memory T-cells against paternal HLA can persist for up to 10 years after birth, even in the absence of antibodies [157], suggesting that transient exposure to paternal HLA via the fetus can shape the maternal T-cell repertoire. Among susceptible women, the interaction between maternal and paternal antigens could contribute to the break in tolerance leading to the production of autoantibodies.

Our findings are intriguing because *DRB1*04:01* is not an allele associated with overall risk of SLE, although it may be associated with a subset of autoantibodies [160-162]. In rheumatoid arthritis patients, *DRB1*04:01* is one of the alleles that comprise the strongest genetic risk factor referred to as the "shared epitope" which are associated with anti-citrullinated protein antibody production [163-165]. Our rationale for including *DRB1*04:01* in the current study was due to its association with an EBV glycoprotein previously identified in a study of rheumatoid arthritis [154]. The association between exposure to a child who carries *DRB1*04:01* and SLE may be due to a form of molecular mimicry, where components of a child's DNA may mimic an infectious agent that leads to the initiation or propagation of an immunologic process that results in SLE. Both molecular mimicry and epitope spreading have been hypothesized to contribute to SLE [166, 167]. Longitudinal studies on the natural course of SLE have shown that autoantibodies appear years before diagnosis and they accumulate and change over time, possibly due to epitope spreading [148, 149, 152].

Exposure to fetal material may also affect the maternal immune system through other pathways. It is estimated that fetal DNA makes up 3% to 6% of total maternal plasma DNA in early and late pregnancy, respectively [168]. Fetal material, including fetal DNA, can contribute to systemic inflammation leading to pregnancy complications such as preeclampsia [169, 170]. Levels of fetal DNA are higher in preeclamptic pregnancies compared to controls [171]. It is possible that shared mechanisms may be involved in some aspects of SLE and preeclampsia. SLE patients suffer have higher rates of preeclampsia compared to the general population [67]. Fetal-maternal genotype combinations of HLA-C and maternal KIR are associated with increased risk of

preeclampsia [172]. These findings support the hypothesis that the fetal genotype may affect the development of SLE through immunologic pathways.

Another possible explanation is that fetal cells trigger an immune response by maternal immune cells or that fetal lymphocytes attack maternal cells. Microchimerism, of presumed fetal origin, has been found more frequently among SLE cases compared to controls. One study identified fetal microchimerism in 68% of SLE cases compared to 33% of controls [91]. Similarly, another study found evidence of microchimerism in 31% of cases compared to only 4% among controls [95]. Two post-mortem studies [100, 101] and a study using renal biopsies [102] found evidence of microchimerism in SLE affected organs compared to controls or to normal tissue in cases. Phenotypically, microchimeric cells have demonstrated multilineage capacity [173] and may resemble hematopoietic stem cells but their function remains unknown.

We were unable to confirm the underlying assumption that fetal genotype affects the characteristics or function of microchimeric cells or any other type of fetal material since we did not quantify either exposure. Fetal microchimerism is associated with some, but not all, female predominant autoimmune diseases [174]. Our study cannot directly elucidate the role of microchimerism in female predominant autoimmune diseases, although it has informed our hypothesis. The association between microchimerism and autoimmune diseases may be due to a variety of factors. Female predominant autoimmune diseases are a heterogeneous group of conditions with complex etiology. The association of a single factor with each disease may vary depending on its relative importance among the many factors that lead to a specific disease [175], despite sharing common causes. We have identified evidence for association between a child's genotype and maternal SLE, which may or may not be mediated by microchimerism. Studies using functional approaches are needed to understand potential biological effects.

Our study has a number of strengths. We have investigated fetal-maternal genetic interactions in a large sample of mothers and their children. We have performed QA/QC measures that increase our confidence in the quality of our data. We have taken into account potential sources of bias in our models by adjusting for confounding variables and by conducting various sensitivity analyses. We minimized the probability that results are false positives by testing a limited number of candidate alleles and by correcting for multiple comparisons.

Some limitations must also be considered. Although our sample size was large, we were unable to rule out chance as an explanation for some results. We tested a limited number of candidate alleles and our study would benefit from a more thorough investigation using a larger sample. The ITMI controls were younger at age at study entry and had fewer live births compared to our cases. The difference in age could impact our results through misclassification of disease status (future cases in the younger control group) and by a reduced exposure to live births. However, our results are unlikely due to these differences for a number of reasons. SLE is a rare condition, and we expect that if any controls subsequently develop the disease, the number is likely to be very small. Further, inclusion of potential cases in our control group would result in a bias towards the null. Differences in maternal age at interview could influence the number of live births and subsequently the number of allele-positive children. However, we did not find

evidence that maternal age affected the proportion of **04:01* allele-positive children when older and younger control groups were compared.

Potential confounding variables for ITMI controls were extracted from electronic medical records, which differed from direct collection methods used for MCIS participants. Although differences in exposure measurement can be a source of bias in case-controls studies, the impact on results are likely to have been minimal. As a genetic study, confounding can result from differences in population structure between cases and controls. Our study was conducted among non-Hispanic white individuals and models were adjusted for genetic ancestry. The validity and reliability of maternal report of live births compared to medical records has previously been reported to be very high (kappa=1.0) [144, 145], therefore minimizing concern that the method of collection contributed to the results.

Our study was conducted in a subset of female patients who had given birth to a child prior to diagnosis, and are therefore not generalizable to the entire SLE patient population. In order to minimize confounding by ancestry, white women of European ancestry were investigated, which also affected the generalizability of study findings. SLE disproportionately affects African Americans who suffer from more severe disease [176-178] and additional populations need to be examined. Compared to white cases from a population-based study, we observed a similar frequency of lupus nephritis, an indicator of disease severity [178]. The similarities increase our confidence that our cases are representative of European-ancestry women with SLE in the United States.

In conclusion, these findings support the hypothesis that exposure to the fetus influences a mother's risk of disease. This is the first study to demonstrate an association between a child's *DRB1* genotype and risk of SLE in the mother.

TABLES AND FIGURES

Table 1. UCSF Mother-Child Immunogenetic Study (MCIS) and Inova Translational Medicine Institute (ITMI) participant characteristics

	Cases	**Controls**
	Mean ± SD or *N* (%)	**Mean ± SD or *N* (%)**
Mothers, n	219	1,085
Age at study enrollment [1, 2]	53.8±11.0	37.7±10.4
Number of live births [2]	2.3±1.2	1.9±1.0
Ever smoker [2]	103 (47.0)	271 (25.0)
Age at diagnosis, per medical records	38.6±12.6	--
Autoantibodies [3]		
Anti-dsDNA	87 (39.7)	--
Missing	7 (3.2)	
Anti-Ro	65 (29.7)	--
Missing	13 (5.9)	
Anti-La	28 (12.8)	--
Missing	14 (6.4)	
Anti-Sm	20 (9.1)	--
Missing	14 (6.4)	
Anti-RNP	33 (15.1)	--
Missing	19 (8.7)	

[1] ITMI controls age at study enrollment is mother's age at birth of child. [2] p-value <0.001 for the difference between cases and controls. [3] Patients may have more than one autoantibody.

Table 2. Association between carrying 1 or more known *DRB1* risk alleles and systemic lupus erythematosus among mothers in the Mother-Child Immunogenetic Study (MCIS)

***HLA-DRB1 alleles* (Mothers)**		***N* =1,304 (cases/controls)**	**SLE OR (95% CI)** [1]	**Bonferroni corrected *p*-value**
**03:01*				
	None	139/832	Reference	0.0004
	1 or 2	80/253	1.9 (1.4-2.6)	
**15:01*				
	None	156/831	Reference	0.36
	1 or 2	63/254	1.3 (1.0-1.8)	
**08:01*				
	None	201/1,038	Reference	0.07
	1 or 2	18/47	2.0 (1.1-3.5)	
**04:01*				
	None	177/920	Reference	0.56
	1 or 2	42/165	1.3 (0.9-1.9)	

[1] OR = odds ratio; 95% CI = 95% confidence interval for the association between carrying one or more of each listed *DRB1* allele compared to none and SLE among mothers.

Table 3. Association between children carrying *DRB1* alleles of interest and mothers' systemic lupus erythematosus status

***HLA-DRB1* allele-positive children**	***N* =1,304 (cases/controls)**	**SLE (mother) OR (95% CI)** [1]	**Bonferroni corrected *p*-value**
***03:01**			
None	147/836	Reference	*1.0*
1 or more	72/249	1.2 (0.8-1.7)	
***15:01**			
None	141/787	Reference	*0.48*
1 or more	78/298	1.3 (0.9-1.9)	
***08:01**			
None	204/1,035	Reference	*1.0*
1 or more	15/50	1.0 (0.5-2.2)	
***04:01**			
None	168/941	Reference	*0.004*
1 or more	51/144	2.1 (1.4-3.1)	

[1] OR = odds ratio; 95% CI = 95% confidence interval for the association between having one or more children with the specified *DRB1* alleles compared to none and SLE among mothers. Each model is adjusted for number of live births and maternal carrier status of the same allele.

Table 4. Association between children carrying *DRB1* alleles of interest and mothers' systemic lupus erythematosus status among allele-negative mothers

***HLA-DRB1* allele-positive children**	***N*** **Allele-negative mothers (cases/controls)**	**SLE (mother) OR (95% CI)** [1]	**Bonferroni corrected *p*-value**
***03:01**			
None	118/729	Reference	*1.0*
1 or more	21/103	1.1 (0.7-1.9)	
***15:01**			
None	127/688	Reference	*1.0*
1 or more	29/143	1.0 (0.7-1.6)	
***08:01**			
None	195/1,014	Reference	*1.0*
1 or more	6/24	1.4 (0.5-3.4)	
***04:01**			
None	155/852	Reference	*0.08*
1 or more	22/66	1.9 (1.1-3.2)	

[1] OR = odds ratio; 95% CI = 95% confidence interval for the association between having one or more children with the specified *DRB1* alleles compared to none and SLE among mothers. Each model is for mothers negative for the allele and is adjusted for number of live births.

Table 5. Association between *DRB1 *04:01* allele-positive children and maternal SLE according to maternal carrier status of any SLE risk-associated *DRB1* allele **03:01, *15:01*, and/or **08:01*

DRB1 *04:01* allele-negative mothers				
	SLE risk allele-positive		**SLE risk allele-negative**	
****04:01* allele-positive children**	***N* (cases/controls)**	**OR (95% CI)** [1]	***N* (cases/controls)**	**OR (95% CI)**[1] **n = 510**
None	100/439	Reference	55/415	Reference
1 or more	17/30	**2.6 (1.4-4.9)**	5/36	1.0 (0.4-2.6)

[1] Adjusted for number of live births. Risk allele-positive is defined as carrying at least one *DRB1*03:01, *15:01*, and/or **08:01* allele and risk allele-negative as not carrying any.

Supplementary material

Genotyping. Cases were genotyped for a panel of 2,360 SNPs in the MHC region using the combined MHC Exon-Centric and Mapping panels from Illumina. Full genome profiling of MCIS controls and all children was conducted using the Illumina 660K SNP array or the Illumina ImmunoChip. QA/QC criteria were applied to all genotype data resulting in the exclusion of SNPs that were genotyped in <80% of samples and/or had a minor allele frequency (MAF) <1%. Whole genome sequencing (WGS) data was available for ITMI control families. We excluded SNPs that were genotyped in <90% of samples and/or had an MAF <1%. ITMI WGS methods have been previously described [179]. We verified familial relationships for all mother-child duos.

Classical HLA allele imputation. In order to investigate mother-child relationships at classical HLA loci, post-QA/QC genotype data was used to impute markers across the extended MHC using SNP2HLA [128]. SNP2HLA uses the Type 1 Diabetes Genetics Consortium (T1DGC) as the reference. After imputation, we excluded variants with a low measure of imputation accuracy (r^2 <0.3). As an additional QC measure, we compared imputation results at the allelic level to typed two-field *DRB1* data available for a subset of MCIS participants (n=2,136) using the method described by Raychaudhuri et al. [46]. The imputation procedure correctly called 93.1% of alleles. Using a chi-square test and a significance threshold of α=0.05, we compared allele frequencies by platform among controls. We did not find statistically significant differences by platform for the *DRB1* alleles investigated (data not shown). Allelic frequencies were within the range reported for individuals of European ancestry as published in the online database www.allelefrequencies.net (Download 10/23/2015).

Population substructure. In order to minimize confounding by genetic ancestry, the MCIS recruited mothers who self-identified as non-Hispanic white. ITMI mothers were selected for having >90% European ancestry using markers across the genome and multidimensional scaling analysis conducted in PLINK (version 1.90p) [180] using the Human Genome Diversity Project as the reference panel [181]. Due to differences in coverage depending on genotyping or sequencing platform, we used the software package STRUCTURE (version 2.3.4) [155] to adjust for potential stratification by ancestry in the combined MCIS-ITMI dataset. We used 271 ancestry informative markers (AIMs) available for the majority of participants in the combined dataset. The markers were a subset of markers that differentiate between northern and southern European populations [48]. AIMs were available for 95% of study participants (n=1,239). In our analyses, we did not find evidence of stratification by ancestry. There were no statistically significant differences (p <0.05) in *DRB1* allele frequencies between control participants with and without AIM data.

Supplementary Table 1. Frequency of 1997 ACR Revised Criteria for Systemic Lupus Erythematosus (SLE) among cases from the UCSF Mother-Child Immunogenetic Study

ACR criteria for classification of SLE, n (%)	**SLE Cases (n=219)**
Antinuclear antibody (ANA) positivity	202 (92)
Photosensitivity	186 (85)
Arthritis	161 (74)
Hematologic disorder	133 (61)
Immunologic disorder	125 (57)
Malar rash	88 (40)
Oral ulcers	58 (27)
Serositis	53 (24)
Renal disorder	40 (18)
Discoid rash	9 (4)
Neurologic disorder	8 (4)

[1] Non-exclusive frequency for each characteristic.

Chapter 4. Increased risk of rheumatoid arthritis among mothers with children who carry *DRB1* risk-associated alleles

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 1,263 mothers (179 cases/1,084 controls) and their respective 1,575 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 carrier status and number of live births.

Results. We found increased risk of RA among mothers who had any child with the SE (OR 3.1; 95% CI, 2.1-4.6), DERAA (OR 1.9; 95% CI, 1.3-2.8), valine (OR 2.5; 95% CI, 1.7-3.6), lysine (OR 2.4; 95% CI, 1.7-3.4) and alanine (OR 2.7; 95% CI, 1.3-5.7) 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 2.1; 95% CI, 1.4-3.1) and alleles encoding lysine at DRB1 position 71 (OR 2.6; 95% CI, 1.5-4.5).

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

Key words: Rheumatoid arthritis, epidemiology, autoimmune disease

Introduction

Rheumatoid arthritis (RA) affects two to three times more women than men [22, 182] for reasons not entirely understood [113]. Various aspects of pregnancy have been investigated due to the observed overall sex dimorphism and to findings indicating this time period is relevant to disease risk. Among disease-free women, an increased risk of onset takes place during the post-partum period [60, 117]. Further, improvement of symptoms during pregnancy among cases has been described [115]. 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 [183]. Fetal cells may persist in small quantities for up to decades after delivery [76]. 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 [40, 41]. At the same positions, the sequence DERAA has been associated with reduced risk of RA [184]. 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 [46]. 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 [46], a well-documented association [185]. Clinically, the SE is associated with anti-citrullinated peptide antibody-positive (ACPA) RA and antibody titers [19], disease severity [186] and mortality [187]. The SE alleles have been shown to be more predictive of ACPA presence than of RA [163]. 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 [96, 97]. 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 [188]. 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 1,263 mothers and 1,575 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. All RA cases met the 1987 revised criteria of the American College of Rheumatology (ACR) [124] 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.

HLA allele imputation. We used SNP2HLA [128] 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 [48], we adjusted for ancestry proportions estimated using STRUCTURE (version 2.3.4) [155]. A detailed description of genotyping, QA/QC steps and imputation methods has been previously published [189].

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 association between allele groups and RA among mothers, 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 a 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 was included in models for the second and third questions.

We tested the robustness of our estimates through a number of sensitivity analyses. 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. After imputation, QA/QC steps resulted in slightly different sample sizes at each of the three genes investigated (*HLA-DRB1, DPB1* and *B*). All participants included in analyses of *DPB1* and *B* alleles are included in analyses of *DRB1*. A complete case analysis of participants with genotypes at all three sites was also conducted to ensure our results were not due to the sample size available for analyses involving *HLA-B* and *DPB1*. We repeated our analyses in the seropositive subgroup and same number of controls. We applied a Bonferroni correction to each of the three questions investigated to correct for multiple testing [133]. Analyses were conducted using Stata 13 (StataCorp, College Station, Texas) and R [134].

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) 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. Results were very similar when seropositive cases were compared to controls (data not shown). The only notable difference was a stronger association between *DRB1* alleles encoding alanine at position 74 and seropositive RA compared to all RA cases compared to controls (OR 5.3; 95% CI, 1.9-14.6 vs. OR 3.8; 95% CI, 1.8-7.9).

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 allele carrier status. We found a three-fold increased risk of RA for mothers who had any child positive for the SE, independent of maternal carrier status (OR 3.1; 95% CI, 2.1-4.6). A two and a half-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 1.9; 95% CI, 1.3-2.8). 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 three-fold increase in risk of maternal RA (OR 3.1; 95% CI, 1.1-8.8). The effect size for *DPB1* alleles was stronger in the seropositive group (OR 8.7; 95% CI, 1.2-63.4) than among seronegative cases (OR 1.3; 95% CI, 0.4-4.3). No other striking differences were found by serostatus (data not shown).

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 1.6 from the three-fold increase in the previous model that included all mothers (Table 3).

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 [46]. In our study, the association between **04:01* and RA among mothers was OR 5.6 (95% CI, 4.0-7.9). Having any **04:01* allele-positive children and adjusting for number of live births and mother's **04:01* resulted in an OR of 2.4 (95% CI, 1.6-3.7). Among allele-negative mothers, the association between **04:01* allele-positive children and RA was similar (OR 2.4; 95% CI, 1.3-4.6).

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 [190, 191], possibly implicating non-host genetic factors in RA pathogenesis.

An increase in risk of RA was also associated with having children with DERAA-encoding alleles. Our findings are in contrast to what we might expect given that the sequence is associated with reduced risk of RA [184]. 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. 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 [192]. 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 [193]. 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 [19, 194-196]. The estimate for maternal SE status and RA was attenuated once we took into account having any SE+ children (OR 5.2; 95% CI, 3.5-7.9). These findings support the hypothesis that a child's genotype can independently contribute to risk of maternal RA.

SE+ women are more likely 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 [19, 194]. 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 a 9% difference in the frequency of SE+ children that may help explain the excess of SE+ microchimerism among cases compared to controls previously reported [96, 97]. 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 [189]. *DRB1*04:01* does not have a strong association with SLE but it does share sequence similarities with the Epstein-Barr Virus (EBV) [154]; EBV is a risk factor for SLE [197]. Studies in RA have demonstrated that ACPAs react with EBV viral sequences and may contribute to disease-associated antibody formation [198]. 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. As a result of QA/QC measures, some participants were missing locus-specific data. All participants with *DRB1* data were included in analyses regardless of whether HLA genotypes were available for the other two loci. The results obtained from a complete case analysis of 1,162 participants (167 cases and 995 controls) with data for all three loci were similar to those presented in all tables. We took into account potential confounding variables in our analyses and corrected for multiple comparisons. 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. Among SE-negative mothers, we had only 10% power to detect an association of the observed magnitude or greater after correcting for multiple comparisons. 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. 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.

TABLES AND FIGURES

Table 1. UCSF Mother-Child Immunogenetic Study (MCIS) participant characteristics

Characteristics	RA Cases Mean ± SD or N (%)	Controls Mean ± SD or N (%)
Mothers, n	179	1,084
Age at study enrollment	57.0 ± 9.9	37.7 ± 10.4
Number of live births	2.2 ± 1.0	1.9 ± 1.0
Age at diagnosis, per medical records	40.3 ± 11.9	--
Seropositive, per medical record[1]	123 (68.7)	

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

Table 2. Case-control association between mothers carrying one or more alleles from each group and rheumatoid arthritis

Risk allele group (Mothers)	Alleles	*N* Cases / Controls	RA (Mother) OR (95% CI)†	Bonferroni corrected *p*-value
	HLA-DRB1	179/1,084		
Shared Epitope +	**04:01, *04:04, *04:05, *04:08, *01:01, *10:01, *01:02*	146/423	6.9 (4.6-10.3)	<0.001
DERAA +	**04:02, *01:03, *11:02, *13:01, *13:02*	19/230	0.4 (0.3-0.7)	<0.001
Valine + (AA position 11)	**04:01, *04:08, *04:05, *04:04, *10:01, *04:03, *04:07, *04:02*	113/315	4.2 (3.0-5.8)	<0.001
Lysine + (AA position 71)	**04:01, *13:03, *03:01*	118/450	2.7 (2.0-3.8)	<0.001
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*	171/920	3.8 (1.8-7.9)	<0.001
	HLA-B	175/1,019		
Aspartic acid + (AA position 9)	**08*	48/232	1.3 (0.9-1.8)	1.0
	HLA-DPB1	174/1,058		
Phenylalanine + (AA position 9)	**02:01, *02:02, *04:01, *04:02, *05:01, *16:01, *19:01, *23:01*	163/995	0.9 (0.5-1.8)	1.0

† 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. AA = amino acid.

Table 3. Case-control association between children carrying 1+ risk alleles and mothers' rheumatoid arthritis status

Risk allele group	*N* Cases / Controls with children carrying alleles	RA (Mother) OR (95% CI)†	Bonferroni corrected *p*-value
HLA-DRB1	179/1,084		
Shared Epitope +	139/418	3.1 (2.1-4.6)	<0.001
DERAA +	50/240	1.9 (1.3-2.8)	0.007
Valine + (AA position 11)	107/297	2.5 (1.7-3.6)	<0.001
Lysine + (AA position 71)	119/420	2.4 (1.7-3.4)	<0.001
Alanine + (AA position 74)	171/935	2.7 (1.3-5.7)	0.06
HLA-B	175/1,019		
Aspartic acid + (AA position 9)	52/229	1.4 (0.9-2.1)	1.0
HLA-DPB1	171/1,058		
Phenylalanine + (AA position 9)	167/988	3.1 (1.1-8.8)	0.21

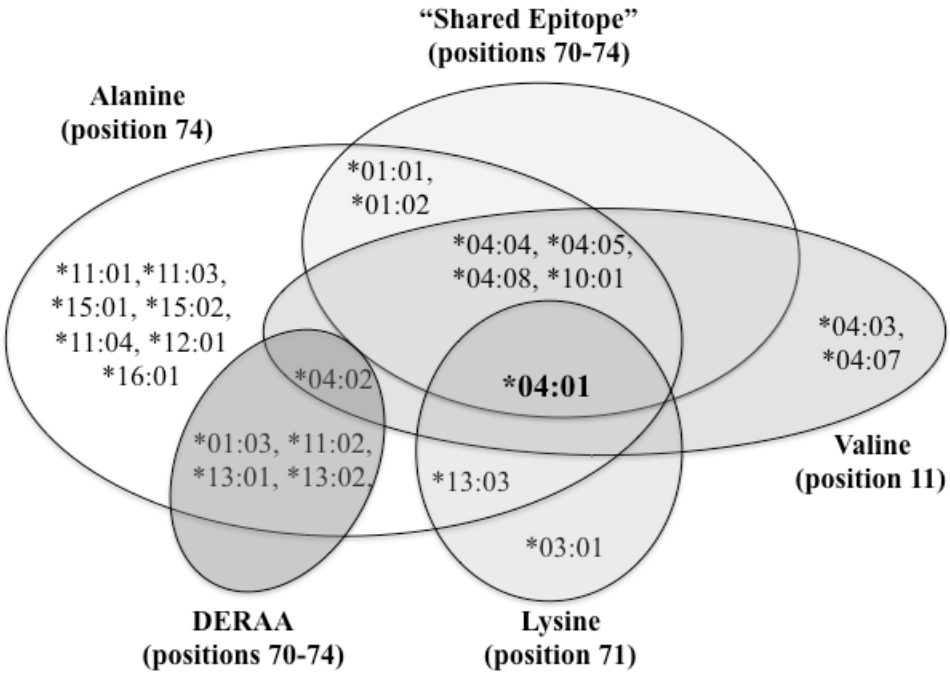
† OR = odds ratio; 95% CI = 95% confidence interval for the association between having at least one child (born prior to diagnosis for cases) with one or two alleles (+) of each risk allele group compared to none and RA among mothers. Estimates adjusted for mother's carrier status of the same allele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

Table 4. Case-control association between children carrying 1+ risk alleles and allele-negative mothers' rheumatoid arthritis status

Risk allele group	Allele-negative mothers		
	N **Cases / Controls with children carrying alleles**	**RA (Mother) OR (95% CI)†**	**Bonferroni corrected p-value**
HLA-DRB1			
Shared Epitope +	(11/33) / (157/661)	1.6 (0.8-3.4)	1.0
DERAA +	(38/160) / (109/854)	2.1 (1.4-3.1)	0.007
Valine + (AA position 11)	(17/66) / (124/769)	1.9 (1.0-3.4)	0.28
Lysine + (AA position 71)	(26/61) / (137/634)	2.6 (1.5-4.5)	0.007
Alanine + (AA position 74)	(6/8) / (106/164)	1.6 (0.3-8.4)	1.0
HLA-B			
Aspartic acid + (AA position 9)	(17/127)/ (86/787)	1.2 (0.7-2.1)	1.0
HLA-DPB1			
Phenylalanine + (AA position 9)	(9/11) / (50/63)	0.8 (0.1-4.6)	1.0

† 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. AA = amino acid.

Figure 1. *DRB1* allele overlap



Supplementary Table 1. 1987 ACR Criteria

1987 ACR criteria, N (%)	RA Cases (n=179)
Morning stiffness	141 (61.6)
Arthritis of 3 or more joint areas	201 (87.8)
Arthritis of hands and joints	227 (99.1)
Symmetric arthritis	214 (93.5)
Rheumatoid nodules	75 (32.8)
Serum rheumatoid factor	156 (68.1)
Radiographic changes	126 (55.0)

Chapter 5. Conclusions

Autoimmune disorders disproportionately affect women [22]. The causes of the gender disparity are likely to include genetic and non-genetic exposures [113]. Pregnancy and pregnancy-related factors have been the focus of numerous investigations in female-predominant autoimmune disorders [60]. One exposure that may be implicated in autoimmunity is the persistence of fetal cells in mothers, or microchimerism, naturally acquired during pregnancy [8]. Interest in microchimerism has stemmed from similarities of clinical manifestations of scleroderma and graft-versus-host disease (GVHD) [118], a potential complication of bone marrow transplantation. As fetal cells may be analogous to a graft, it is logical to hypothesize that Human Leukocyte Antigen (HLA) relationships between mothers and children influence risk of autoimmune disease. Studies of mother-child histocompatibility and risk of autoimmune disease are few and have only investigated histocompatibility at a limited number of HLA loci [107-112]. The objective of this dissertation was to investigate mother-child HLA relationships and their association with risk of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), two female-predominant autoimmune disorders.

Chapter 2 investigated the association between RA and SLE and mother-child compatibility. Histocompatibility was defined from the perspective of the mother at classical HLA loci. Data from the University of California San Francisco Mother Child Immunogenetic Study (MCIS) and studies at the Inova Translational Medical Institute (ITMI) were used for these analyses. Histocompatibility at *HLA-B* and *DPB1* was associated with increased risk of RA among mothers. Risk of SLE was lower among women who on average had a lower sequence similarity matching (SSM) score indicative of greater amino acid similarity between mismatched alleles relative to less similarity (1^{st} versus 4^{th} quartiles of SSM) at *DPB1*. Among mothers positive for *HLA-B*07:02*, compatibility at ZAPHIR, a minor histocompatibility antigen (mHag), was associated with reduced risk of SLE. Further analyses among mothers who were compatible at the respective HLA restriction locus for a given mHag revealed that SLE cases were more likely to have compatible children for both *HLA-A* and LB-WNK1 and less likely to have compatible children at *HLA-B* and ZAPHIR.

Chapter 3 investigated the association between maternal SLE and having children who carried SLE-associated *DRB1* alleles **03:01, DRB1*15:01* and **08:01*. To test the hypothesis that fetal peptides could mimic viral epitopes, *DRB1*04:01* was included in the list of alleles due to its shared sequence similarity with Epstein-Barr Virus (EBV), a risk factor for SLE. An increase in risk of maternal SLE was associated with exposure to children who inherited *DRB1*04:01* from their father, among mothers negative for the allele but positive for one of the SLE-associated alleles. Among genetically susceptible women, exposure to a paternally inherited allele of the child increased risk of SLE in the mother.

Chapter 4 investigated the association between RA and having any children with alleles encoding the "shared epitope" (SE) and DERAA AA sequences at positions 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. Increased risk of RA among mothers was associated with having any children who carried alleles encoding the SE, DERAA, valine, lysine and alanine at the DRB1 molecule, independent of maternal carrier status. Among mothers who did not carry the alleles for each amino acid or amino acid sequence designation, increased risk of RA was associated with having a child who carried DERAA as well as alleles encoding lysine at DRB1 position 71.

Conclusions and future research directions

The results from this dissertation provide limited evidence for a role of mother-child histocompatibility in RA and SLE among mothers. The results from Chapter 2 show contrasting associations between compatibility at *DPB1* and RA and SLE. The results suggest that the causes underlying the association with histocompatibility are unlikely due to a shared mechanism, such as in bone marrow transplantation. The finding that among mothers with *HLA-A*02:01*, SLE cases were more likely to have an HLA-compatible/mHag-compatible child compared to controls does provide some support for the compatibility hypothesis. Results from analyses of mHag compatibility at ZAPHIR are consistent with a hypothesis based on exposure to fetal antigens, where HLA compatibility is not a factor but mismatch on ZAPHIR is associated with increased risk of SLE, regardless of histocompatibility at *HLA-B*. Minor histocompatibility antigen mismatch is an immunogenic exposure. As only a small number of mHags were investigated, studies of other known mHags should be conducted. Their immunogenic potential has been realized in the context of bone marrow transplantation as has their curative potential in hematopoietic malignancies [135]; a similar opportunity might exist in autoimmunity.

The results from subsequent chapters further support the fetal-antigen hypothesis where mismatch is the risk factor. Chapter 3 finds an association between having children who carry *DRB1*04:01* and risk of maternal SLE among mothers negative for *DRB1*04:01* and positive for SLE-associated *DRB1* alleles. *DRB1*04:01* was investigated due to its shared sequence similarity with Epstein-Barr virus, a probable risk factor for SLE. These findings are in support that fetal antigens influence maternal immunity.

Results from Chapter 4 provide more evidence that it is not histocompatibility *per se* but rather specific amino acid sequences possibly presenting as antigens. In Chapter 2, there was no association between compatibility (or incompatibility) at *DRB1* between mothers and children and risk of RA (or SLE). Findings from Chapter 4 suggest that some, but not all, amino acid sequences are associated with risk of disease. The association between having children positive for risk-associated amino acid sequences and amino acids encoded by *DRB1* alleles increased risk of RA among mothers. The most intriguing finding was the association between increased risk of RA and having children who are positive for alleles encoding the DERAA amino acid sequence among mothers without the alleles. DERAA is generally associated with decreased risk of RA. This finding suggests that peptides from these alleles, when not presented natively in the

mother, have a different function. Finding the association among allele-negative mothers further supports the hypothesis that mismatch of immunogenic peptides from the fetus increase risk of autoimmunity among mothers.

Future research should investigate a more thorough list of potentially immunogenic fetal antigens. A comprehensive evaluation of the role of minor histocompatibility mismatch is a justified next step in the context of this limited investigation. Functional studies to determine if specific fetal antigens trigger immune responses by maternal lymphocytes would contribute to the mechanistic understanding of how exposure to fetal material during pregnancy (fetal cells, placental vesicles or cell-free DNA) could contribute to maternal immune processes relevant to autoimmunity. Cross-reactivity with viral epitopes should also be considered. These analyses were conducted in women of European origin. SLE disproportionately affects persons of African ancestry and other non-white populations [33-36]. It is possible that in admixed populations, the risk of mismatch is increased. These results provide the rationale for investigating the role of mismatch in admixed populations such as African Americans and Hispanics. Finally, large studies are needed to understand the role of disease subtypes in these findings.

In conclusion, we have found evidence that mother-child HLA relationships are associated with RA and SLE. These relationships are possibly mediated by exposure to fetal antigens during pregnancy. Further investigation of the immunogenicity of fetal antigens is warranted as better understanding of these relationships could lead to new therapies for immune-cell mediated conditions.

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