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Maternal–fetal HLA sharing and preeclampsia: Variation in effects by seminal fluid exposure in a case–control study of nulliparous women in Iowa

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Abstract

Whereas histocompatibility is critical for transplantation, HLA histo*in*compatibility is associated with successful pregnancy. Literature on HLA sharing and preeclampsia has been inconsistent; most studies focused on maternal–paternal rather than maternal–fetal sharing. This study examines whether maternal–fetal histocompatibility is associated with preeclampsia, and whether effects vary by semen exposure history. This case–control study of nulliparous women was designed to examine associations among HLA sharing, semen exposure, and preeclampsia. 258 preeclampsia cases and 182 normotensive controls met the eligibility criteria. HLA typing for mother and baby was performed for HLA-A, -B, -C, -DRB1, and -DQB1. We further restricted our study sample to 224 mother–baby pairs who had complete HLA typing for all five genes. Seminal fluid exposure indexes incorporated information on type of practice, frequency, contraceptive use (for vaginal exposure) and ingestion practices (for oral exposure). Multivariate models were adjusted for BMI and education. HLA-A matching, Class I matching, and combined Class I and II matching were associated with increased odds of preeclampsia. Among women with low semen exposure, effects of Class I matching were amplified (HLA-A matching, OR=6.27, 95%CI=1.04, 37.97; Class I matching, OR=4.49 per one-match increase, 95%CI=1.89, 14.50). With moderate to high semen

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exposure, Class II matching effects predominated (HLA-DQB1, OR=3.22, 95%CI=1.04, 9.99; Class II, OR=1.76 per one-match increase, 95%CI=1.05, 2.98; and total matches, OR=1.45 per one-match increase, 95%CI=1.02, 2.06). We found consistent evidence that maternal–fetal HLA sharing was associated with preeclampsia in a pattern influenced by prior vaginal exposure to paternal seminal fluid.

Keywords

Preeclampsia; HLA sharing; maternal-fetal histocompatibility; immunology; seminal fluid

1. Introduction

Preeclampsia is a potentially life-threatening hypertensive complication of pregnancy of placental origin. An estimated 3% of deliveries in the United States (Wallis et al., 2008) are complicated by preeclampsia, which is a leading cause of severe maternal and perinatal morbidity and mortality worldwide. Despite many years of clinical and basic science research, the only effective means of treating preeclampsia remains delivery of the baby, the placenta and all products of conception.

An accumulating body of epidemiological evidence points convincingly toward an immune-based susceptibility to preeclampsia that strongly implicates a paternally mediated fetal role (Saftlas et al., 2005; Redman and Sargent, 2010). Although a completed pregnancy is the best-established protective factor (MacGillivray, 1958), it is now known that the protection conferred by a previous birth or abortion (Eras et al., 2000) is lost if the woman's next pregnancy is with a new partner (Li and Wi, 2000; Saftlas et al., 2003; Mostello et al., 2008; Wikström et al., 2012), suggesting that prior exposure to paternal/fetal antigens may be protective. Further evidence in support of the paternal mediation of preeclampsia risk comes from studies of gamete-donated pregnancies, where there is limited prior exposure to the partner's DNA (Sahla et al., 1999; Saito et al., 2007), and from studies assessing the degree of exposure to paternal seminal fluid (Marti and Herrmann, 1977; Klonoff-Cohen et al., 1989; Dekker and Robillard, 2007) and the duration of sexual cohabitation with the baby's father (Robillard et al., 1994).

Whereas successful organ transplantation often depends on close HLA matching of donors to recipients (i.e., histocompatibility), HLA histo*in*compatibility is associated with successful pregnancy. Mouse studies demonstrate that most offspring from brother/sister matings are heterozygous for MHC antigens, suggesting the selective elimination of histocompatible progeny (Beer and Need, 1985). Similarly, genetic studies of human population isolates throughout the world reveal a deficiency of HLA homozygotes (e.g., Kil Kummer Tauregs, Havasupai, and Brazilian Amerindians) (Gill, 1996), which may reflect a reduced overall fertility among couples with matching HLA haplotypes or loci (Ober et al., 1998).

Although the general literature examining HLA and preeclampsia risk is inconsistent, studies that focused specifically on maternal–fetal antigen sharing were more consistent, with most finding increased preeclampsia risk associated with HLA sharing at the HLA-A,

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B, or DR loci (Saftlas et al., 2005; Biggar et al., 2010). These studies, however, did not consistently implicate one particular locus and suffered from many methodological deficiencies (Saftlas et al., 2005).

The objective of this study is to determine whether women who develop preeclampsia while pregnant with their first child are more likely to be histocompatible with their infants (i.e., to share HLA antigens) than women with normotensive pregnancies. We hypothesize that maternal–fetal HLA-sharing impairs maternal recognition of the fetal allograft as a pregnancy, compromising maternal immune tolerance and implantation of the placenta. Specifically, we assess simultaneous effects of sharing allele groups at each of five HLA genes (A, B, C, DR, and DQ), as well as generalized sharing of allele groups across all five genes combined. In addition, we examine whether the effects of maternal–fetal HLA sharing vary by seminal fluid exposure history.

2. Materials and methods

2.1 Study population and design

The Study of Pregnancy Hypertension in Iowa (SOPHIA) is a population-based case–control study designed to determine the influence of maternal–fetal HLA sharing and exposure to paternal seminal fluid on the risk of preeclampsia. The study population comprised women from 42 counties in Iowa who delivered a live birth over the period August 2002 through May 2005. Using electronic birth certificates as the sampling frame, potential preeclampsia cases were selected as primiparous women who had a notation of either “pregnancy-induced hypertension” or “eclampsia” on a check box listing of maternal conditions. Potential controls were randomly selected as primiparous women who had no indication of hypertension on the birth certificate. For both cases and controls, exclusion criteria included: age <18 years at delivery; non-English-speaking; history of an autoimmune disease (e.g., systemic lupus erythematosus, insulin-dependent diabetes mellitus, rheumatoid arthritis); recurrent spontaneous abortion (at least three sequential pregnancy losses); chronic hypertension or renal disease; plural gestations; major congenital anomalies; infant death or seriously ill infant. Although we may have missed some of the most severe cases of preeclampsia by excluding deaths, severe congenital anomalies, and serious neonatal illnesses, it was essential to collect biological samples from both mother and offspring. Women less than 18 years old were excluded because they are not of legal age in Iowa, and the interview included some detailed questions about reproductive history and sexual practices with the baby’s father.

Women selected from birth certificates were sent an introductory letter detailing the study. Study personnel later contacted women via telephone and screened interested subjects for their potential eligibility. Women who met the initial eligibility criteria were invited to participate in completing a 20 to 25-min computer-assisted telephone interview collecting information on maternal and sociodemographic characteristics (e.g., age, education, race, pre-pregnancy body mass index [BMI], smoking in pregnancy, prior pregnancies), family history of cardiovascular and renal diseases and preeclampsia, and a detailed sexual history with the baby’s father. Buccal cell samples for HLA genotyping were provided from mother and baby by mail, along with medical release forms and consent forms. Medical records

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were abstracted using a structured abstraction form collecting detailed information on blood pressure and urinary protein values during the prenatal, intrapartum, and immediate postpartum periods, along with information on potential exclusion criteria (e.g., diabetes, SLE, preexisting hypertension, etc.) for both cases and controls.

A total of 1,188 potential cases and controls were interviewed for the SOPHIA study. Of these, 982 (82%) returned signed medical release forms, and 97% of their charts were successfully abstracted. Based on chart review, 258 preeclampsia cases and 182 normotensive controls met the study's strict case and control criteria (see below). We further restricted our study sample to the 224 mother–baby pairs (118 cases and 106 controls) who had complete HLA typing for all five alleles for both mother and baby.

2.2 Criteria for classifying preeclampsia cases and controls

Preeclampsia was defined according to strict National Heart, Lung and Blood Institute (NHLBI) guidelines (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000). The criteria required:

1. *De novo* hypertension (≥140 mm Hg systolic or ≥ 90 mm Hg diastolic on two or more occasions at least six hours apart beginning after the 20th week of gestation;
2. Accompanying proteinuria, defined as urinary protein concentrations of 30 mg/dl or greater (equivalent to a dipstick value of 1+ from two or more specimens collected at least four hours apart, or one or more urinary dipstick values of 2+ near the end or pregnancy, or one or more catheterized dipstick values of 1+ during delivery hospitalization, or 24-h urine collection with protein of ≥ 300 mg.

Potential controls were excluded if their medical records included any indication of high blood pressure in the prenatal or postpartum period, or two or more high blood pressure readings in the intrapartum period, or any indication of proteinuria (1+ on a dipstick) during pregnancy.

2.3 Collection of buccal cell samples

A package containing buccal cell collection kits for the woman and her baby, medical record release forms, and consent forms was mailed to the woman. Buccal cells were collected from study subjects using Cytosoft Brushes (catalog no. CP-5B; Medical Packaging Corporation, Camarillo, CA, USA) as previously described (Saftlas et al., 2004). Mothers were instructed to twirl and rub each of two brushes for 30 s in the upper and then the lower gutter area (the space where the gums join the inner cheek/lip area along the sides and front of the mouth). They were also instructed to collect buccal samples from their infant using four Cytosoft Brushes in the cheek and gutter areas. After collection, the cytobrush samples were placed in separate Kraft paper envelopes for mother and baby samples and mailed inside a Tyvek bubble lined envelope (Quality Park Products), which served as the outer mailing envelope. Upon receipt, samples were stored at 4° C until processing, generally within 5–12 days of the collection date. The mother was asked to return the signed forms and buccal cell samples as soon as possible in the pre-paid postage envelope.

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2.4 HLA genotyping of subjects

The DNA was isolated using the PUREGENE DNA isolation kit (Qiagen) according to the manufacturer's specifications with modifications. The brush portion of the Cytobrush was clipped off and placed into a 1.5-ml microfuge tube containing 550 μl Cell Lysis Solution or the equivalent (100 mM NaCl; 10 mM Tris-Cl, ph8; 25 mM EDTA, pH 8; 0.5% SDS), and samples were incubated with proteinase K overnight in a 55–65°C water bath. After carefully removing the brush, the remaining cell lysate was treated with RNase A Solution, the tubes were gently mixed and then incubated at 37°C for 40 to 60 min. To precipitate protein, the sample was cooled at 4° C for 10 min, 250 μl Protein Precipitation Solution (7.5M NH_4Acetate) was added, the sample was vortexed vigorously at high speed for 20 s, then incubated at 4° C for at least 10 to 30 min, and centrifuged at 13,000 × g for 5 min. The supernatant containing the DNA was harvested and transferred into a clean 1.5-ml microfuge tube containing 400 μl 100% Isopropanol (2-propanol) and 2 μl Gentra Glycogen Solution (20 mg/ml). Tubes were mixed by gentle inverting for 1 min, kept at room temperature for at least 30 to 40 min, and then centrifuged at 13,000 × g for 5 min. The pelleted DNA was washed with 70% Ethanol, and tubes were centrifuged at 13,000 × g for 1 min. The supernatant was removed, and the pellet was air-dried. DNA was rehydrated with up to 50 μl DNA Hydration Solution (Tris-EDTA) by incubation at room temperature. After concentration and purity were determined, DNA samples were stored at 2–8°C until genotyping was completed and at −70° C for long-term storage.

Genotyping of *HLA-A, -B, -C, -DRB1*, and *-DQB1* was performed using LABType® SSO reagents (One Lambda, Canoga Park, CA, USA), which uses sequence-specific oligonucleotide probes (SSO) bound to fluorescently coded microspheres to identify alleles homologous to the probes, as per the manufacturer's specifications. Samples were analyzed using the LABScan 100 and the LABScan™ 100 Luminex XY Platform. HLA typing was assigned using the software HLA Visual 2.1.0 (One Lambda), and typing assignments were independently verified by a primary and secondary reviewer.

2.5 HLA exposure variables

Maternal–fetal HLA sharing was measured for three classical HLA Class I genes (*HLA-A, -B,* and *-C*) and two Class II genes (*HLA-DRB1*, and *-DQB1*). Since the mother contributes one allele to the baby, there is a minimum of one match for a mother–baby pair; for each gene, the number of matches ranges from one to two. The combined number of Class I matches ranged from 3 to 6, the combined Class II matches ranged from 2 to 4, and the total number of matches (Class I and II combined) ranged from 5 to 10.

2.6 Seminal fluid exposure and other co-variables

The seminal fluid exposure variables are described in detail in Saftlas et al. in this issue of the *Journal of Reproductive Immunology*. Briefly, information on sexual and contraceptive practices was self-reported by women through detailed questions covering each six-month period in the year prior to pregnancy and at each 12-month period back through the beginning of the sexual relationship with the baby's father. Information included source of seminal fluid exposure (i.e., vaginal vs. oral), frequency of exposure, use of contraceptives, and ingestion of semen. These data were used to construct an index for vaginal seminal fluid

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exposure and an index for oral seminal fluid exposure.. Because our findings in the paper by Saftlas et al. demonstrated that increased vaginal exposure was significantly protective against preeclampsia while oral exposure was not we focused our analyses in this manuscript exclusively on vaginal sperm exposure. Vaginal exposures were weighted based on the contraceptive method used (e.g., weight for no contraception use=1.0, condom use=0.1, and intermediate weights for various other methods). The seminal fluid exposure index was categorized into tertiles of exposure; low exposure was defined as the lowest tertile and moderate to high exposure as the second and third tertiles combined.

2.7 Statistical analysis

Distributions of study population characteristics were compared between cases and controls and between HLA-sharing variables. Univariate associations between each individual HLA sharing variable and preeclampsia provide unadjusted measures of association. Multivariate models considered sharing variables jointly. Model 1 included sharing (2 vs. 1) at each of the five HLA genes (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1) simultaneously. Model 2 included the sum (continuous) of Class I matches (ranging from 3 to 6) and Class II matches (ranging from 2 to 4) simultaneously. Model 3 included the single variable for total number of matches across Class I and Class II genes (ranging from 5 to 10). Factors associated with both preeclampsia and HLA sharing exposure variables at $P<0.25$ were included in multivariate models. In addition, we ran models that were fully adjusted for any potential confounder associated with either preeclampsia or HLA sharing at $P<0.10$. Since the results were consistent, we present the more parsimonious models.

3. Results

Table 1 displays the study population characteristics by case–control status and their associations with preeclampsia. Study participants tended to be under the age of 30, college-educated, and of white race. BMI was the only characteristic that was significantly associated with case–control status: obese women had a 3.6-fold higher (95% CI 1.63, 7.96) risk of preeclampsia than women of low to normal BMI.

The distribution of maternal–fetal HLA sharing at each HLA locus examined is shown according to characteristics of the entire study population (i.e., cases and controls combined) in Table 2. Overall, complete HLA sharing (two shared allele groups between mother and baby) ranged from a low of 8.3% for HLA-B to a high of 25.7% for HLA-DQB1 (Table 2). Significant differences in complete HLA sharing were noted in association with the following maternal characteristics: BMI with HLA-B sharing; maternal education with HLA-A sharing; and maternal race with sharing at the HLA-DRB1 and -DQB1 loci.

The univariate associations of HLA-sharing with odds of preeclampsia for each HLA locus separately, and for the number of Class I matches, Class II matches, and the total number of combined Class I and Class II matches are shown in Table 3. While maternal–fetal HLA matching at each of the individual HLA loci was not significantly associated with odds of preeclampsia, odds were directly associated with the number of Class I matches (p trend=0.05). Neither the number of Class II matches nor the number of total matches was associated with the risk of preeclampsia in the univariate analyses.

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Multivariate logistic regression analysis of the associations between HLA sharing variables and risk of preeclampsia were conducted among all subjects (Table 4). All models are adjusted for pre-pregnancy BMI and maternal education and the other HLA-sharing variables in the model. In model 1, which simultaneously includes the matching variables for each of the five HLA loci, subjects who completely matched on HLA-A had 2.35 times higher (95% CI: 0.94, 5.86) odds of preeclampsia than those who did not completely share HLA-A genes with their offspring. Although case–control status was not significantly associated with matching at any of the other HLA genes, preeclampsia subjects had 66% higher odds of HLA-B sharing and 65% higher odds of HLA-HLA-DQB1. In model 2, which included the variable for the number of Class I matches and that for the number of Class II matches, we found that each additional Class I match was significantly associated with a 59% increase in the odds of preeclampsia. We found no significant association between increased sharing of Class II genes and the odds of preeclampsia, although the association was inverse when adjusting for Class I sharing. In model 3, the total number of Class I and Class II matches combined was associated with significantly increased odds of preeclampsia (OR=1.34, 95% CI: 1.01, 1.79).

After stratifying by low and moderate to high seminal fluid exposure (Table 4), we found some notable differences in the effects of HLA sharing on the odds of preeclampsia between these two groups. Among women in the lowest tertile of seminal fluid exposure, the odds of preeclampsia were amplified in association with increased numbers of HLA-A and Class I allele group matches. In model 1, matching at HLA-A was associated with over 6-fold higher odds of preeclampsia (OR=6.27, 95% CI: 1.04–37.97). Similarly, in model 2, each incremental increase in matching on Class II genes was associated with more than a 4-fold increase in the odds of preeclampsia (95% CI: 1.89, 14.50). In contrast, there was no association between the number of Class II gene matches and the odds of preeclampsia. In the moderate to high seminal fluid exposure groups, neither matching at the HLA-A locus nor the number of Class I matches was significantly associated with preeclampsia. The number of Class II gene matches, however, was significantly associated with increased risk: cases were 3.22 times more likely (95% CI: 1.04, 9.98) to completely match HLA-DQB1 compared with controls. Similarly, there was a 76% increase in the odds of preeclampsia for every one-match increase in Class II matches and a 45% increase in odds for every one-match increase in total number of Class I and Class II matches.

4. Discussion

Findings from this case–control study of nulliparous women indicate that maternal–fetal HLA sharing is associated with the risk of preeclampsia; furthermore, these risks varied as a function of a woman’s cumulative vaginal exposure to paternal seminal fluid. In particular, there were increased odds of preeclampsia associated with complete HLA-A sharing, having an increased number of shared Class I genes, as well as increased generalized sharing across the five HLA genes combined. Among women with the lowest tertile of vaginal semen exposure, associations between Class I genes and preeclampsia were amplified. Among women with moderate to high seminal fluid exposure, the Class I associations disappeared, while increased sharing at HLA-DQB1 and generalized sharing across the five genes were associated with increased odds of preeclampsia.

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A relatively small body of clinical studies, most conducted in the 1970s, 1980s and 1990s, evaluated the effects of HLA sharing among couples; and even fewer studies directly assessed associations between maternal–fetal HLA sharing and preeclampsia (Saftlas et al., 2005). Collectively, these studies varied considerably according to the composition and size of their study populations, the case definitions applied, the HLA loci examined, and the typing methodologies used. Eight studies examined the association between couple HLA sharing and preeclampsia, with disparate findings. Some (Jenkins et al., 1978; Fujisawa, 1985; Bolis et al., 1987; Kilpatrick et al., 1989; de Luca Brunori et al., 2000; Ooki et al., 2008) found an increased risk of preeclampsia with increased HLA sharing. However, since the fetus can inherit one of two alleles from the father, it is more likely that the HLA of the fetus rather than that of the father is most important. The handful of studies that have examined maternal–fetal HLA sharing and risk of preeclampsia have also yielded mixed findings (Kilpatrick et al., 1987, 1990; Hoff et al., 1992; Schneider et al., 1994; Wu et al., 1997). The largest and most recent study to date (Biggar et al., 2010) found no association among HLA-A, -B, or -DR sharing in 201 cases and 195 controls. However, this study, which included both multiparous as well as nulliparous women, did not control for parity and did not consider the simultaneous effects of the sharing of multiple alleles. Moreover, the study did not examine HLA-C, which is potentially important in the maternal immune response to pregnancy because HLA-C is expressed on placental trophoblasts (King et al., 2000).

Despite the fact that the fetus is a semi-allograft, an increasing body of evidence from both animal and epidemiological studies suggests that histo*in*compatibility rather than histocompatibility appears necessary for adequate placentation and successful pregnancy. Studies in mice have found a higher than expected number of offspring from brother/sister matings that are heterozygous for MHC antigens, suggesting that a selective elimination of histocompatible progeny may occur (Beer and Need, 1985). Similarly, genetic studies of human population isolates in different parts of the world have reported a relative deficiency of HLA homozygosity in progeny (e.g., Kil Kummer Tauregs, Havasupai and Brazilian Amerindians) (Gill, 1996). In a 10-year, prospective study of the Hitterite population, Ober et. al. found significantly increased rates of fetal loss and reduced overall fertility among couples with matching HLA haplotypes or loci (Ober et al., 1998). Our study supports this tenet of selection against histocompatible fetus.

When comparing all subjects, the risk of preeclampsia is shown most strongly for the Class I matches in model 2. Furthermore, our study shows that the effect of histocompatibility at Class I is modified by prior vaginal exposure to paternal seminal fluid. The risk of preeclampsia is most pronounced for HLA Class I-compatible fetus in women with low prior vaginal sperm exposure (Table 4, models 1 and 2), and this risk is attenuated by normal to high sperm exposure. These data suggest that no or low exposure to HLA Class I-compatible paternal antigens in the vaginal mucosal tissue favors rejection of an HLA Class I-compatible fetus, while prolonged or frequent exposure to HLA Class I-compatible paternal antigens favors healthy implantation of the HLA class I-compatible fetus.

Robertson speculated that exposure of the reproductive tract to paternal HLA alloantigens in the context of immune-deviating cytokines, such as TGF-β, might facilitate tolerance to

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pregnancy (Robertson et al., 2003). Following intercourse in women, a local immune reaction ensues with an increase in antigen-presenting cells (i.e., tissue dendritic cells) that can sample paternal antigens (Sharkey et al., 2012a, 2012b). Based on experiments in mice (Robertson et al., 2009), it is plausible that these APCs migrate to the uterine lymph nodes where they induce differentiation of tolerogenic regulatory T cells. Both Class I-a HLA antigens and TGF-β are present in large quantities in seminal fluid (Loras et al., 1999; Koelman et al., 2000). Thus, repeated 'seminal priming' in a TGF-B-enriched environment would favor progressive differentiation of paternal alloantigen-specific regulatory T cells (Robertson et al., 2013), which would facilitate healthy implantation in part by suppressing pro-inflammatory Th17 cells (Darmochwal-Kolarz et al., 2012). We emphasize that this model has been evoked for the histoincompatible fetus. Indeed, Shima et al. reported in mice that depletion of CD4+CD25+ Treg cells between days 2.5 and 7.5 post coitus blocked implantation and induced early loss of fetuses from fully histoincompatible matings, but did not affect fertility of completely histocompatible matings (Shima et al., 2010). Further studies of congenic matings would be necessary to determine whether Treg expansion is important for protection against preeclampsia of the fetus that is histocompatible at different Class I or Class II loci. Nonetheless, our findings of reduced preeclampsia with increased vaginal exposure to paternal seminal fluid at the least suggests that repeated vaginal priming with paternal antigen also plays a role in the healthy implantation of the histocompatible fetus.

The question of why preeclampsia is increased for histocompatible rather than histoincompatible fetuses in the setting of low antigen exposure remains. Perhaps the histocompatible fetus, with its relative paucity of HLA differences, would require a higher threshold of vaginal antigen exposure to trigger sufficient protective Treg populations. Alternatively, the innate immune system, specifically NK cells, may function to mitigate preeclampsia in the setting of low antigen exposure for a histoincompatible fetus, but not a histocompatible fetus.

Human NK cells express a complement of KIR (Killer Ig-like receptors) on their surface. These include activating and inhibitory receptors. The ligands for the KIR receptors are the HLA Class I molecules, including one of two groups of HLA-C, the public epitope Bw4, on a group of HLA-B molecules, and certain HLA-A molecules. All NK cells express at least one inhibitory receptor that recognizes a self-HLA Class I molecule. NK cells function in innate immune surveillance, normally held in check when an inhibitory receptor binds to its ligand, but activated to kill cells that lack a signature of "self" MHC—i.e., "missing self" MHC Class I of infected cells or transformed cells that have downregulated HLA Class I. However, reports that preeclampsia is associated with maternal inheritance of insufficient activating KIRs in the context of fetal inheritance of the respective ligand for self HLA-C (Hiby et al., 2004, 2010) suggested that HLA incompatible KIR ligands on extra villous trophoblast cells (e.g., HLA-C) activate specialized uterine NK cells to promote placentation and remodeling of maternal spiral arteries in the developing placenta (Parham and Guethlein, 2010). This would disadvantage placentation of fetuses that are too well matched. Our findings are compatible with a model in which HLA-A and -B KIR ligands may also function to disadvantage placentation of a highly matched fetus. Further research is needed to explore this possibility.

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A somewhat unexpected finding was the increase in odds of preeclampsia by Class II HLA sharing only among women with moderate to high seminal fluid exposure. Class II HLA appears to be present in low levels on human sperm cells (Ohashi et al., 1990), but not in seminal plasma. Findings from the infertility literature suggest that exposure to sperm cells is also important for successful pregnancy. Wang et al. found that women who were treated with ICSI using surgically obtained sperm (i.e., exposed to seminal plasma but never exposed to partner's sperm) had three times the risk of preeclampsia as those exposed to IVF or ICSI with ejaculated sperm (i.e., exposed to both seminal plasma and sperm cells) (Wang et al., 2002). This could support the possible role of Class II HLA sharing, even in the presence of seminal plasma exposure. However, the HLA-DQ protein differs from HLA-A, -B, -C, and -DR, in that both the alpha and the beta chains are polymorphic. Because both chains contribute to the antigenic epitope of HLA-DQ, additional HLA typing of the DQA chain would be needed to confirm whether there truly is complete compatibility at the DQ locus.

Our study has several notable strengths. This study was designed specifically to examine HLA sharing between mother and fetus, rather than focusing on couple-sharing, and it provides to our knowledge the first examination of the effects of maternal–fetal HLA sharing in the context of history of exposure to seminal fluid. Given that the fetus could have inherited one of two alleles from the father, examining fetal rather than paternal genes is likely more relevant for any potential pathogenesis. In addition, we collected detailed data from women regarding their sexual frequency and practices with the baby's father from the beginning of the sexual relationship through to the date of conception. This detailed history provides a more accurate measure of cumulative seminal fluid exposure than other proxy measures (e.g., length of sexual cohabitation), which many prior studies have heavily relied upon. Excluded from the study were multiparous women and those with a history of recurrent spontaneous abortion. By including only nulliparous women, the preeclampsia phenotype is likely to be more homogeneous; and in addition, different risk factors may be involved in the etiology of preeclampsia in multiparous versus nulliparous women. Our use of birth certificate data to identify potential cases and a population-based sample of potential controls is a further strength of the study design. Potential cases underwent medical chart review to ensure that cases met strict preeclampsia criteria. Likewise, all potential controls underwent chart review to ensure that they had no indication of elevated blood pressure or urinary protein that could lead to misclassification. In addition, we examined sharing at all five HLA loci simultaneously, which is a key strength of our analytic approach. Effects of sharing at one gene may be influenced by sharing at other genes; thus, joint effects are important to consider. Given the relatively modest correlations between sharing at each of the five HLA genes (Spearman correlations ranging from −0.02 to 0.38 with the exception of those for sharing at the HLA-DRB1 and HLA-DQB1 loci with a correlation of 0.64), it is reasonable to look jointly rather than individually at the sharing variables.

There are also some study limitations. First, our HLA typing data were only at an intermediate level of resolution; this may have limited our ability to distinguish complete matching at a higher level of resolution. These findings should be replicated at a higher level of resolution to determine whether there are patterns of specific sharing that could more clearly define those at highest risk of preeclampsia. In addition, data were missing on at least

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one of the HLA genes for a number of mother–baby pairs; however, we would not expect the missing HLA data to differ systematically between cases and controls. The lack of racial diversity in our mostly white study population could be considered a potential weakness. For this reason we considered restricting the analysis to white women only; however, we obtained similar results, with somewhat less precision, after excluding non-white women. There is also the possibility of residual confounding by BMI, as there were very few underweight women in the study population and they were grouped with women of normal BMI. Similar results, however, were obtained when the analysis was restricted to women of normal BMI. Another potential limitation is that paternity has not been confirmed in this study. It is likely that at least in a small proportion of women, the father of the baby will be a different male than the male for whom the primary sexual relationship was reported.

5. Conclusions

This study provides strong evidence of an association between HLA sharing and preeclampsia when jointly considering sharing at multiple genes. Most intriguing is the finding that the effects of sharing vary considerably by seminal fluid exposure. Class I sharing strongly increases the odds of preeclampsia in the context of low seminal fluid exposure, but moderate to high exposure attenuates this risk. Class II sharing increases odds of preeclampsia only in the context of moderate to high seminal fluid exposure. While the findings from this study are compelling and may link together prior HLA sharing and semen exposure findings, it will be important to replicate this research in larger studies. Furthermore, understanding biological mechanisms underpinning the associations will be important in developing appropriate interventions to help couples avoid preeclampsia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of the SOPHIA study population by case–control status

	Controls		Cases			
	N[a]	%	N[a]	%	OR	95% CI
Maternal age						
19–24	25	24.0	31	27.0	ref	
25–29	50	48.1	48	41.7	0.77	0.40, 1.50
30–34	16	15.4	25	21.7	1.26	0.56, 2.86
≥35	13	12.5	11	9.6	0.68	0.26, 1.78
Maternal BMI						
Low/Normal BMI (<25)	72	69.9	60	52.2	ref	
Overweight (<30)	21	20.4	25	21.7	1.43	0.73, 2.80
Obese (≥30)	10	9.7	30	26.1	3.60	1.63, 7.96*
Maternal education						
High school or lower	20	19.4	15	13.0	ref	
Some college	30	29.1	49	42.6	2.18	0.97, 4.89#
College degree or higher	53	51.5	51	44.4	1.28	0.59, 2.78
Race						
White	97	94.2	106	92.2	ref	
Non-white	6	5.8	9	7.8	1.37	0.47, 4.00
Prior pregnancies						
No prior pregnancy	73	74.5	81	75.0	ref	
Prior pregnancy with same father	17	17.4	14	13.0	0.74	0.34, 1.61
Prior pregnancy, but not with same father	8	8.2	13	12.0	1.46	0.57, 3.73
Smoked in pregnancy?						
No	80	77.7	94	81.7	ref	
Yes	23	22.3	21	18.3	0.78	0.40, 1.51

[a] N values may not sum to total due to missing values

* p<0.05

# p<0.10

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Table 2

Distribution of study population characteristics by HLA sharing status

	N[a]	Matched HLA-A (%)	Matched HLA-B (%)	Matched HLA-C (%)	Matched HLA-DRB1 (%)	Matched HLA-DQB1 (%)
Overall	218	12.8	8.3	17.9	13.3	25.7
Maternal age						
19–24	56	7.4	1.8	14.3	12.5	17.9#
25–29	97	15.3	9.3	15.5	5.0	24.0
30–34	41	9.8	12.2	26.8	17.1	41.5
≥ 35	24	20.8	12.5	20.8	12.5	20.8
Maternal BMI						
Low/Normal BMI (<25)	130	15.4	6.1*	13.7	14.4	24.6
Overweight (<30)	46	10.9	17.8	23.9	10.9	21.7
Obese (≥30)	40	7.5	2.5	25.0	10.0	30.0
Maternal education						
High school or lower	33	0.0*	5.7	11.4	8.6	14.3
Some college	79	19.0	9.0	17.9	12.7	24.4
≥ College degree	104	12.5	7.7	20.1	14.4	29.1
Race						
White	202	13.9	13.3	18.3	11.3*	22.9*
Non-white	14	0.0	7.4	13.3	33.3	53.3
Prior pregnancies						
None	153	13.2	9.1	18.3	14.9	27.4
Prior with same father	31	9.7	6.4	16.1	6.4	23.3
Prior with different father	21	19.1	5.0	19.1	14.3	23.8
Smoked in pregnancy?						
No	173	12.7	9.2	17.8	13.8	25.4
Yes	43	13.9	2.3	18.6	9.1	23.3

[a] χ^2 p values <0.05 are bolded

* $p<0.05$

$^{\#}p<0.10$

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Table 3

Univariate associations between HLA sharing variables and case–control status

	Controls (n=106) %	Cases (n=118) %	OR	95%CI
Complete match - HLA-A	9.5	16.2	1.84	0.81, 4.16
Complete match - HLA-B	5.7	10.2	1.87	0.67, 5.17
Complete match - HLA-C	14.3	20.3	1.53	0.76, 3.11
Complete match - HLA-DRB1	22.6	27.6	0.96	0.44, 2.09
Complete match - HLA-DQB1	13.2	12.7	1.30	0.71, 2.40
Class I matches[a]				
3 matches	74.8	66.7	*ref*	
4 matches	21.4	23.1	1.21	0.64, 2.31
5 matches	3.9	6.8	1.97	0.57, 6.83
6 matches	0.0	3.4	--	--
p trend			*1.51*	*0.99, 2.30*[#]
Class II matches[a]				
2 matches	76.4	72.4	*Ref*	
3 matches	11.3	14.7	1.11	0.50, 2.48
4 matches	12.3	12.9	1.37	0.61, 3.04
p trend			*1.01*	*0.75, 1.61*
Total # matches[a]				
5 matches	57.3	47.8	*Ref*	
6 matches	23.3	29.6	1.52	0.80, 2.88
7 matches	15.5	14.8	1.14	0.52, 2.47
8 matches	3.9	3.5	1.07	0.26, 4.50
9 matches	0.0	2.6	--	--
10 matches	0.0	1.7	--	--
p trend			*1.25*	*0.95, 1.64*

[a] Mother donates one allele to the baby so there must be at least one shared allele for each HLA gene; for example, class I includes 3 HLA genes; thus, the minimum number of shared alleles is 3

[#] $p<0.10$

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Table 4

Logistic regression models of associations between HLA sharing and preeclampsia among all subjects and stratified by sperm exposure

	All subjects		Low vaginal sperm exposure[e]		Normal to high vaginal sperm exposure	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Model 1[a, b]						
HLA-A match	2.35	0.94, 5.86#	6.27	1.04, 37.97*	1.29	0.38, 4.42
HLA-B match	1.66	0.49, 5.65	*f*	--	1.16	0.27, 5.01
HLA-C match	1.22	0.54, 2.77	1.91	0.34, 16.29	1.14	0.40, 3.22
HLA-DRB1 match	0.70	0.23, 2.10	0.31	0.02, 2.87	0.78	0.19, 3.14
HLA-DQB1 match	1.65	0.69, 3.94	0.56	0.06, 2.52	3.22	1.04, 9.99*
Model 2[a, c]						
# Class I matches	1.59	1.02, 2.47*	4.49	1.89, 14.50*	1.19	0.72, 1.08
# Class II matches	1.16	0.77, 1.73	0.49	0.20, 1.16	1.76	1.05, 2.98*
Model 3[a, d]						
Total # matches	1.34	1.01, 1.79*	1.13	0.65, 1.96	1.45	1.02, 2.06*

[a] All models adjusted for pre-pregnancy BMI and maternal education and other HLA variables listed in the model

[b] Reference for each HLA gene match term is one match; all 5 HLA gene matching variables included in same model

[c] Number of Class I and II matches; Class I matches range from 3 to 6; Class II matches range from 2 to 5; OR for each one-match increase

[d] Total number of matches ranges from 5 to 10 matches; OR for each numerical match increase

[e] Low sperm exposure is the lowest tertile of vaginal sperm exposure

[f] Odds ratio could not be calculated because all who had complete matches for HLA-B were cases

* $p<0.05$

# $p<0.10$