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Maternal-fetal HLA compatibility and trophoblast-immune interactions in healthy and preeclamptic pregnancy: elegance in complexity

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3

Maternal-fetal HLA-DQB1 incompatibility is associated with pregnancy-induced hypertensive disorders in a genetically isolated population

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ABSTRACT

In pregnancy, semi-allogenic fetal trophoblasts express a specific HLA profile mediating maternal leukocyte contact, crucial for placentation. Paradoxically, maternal immunomodulation requires fetal antigen recognition, especially involving certain HLA molecules. Preeclampsia, a severe hypertensive complication, has been linked to antigenic similarity. Previously, we showed no selection for HLA (in)compatibility in uncomplicated naturally conceived pregnancies. However, preeclamptic pregnancies were associated with increased total maternal-fetal HLA and HLA-C matching. These associations suggest a role for HLA mismatches in immune regulation leading to an uncomplicated pregnancy. To better understand HLA homozygosity in human reproduction, we aimed to determine if there is a preferential selection for HLA compatibility in a genetically isolated population, and its relation to hypertensive complications. A nested case-control study, comprising 125 uncomplicated pregnancies and 50 with hypertensive complications (29 with pregnancy-induced hypertension, 21 with preeclampsia) was conducted in a genetically isolated Dutch population (F_{ROH} 1.3-3.1). Maternal and fetal HLA-A, -B, -C, -DRB1, -DQA1, -DQB1, and maternal KIR genotyping were performed. Maternal-fetal HLA (mis)match counts were compared to expected values from randomization of paternal HLA haplotypes over maternal haplotypes of the fetuses. Mismatched $CD4^+$ T-cell epitopes presented by maternal HLA class II were predicted using the PIRCHE-II algorithm. In uncomplicated pregnancies, no difference was found between observed and expected maternal-fetal HLA (mis)matches. However, pregnancies with hypertensive complications showed significantly higher observed HLA-DQB1 mismatches, reflected in PIRCHE-II scores. No significant differences were found in KIR/HLA-C frequencies. Interpretation is limited by the small sample size and the grouping of distinct hypertensive disorders. Nonetheless, maternal-fetal HLA-DQB1 mismatch seems to play a role in the etiology of hypertensive complications during pregnancy in this population.

INTRODUCTION

Preeclampsia is a common but progressive and potentially severe pregnancy-induced hypertensive disorder characterized by new-onset hypertension and organ dysfunction. It is a leading cause of fetal and maternal morbidity and mortality worldwide. (1) While the clinical presentation of preeclampsia is in the third trimester, the underlying pathophysiology is related to preceding placental dysfunction that starts early in gestation. There is growing evidence that immune recognition is involved in the etiology of the disease. (2-4)

Successful development of the semi-allogeneic fetus and placenta within the uterus requires modulation of the maternal immune system. Fetal extravillous trophoblasts (EVT) encounter maternal immune cells at the maternal-fetal interface (5-7) and express a specific HLA profile that constitutes classical polymorphic HLA-C as well as non-classical oligomorphic HLA-E, -F, and -G. (8) The interaction of maternal uterine NK (uNK) cells with the invading EVT is suggested to be essential for implantation and placentation. (9, 10) The killer-cell immunoglobin-like receptor (KIR) on uNK cells directly interacts with HLA-C molecules on the surface of EVTs to facilitate spiral artery remodeling. Certain combinations of maternal KIR and (paternally inherited) fetal HLA-C are associated with either enhancement or disruption of placentation, potentially resulting in an uncomplicated or preeclamptic pregnancy, respectively. (11, 12) Furthermore, pregnancies with an HLA-C mismatched child have been associated with an increased regulatory T cell population in the placenta. (13)

The degree of complete HLA compatibility between mother and child has been associated with pregnancy outcome and risk of complications. (14) Adequate maternal immunomodulation does require a certain extent of recognition of paternal antigens and therefore histo-incompatibility. Indeed, antigenic similarity among couples has been associated with an increased risk of preeclampsia, preterm stillbirth, and recurrent pregnancy losses. (15-17) Even though the trophoblast has a limited profile of HLA expression, indirect recognition of HLA peptides presented by maternal HLA class II may also play a role in T cell mediated immunomodulation. (18)

We previously showed no preferential selection of maternal-fetal HLA compatibility in uncomplicated naturally conceived pregnancies. (14) In contrast, the same study showed that increased total HLA, HLA class I and, especially, HLA-C compatibility is associated with preeclampsia. This suggests a role for HLA mismatches in immune regulation leading to uncomplicated pregnancy. However, to increase understanding of the biological mechanisms underlying maternal-fetal HLA (in)compatibility in human reproduction, studies on parental HLA sharing between closely related individuals, rather than larger random populations, are necessary. (19)

Hence, to further investigate the optimal degree of HLA compatibility for a successful pregnancy and the (clinical) consequences of increased HLA compatibility, we aimed to determine whether there is a preferential selection for HLA compatibility and specific KIR/HLA-C combinations in a Dutch genetically isolated population with a high inbreeding coefficient and its relation to pregnancy outcome, especially preeclampsia.

MATERIAL AND METHODS

Study design

This study was conducted as a case-control study nested within the DNA Analysis of Residents Within an Isolate in the Netherlands (DARWIN) cohort, a Dutch genetically isolated population. The village, founded in the 14th century by 7 to 20 families, has remained largely isolated due to strong social and religious cohesion. This isolation has resulted in a high degree of genetic homogeneity, with an inbreeding coefficient of F_{ROH} 1.3 (recent) & 3.1 (ancient), which is 6-14x greater than the F_{ROH} of the overall Dutch population. (20) The current population consists of approximately 22,500 inhabitants, with around 250 births annually.

The genetic homogeneity has led to an increased prevalence of rare genetic diseases caused by founder mutations, such as pontocerebellar hypoplasia type 2 (PCH2), fetal akinesia deformation sequence (FADS), rhizomelic chondrodysplasia punctata type 1 (RCDP1), and osteogenesis imperfecta type IIB/III (OI type IIB/III). (21, 22) Since 2012, inhabitants have been able to attend an outpatient clinic of the Department of Clinical Genetics at the Amsterdam University Medical Centers (Amsterdam UMC) for (preconception) carrier screening for these diseases.

Subjects

All women who attended the outpatient clinic between 2012 and 2022 were invited to participate. Exclusion criteria included pregnancies with children affected by the aforementioned hereditary diseases, twin pregnancies, pre-existing hypertension and pregnancies conceived via embryo-, sperm-, or oocyte donation.

The preconception care consultation included ancestry tracing up to 4 generations, resulting in a total of 16 ancestors per individual. The descent of the included children was quantified by counting the total number of the 16 ancestors that originated from the founder population. Women-child couples were selected if the combined founder descent mother and father of the child were at least 8/16 + 8/16 (i.e., half from each parent) or 4/16 + 16/16 (i.e., one parent entirely from founder descent and the other partially so).

We included the first ongoing pregnancy of all women with the 259 eligible pregnancies; this resulted in 175 pregnancies. The 125 uncomplicated pregnancies were defined as a pregnancy with no pregnancy-induced hypertension (PIH), preeclampsia, HELLP syndrome (pregnancy-associated syndrome characterized by hemolysis, elevated liver enzymes and low platelet count), preterm birth, fetal growth restriction, or intrauterine infection. We included 29 pregnancies complicated by PIH and 21 pregnancies complicated by preeclampsia. Preeclampsia was defined as previously described by the International Society for the study of Hypertension in Pregnancy (ISSHP), namely a new onset diastolic blood pressure ≥90 mmHg after 20 weeks of gestation or worsening of pre-existing hypertension together with proteinuria (>0.3g/L/24h, or EKR >30, or 2 times ++ on the qualitative dipstick) and/or organ dysfunction. (23) PIH was defined as the new onset of hypertension (blood pressure ≥140 mmHg systolic or ≥90 mmHg diastolic) at or after 20 weeks of gestation, in the absence of proteinuria or other findings suggestive of preeclampsia. We combined the cases with PIH or preeclampsia, classified as hypertensive complications. The flowchart of patient inclusion is depicted in Figure 1. All medical records were reviewed, and clinical data summarized. The study protocol was approved by the ethics committee of the LUMC (23-3033), and informed consent from all women was obtained.

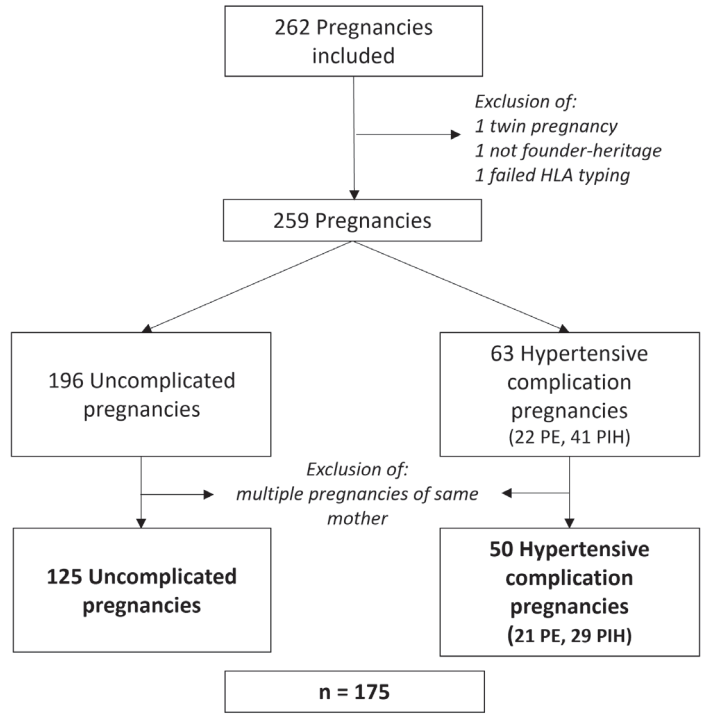

Figure 1. Flowchart of the patient inclusion process. *PE; preeclampsia, PIH; Pregnancy-induced hypertension.*

HLA and KIR typing

Maternal DNA samples, isolated from peripheral blood samples collected at the outpatient clinic for carrier screening, were stored at the department of Clinical Genetics at Amsterdam UMC. These maternal DNA samples were transferred to the Leiden University Medical Center (LUMC) for HLA typing. Saliva of the children was collected at a temporary outpatient clinic at the local midwifery practice from November 2022 to January 2023, using the Oragene DNA OG-500 and OG-575 collection kits for human DNA from saliva (DNA Genotek, Canada).

HLA genotyping was performed on the maternal and children's DNA samples as previously described by Van 't Hof et al. (14) DNA was isolated from the children's saliva samples using QIAsymphony (Qiagen, Germany). A Luminex bead-based Reverse Sequence Specific Oligonucleotides DNA-typing technique was used to type the samples for HLA-A, -B, -C, -DR, -DQA1, and -DQB1 loci (LIFECODES typing kits, Immucor, USA). The number of maternal-fetal HLA (mis)matches was calculated at the Dutch National Reference Laboratory for Histocompatibility Testing at the LUMC. HLA class I and class II compatibility was defined at the first field level. The total number of observed antigen matches, calculated on first field level, ranged from 5 to 10. HLA loci showing homozygosity in both mother and child were counted as respectively 1 match and 0 mismatch.

KIR genotyping was performed for all maternal DNA samples using RT-qPCR with sequence specific primers. DNA was amplified using the SYBR green protocol (Bio-Rad, USA). The following KIR genes were typed: 2DL1, 2DL2, 2DL3, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, and 3DS1. We assigned the KIR B haplotype in presence of one or more of the following genes: 2DL2, 2DL5, 2DS1, 2DS2, 2DS3, 2DS5 or 3DS1; group A was assigned when these genes were absent. The HLA-C1 or HLA-C2 group was assigned on basis of the presence of SER77ASN80 (C1) and ASN77LYS80 (C2).

PIRCHE-II analysis

Using the multi-patient solid organ transplantation module of the Predicted Indirectly ReCognizable HLA Class II Epitopes (PIRCHE-II) algorithm version 3.3.30 (PIRCHE AG, Berlin, Germany), both HLA class I and II peptides of the 'donor' (child) can be identified that could be presented by HLA class II of the 'recipient' (mother). In this case, by calculating the number of fetal HLA class I- and class II-derived peptides that could be presented on maternal HLA class II, fetal HLA-derived epitopes were identified. HLA-A, -B, -C, -DRB1, -DRB3/4/5, and -DQB1 were taken into consideration as presented loci.

PIRCHE-II peptides and their weights were calculated based on the 2011 NMDP super population. (24) The PIRCHE-II score of an individual is equal to the number of predicted

PIRCHE-II peptides. PIRCHE-II scores were determined by calculating the sum of all estimated PIRCHE-II peptides.

Statistical analysis

Genotypic frequencies of maternal and fetal HLA were examined for Hardy-Weinberg equilibrium (Hardy, 1908; Weinberg, 1963) with Pypop Software 0.7.0. (25) All other statistical analyses were performed using SPSS Statistics 25 (IBM SPSS Software).

The expected number of HLA-(mis)matches for each pregnancy was determined by randomization of the paternal HLA genotype, as schematically illustrated in Figure 2. First, the paternal allele was deduced by comparing the maternal- and fetal genotype for HLA-A, -B, -C, -DR, and -DQ loci. In case of identical heterozygous HLA genotypes for both mother and child, the classification between maternally or paternally inherited gene was randomized. Second, the paternally inherited haplotype of one pregnancy was randomly assigned to another pregnancy while keeping the maternal genotype and maternally inherited fetal alleles unchanged. This process was repeated for all cases and performed in triplicate for each child, thus generating three "artificial" fetal genotypes per case, each combining the original maternal contribution with a randomly assigned paternal haplotype derived from another fetus in the cohort. Third, the number of HLA (mis)matches of the three complete artificial fetal HLA genotypes, in relation to the maternal genotype, were calculated by direct counting. Finally, the expected number of HLA (mis)matches was determined as the average of the HLA (mis)matches of the three artificial genotypes. The data of observed and expected HLA compatibility was analyzed per locus separately.

The analysis described was repeated for the KIR/HLA-C combinations; maternally and paternally inherited HLA-C genotypes were randomly divided in triplicate over the cases and combined with the fixed KIR genotypes. Expected values were calculated for all possible KIR/HLA-C combinations and for paternally inherited HLA-C genotypes only. The Chi-squared test was used to examine the differences between the expected and observed degree of HLA (in)compatibility and KIR/HLA-C combinations. The Chi-squared test, Mann–Whitney-U test, or Fisher's exact test were used where appropriate to evaluate differences between the groups. The Bonferroni method was applied to correct p-values for multiple testing. A p-value of <0.05 was considered statistically significant.

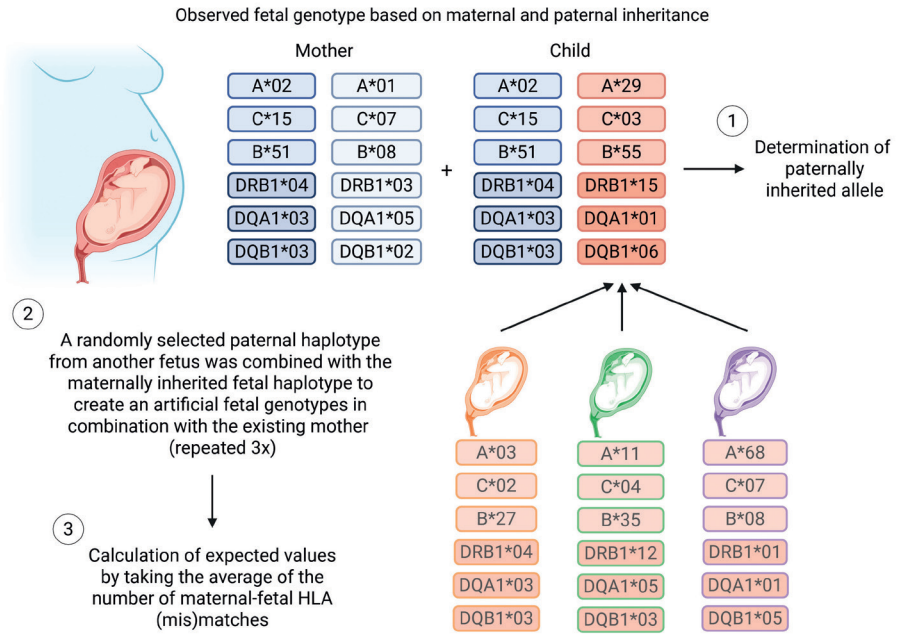

Figure 2. Visual presentation of the randomization-based generation of expected maternal-fetal HLA (mis)matching values. Paternally inherited alleles were determined for all but two cases. This analysis was repeated for KIR/HLA-C combinations, using triplicate randomization of HLA-C genotypes combined with fixed maternal KIR types. Expected values were calculated for all combinations and for paternal HLA-C only. Figure created in https://BioRender.com.

RESULTS

Demographic and baseline characteristics

Demographic and baseline characteristics of the included pregnancies (n=175) are depicted in Table 1. The gestational age, highest diastole and birth weight were significantly different for pregnancies with hypertensive complications compared to uncomplicated pregnancies; inherent to the clinical course of PIH and/or preeclampsia. The age distribution of uncomplicated pregnancies was slightly wider and shifted to older age. A high frequency of (recurrent) pregnancy losses was observed in the included women; the proportion of women with a history of one or more pregnancy losses was 38.9% among the total 175 women. Of these, 35.3% had experienced recurrent pregnancy losses (≥2). This corresponded to 13.7% of the total study population (24 out of 175 women). No differences were observed in the number of total pregnancy losses, the occurrence of one or more losses, or recurrent pregnancy losses between women included with uncomplicated pregnancies and those with hypertensive complications.

Table 1. Demographic and baseline characteristics.

		Uncomplicated (n = 125)	**Hypertensive complications (n = 50,** including preeclampsia**)**	***Preeclampsia (n=21)***	**P-value (Hypertensive complications vs. Uncomplicated)**
Pregnancy					
Gestational Age in days, median (range)		280 (259-293)	270.5 (203-291)	*261 (203-289)*	< 0.001[$]
Mode of delivery, amount (%)					
Spontaneous		102 (81.6)	36 (72)	*14 (66.7)*	0.194[#]
Primary caesarean section		14 (11.2)	6 (12)	*4 (19)*	
Secondary caesarean section		9 (7.2)	8 (16)	*3 (14.3)*	
Highest diastole (mmHg), median (range)		76 (60-100)	100(74-115)	*100 (80-115)*	< 0.001[$]
Mother					
Maternal age (years), median (range)		29 (22-41)	28 (22-39)	*27 (22-34)*	0.006[$]
BMI 1st trimester, median (range)		24 (17.8-38.5)	24.8 (18.25-40)	*25 (18-37)*	0.184[$]
Gravidity, median (range)		1 (1-5)	1 (1-4)	*1 (1-3)*	0.297[$]
Parity, median (range)		0 (0-3)	0 (0-3)	*0 (0-3)*	0.448[$]
Pregnancy losses					
Total pregnancy losses	38.9%	45	22		0.159
Women with 1 or more pregnancy losses	35.3%	45	23		0.146
Recurrent pregnancy losses (≥2)	58.2%	16	8		0.584
Primary	41.8%				
Secondary					
Child					
Gender, %		43.2	48	*47.6*	0.564[#]
Female		56.8	52	*52.4*	
Male					
Birth weight (gram), median (range)		3524 (2555-4925)	3148 (870-4426)	*2920 (870-3982)*	< 0.001[$]
Birth weight below 5th percentile, %		0.0	14	*33.3*	

Table 1. Continued.

	Uncomplicated (n = 125)	Hypertensive complications (n = 50, including preeclampsia)	*Preeclampsia (n=21)*	P-value (Hypertensive complications vs. Uncomplicated)
Heritage$^{\Omega}$, %				
16/16	61.6	48	*47.6*	0.096$^{\#}$
14/16	8	22	*28.6*	
12/16	19.2	28	*19*	
8/16	4.8	2	*4.8*	

#p-value calculated with Chi-square test.
$p-value calculated with Mann-Whitney-U test. p < 0.05 is considered significant.
ΩHeritage being the amount of ancestors originating from the founder village until 4th generation.

Genotypic frequencies

Genotypic frequencies of the HLA alleles (including the HLA-C1 and -C2 group) from all mothers and children were tested for the Hardy-Weinberg equilibrium. There was no significant deviation from the Hardy-Weinberg equilibrium, suggesting a random appearance of the genotypic frequencies of all the HLA alleles in this population (data not shown). The children from the uncomplicated pregnancies showed an increased percentage of homozygosity for HLA-DQB1 compared to their mothers, resulting in an odds ratio of 2.70 (Bonferroni-corrected P=0.011, Supplementary Table 1). The homozygous HLA frequencies of the mother with uncomplicated pregnancies of the genetically isolated population was compared to mothers with uncomplicated pregnancies of a larger general Dutch population studied previously (Supplementary Table 2). (14) All expected percentages of homozygosity were higher in the genetically isolated population compared to the larger general population. The Hardy-Weinberg equilibrium and homozygosity analyses were repeated for only those pregnancies of which the child had 16/16 founder heritage, in which no significant differences were observed (data not shown).

Maternal-fetal HLA compatibility in (un)complicated pregnancies shows no deviation from expected patterns

We explored the possibility of a preferential selection for maternal-fetal HLA matching in this genetically isolated population by comparing the amount of observed HLA matches to the amount of expected HLA matches for the uncomplicated pregnancies and pregnancies with hypertensive complications (Table 2). We did not observe significant differences within or between the pregnancy groups, including sub-analyses for preeclamptic cases only (data not shown).

Table 2. Observed and expected-by-chance amount of maternal-fetal HLA matches in uncomplicated pregnancies and pregnancies with hypertensive complications.

	Uncomplicated (n = 125)					Hypertensive complications (n = 50)					
	Observed (nr. of matches)		**Expected** (nr. of matches)		***P-value****	**Observed** (nr. of matches)		**Expected** (nr. of matches)		***P-value****	***P-value*** (observed vs. observed)$^{\#}$*
	1	2	1	2		1	2	1	2		
HLA-A	82.4%	17.6%	86.1%	13.9%	*1.000*	94.0%	6.0%	85.3%	14.7%	*0.583*	*0.333*
HLA-B	93.6%	6.4%	88.5%	11.5%	*0.528*	88.0%	12.0%	90.7%	9.3%	*1.000*	*1.000*
HLA-C	87.2%	12.8%	83.2%	16.8%	*1.000*	86.0%	14.0%	84.0%	16.0%	*1.000*	*1.000*
HLA-DRB1	87.2%	12.8%	86.1%	13.9%	*1.000*	88.0%	12.0%	86.0%	14.0%	*1.000*	*1.000*
HLA-DQB1	80.8%	19.2%	75.7%	24.3%	*1.000*	88.0%	12.0%	76.0%	24.0%	*0.329*	*1.000*
HLA-DQA1	81.6%	18.4%	78.4%	21.6%	*1.000*	78.0%	22.0%	82.0%	18.0%	*1.000*	*1.000*

*p-values calculated by Chi-square analysis. P-values are corrected for multiple analysis (six HLA loci typed and C1/C2 group analysis) using the Bonferroni method.
Comparing uncomplicated pregnancies with hypertensive complications. p < 0.05 is considered as significant.

Pregnancies with hypertensive complications are associated with higher maternal-fetal HLA-DQB1 mismatching

In contrast to maternal-fetal HLA matches, a significantly higher amount of mismatches for HLA-DQB1 was observed than was expected-by-chance (Bonferroni-corrected P=0.017) in pregnancies with hypertensive complications (Table 3). The same trend was seen in a sub-analysis for pregnancies complicated by preeclampsia only. P-values were not calculated due to sample size (n=21, Supplementary Table 3). No differences were found between the groups or for the uncomplicated pregnancies when comparing the observed and expected-by-chance numbers of HLA mismatches.

Table 3. Observed and expected-by-chance amount of maternal-fetal HLA mismatches in uncomplicated pregnancies and pregnancies with hypertensive complications.

	Uncomplicated (n = 125)					Hypertensive complications (n = 50)					
	Observed (nr. of mismatches)		**Expected** (nr. of mismatches)		***P-value****	**Observed** (nr. of mismatches)		**Expected** (nr. of mismatches)		***P-value****	***P-value**** *(observed vs. observed)#*
	0	1	0	1		0	1	0	1		
HLA-A	36.8%	63.2%	32.8%	67.2%	*1.000*	28.0%	72.0%	37.3%	62.7%	*1.000*	*1.000*
HLA-B	24.0%	76.0%	21.1%	78.9%	*1.000*	22.0%	78.0%	20.7%	79.3%	*1.000*	*1.000*
HLA-C	36.0%	64.0%	40.8%	59.2%	*1.000*	38.0%	62.0%	39.3%	60.7%	*1.000*	*1.000*
HLA-DRB1	28.0%	72.0%	26.1%	73.9%	*1.000*	24.0%	76.0%	28.0%	72.0%	*1.000*	*1.000*
HLA-DQB1	50.4%	49.6%	48.3%	51.7%	*1.000*	34.0%	66.0%	55.3%	44.7%	*0.017*	*0.344*
HLA-DQA1	52.0%	48.0%	50.4%	49.6%	*1.000*	50.0%	50.0%	50.7%	49.3%	*1.000*	*1.000*

*p-values calculated by Chi-square analysis. P-values are corrected for multiple analysis (six HLA loci typed and C1/C2 group analysis) using the Bonferroni method.
Comparing uncomplicated pregnancies with hypertensive complications. p < 0.05 is considered as significant.

KIR/HLA-C combination frequencies do not significantly differ within and between pregnancy groups

We analyzed maternal KIR and fetal HLA-C genotype frequencies in both pregnancy groups to determine if certain combinations occurred differently than what would be expected-by-chance (Table 4). Overall analysis of KIR/HLA-C genotype combinations, including paternally inherited HLA-C alleles only, showed no differences in observed compared to expected-by-chance frequencies for both pregnancy groups and in comparing observed values between the groups. No differences were seen in the comparison between uncomplicated and preeclamptic pregnancies for KIR/HLA-C genotype combination frequencies and frequencies of activating and inhibiting maternal KIR receptors (Supplementary Table 4 and 5).

Table 4. Observed and expected-by-chance KIR/HLA-C combinations for uncomplicated pregnancies and pregnancies with hypertensive complications.

	Uncomplicated (n = 122)$			Hypertensive complications (n = 50)			
	Observed (%)	**Expected** (%)	***P-value****	**Observed** (%)	**Expected** (%)	***P-value****	***P-value**** *(observed vs. observed)#*
Genotype							*0.794*
KIR AA/HLA C1	23.8	22.1	*0.900*	18.0	21.3	*0.970*	
KIR AA/HLA C2	18.9	18.3		20.0	19.3		
KIR Bx/HLA C1	58.2	61.5		62.0	60.0		
KIR Bx/HLA C2	41.8	54.3		50.0	52.0		
Paternally inherited C1/C2 only							*0.452*
KIR AA/HLA C1	17.9	20.7	*0.982*	10.0	14.0	*0.643*	
KIR AA/HLA C2	9.8	10.6		16.0	12.0		
KIR Bx/HLA C1	41.5	42.0		42.0	38.0		
KIR Bx/HLA C2	30.9	30.6		32.0	36.0		

*p-values calculated by Chi-square analysis. P-values are uncorrected, since all p-values are non-significant correction has been omitted.
Comparing uncomplicated pregnancies with hypertensive complications.
$ For 2 samples, no C1 or C2 group could be allocated and for 1 sample KIR A/B count not be determined; those were excluded for the KIR/HLA analysis.

The increased maternal-fetal HLA-DQB1 mismatches in pregnancies with hypertensive complications is reflected in the PIRCHE-II scores

To predict the potential immunogenic effect of the maternal-fetal HLA mismatches and the role of T cell epitope recognition in pregnancies with hypertensive complications, the PIRCHE-II scores of the immunizing paternal haplotypes were compared with uncomplicated pregnancies (Figure 3). The PIRCHE-II scores of HLA-DQB1 (Bonferroni-corrected P=0.059) and HLA class II (Bonferroni-corrected P=0.054) tended to be higher in the group with hypertensive complications, while this was not observed for other HLA loci, HLA class I or total HLA.

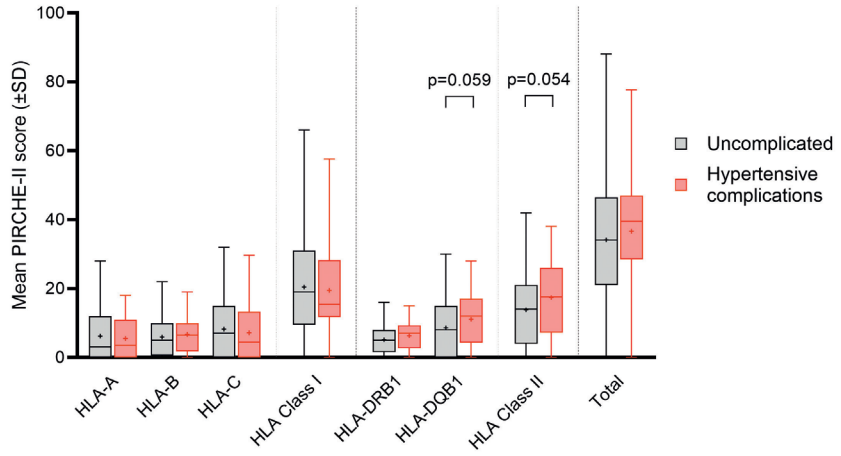

Figure 3. Comparison of PIRCHE-II scores between pregnancy groups per HLA locus. P-values are calculated using the Mann-Whitney-U test. P <0.05 is considered as significant.

DISCUSSION

The complex nature of the interaction between the maternal immune system and the semi-allogenic fetus during pregnancy and how this relates to reproductive complications continues to be a puzzling phenomenon. In the present study we investigated whether there is a preferential selection for maternal-fetal HLA compatibility in pregnancy and its contribution to pregnancy-induced hypertension and/or preeclampsia in a Dutch genetically isolated population. No preferential selection for maternal-fetal HLA compatibility was observed in the uncomplicated pregnancies. A significant increase in observed versus expected maternal-fetal HLA-DQB1 mismatches was found in pregnancies with hypertensive complications. In line with this finding, higher PIRCHE-II scores (prediction of T cell epitopes; mismatched fetal HLA-derived peptides that can be presented by maternal HLA class II) were observed for HLA-DQB1 and HLA class II in pregnancies with hypertensive complications compared to uncomplicated pregnancies. No differences were found in analyses on KIR/HLA-C combination frequencies, including paternally inherited HLA-C alleles only.

Alongside our finding of an association between maternal-fetal HLA-DQB1 mismatching and hypertensive complications in pregnancy within this genetically isolated population, there was a significantly higher prevalence of homozygosity for HLA-DQB1 among the children of the uncomplicated pregnancies, thus linking HLA-DQB1 compatibility to favorable pregnancy outcomes. Several other studies have reported a link between HLA-DQ alleles and hypertensive pregnancy complications. Honda et al. found a higher frequency of

HLA-DQB1*04 in preeclamptic women of Japanese origin compared to controls and they speculated that HLA-DQB1*04-related amino acid residues may induce Th1 predominance; a characteristic of preeclampsia. (26, 27) In the present study, only 5 women were included with the HLA-DQB1*04 allele, and therefore analysis of its association with preeclampsia could not be confirmed. In addition, HLA-DQB1*02 has been suggested to be in linkage disequilibrium with an HLA-G polymorphism, which is associated with preeclampsia and recurrent pregnancy loss and affects the immunomodulatory function of HLA-G in the placenta. (28)

Our study shows a significant difference in observed and expected maternal-fetal compatibility in pregnancies with hypertensive complications for the HLA-DQB1 locus, but not for HLA-DRB1 or HLA-DQA1, despite the strong linkage disequilibrium of these loci. Similarly, in studies on solid organ transplantation, HLA-DQ mismatching is the most immunogenic and potentially most pathogenic mismatch compared to other targets. (29) This may be due to both the α and the β chains being polymorphic, in contrast to HLA-DR, which leads to increased HLA-DQ antibody production. (29) Interestingly, PIRCHE-II scores for HLA class II were similarly increased to those of HLA-DQB1. This suggests that there is no direct translation from HLA match analysis to epitope presentation, as certain HLA-derived epitopes—specifically HLA-DQ—are more immunogenic than others. (30, 31)

Other studies have found associations between other HLA class II alleles and preeclampsia. In a Danish cohort, the HLA-DPB1*04:01:01G allele was more frequent in preeclamptic women, while their offspring showed a lower frequency of the DQA1*01:02:01G allele. (32) DPB1*04:01 is predicted to have low immunogenicity, meaning it has low T-cell epitope self-tolerance. Paradoxically, this makes the maternal immune system more likely to recognize mismatched T-cell epitopes in the child as foreign, potentially increasing the risk of an immunogenic response. (33) While trophoblasts normally do not express HLA class II molecules, aberrant expression of HLA-DR by the syncytiotrophoblast has been observed in placentas of women with preeclampsia. (34) A consequent systemic effect might be enforced as the same research group found circulating syncytiotrophoblast-derived extracellular vesicles with HLA-DR in preeclamptic women. (35) In response to placental inflammation, upregulated IFN-γ may induce HLA class II expression. (36)

Even when trophoblasts do not express HLA class II molecules, the mismatched HLA can still be presented by antigen-presenting cells to maternal $CD4^+$ T cells through epitopes presented by maternal HLA class II. For this reason, the potential effect of such T cell epitopes was explored, reflected by the PIRCHE-II scores. A clear trend of increased PIRCHE-II scores for HLA class II, and HLA-DQB1 specifically, was observed in pregnancies with hypertensive complications. Following kidney transplantation, PIRCHE-II

scores for class II-derived peptides have been associated with T-cell mediated rejection. (37) Furthermore, it has been shown that antibodies to HLA mismatches are specific for epitopes rather than antigens. (38) We hypothesized that maternal-fetal HLA-DQB1 mismatches can result in increased indirect $CD4^+$ T-cell allorecognition and negatively affecting the balance of effector and regulatory T cell populations. Multiple studies have shown that HLA mismatching affects $CD4^+$ T cell populations in pregnancy. For instance, maternal-fetal HLA-C mismatching is associated with increased numbers of $CD4^+CD25^{dim}$ activated T cells and functional $CD4^+CD25^{bright}$ regulatory T cells at the maternal-fetal interface. (13) T cells with direct specificity for paternal allogeneic MHC, expressed by mouse trophoblast cells, have been observed in mice. (39) Interestingly, whether a mismatched T cell epitope leads to increased effector or regulatory T cells may depend on the specific mismatch. (30) Although we did not find a clear relationship between HLA-C matching and preeclampsia in this genetically isolated population, this may be due to factors such as the broader classification of hypertensive complications, which groups gestational hypertension and preeclampsia together, as well as the relatively small sample size of preeclampsia cases. These limitations could have diluted the results, making it harder to detect a robust association. Additionally, the heterogeneity of preeclampsia itself should be considered. (40) However, the present study showed similar trends to previous research in (paternally-derived) HLA-C2. (11, 12, 14) Larger cohort studies and replication in other inbred populations are needed to confirm these findings. It is hypothesized that the difference between the potential advantageous or disadvantageous effect of HLA mismatching strongly depends on the HLA loci and the corresponding effect on the maternal immune system. Disproportional populations of regulatory and effector T cells are a well-recognized features of preeclampsia. (41, 42) T cell responses to maternal-fetal HLA incompatibility appear to be crucial in its pathophysiology, emphasizing the importance of T cells developing into the appropriate phenotype to prevent an aberrant immune response.

It remains unknown if increased predicted $CD4^+$ T cell recognition leads to B cell activation and subsequent antibody production. Oocyte donation (OD) pregnancies, characterized by higher HLA mismatching and an increased risk of PIH and preeclampsia, provide a model to study maternal-fetal interactions. (43) While uncomplicated OD pregnancies show increased HLA antibody production related to HLA-DR mismatches, preeclamptic cases were linked to HLA class II mismatches without corresponding antibody levels, suggesting a cellular rather than humoral immune mechanism. (44, 45) Compared to the current genetically isolated population, OD introduces the artificial scenario of high HLA incompatibility. Interestingly, in a comparable study on OD pregnancies, we observed higher maternal-fetal HLA matching than expected-by-chance and similar to that seen in naturally conceived pregnancies, which was associated with uncomplicated pregnancies.

(46) Thus, a certain degree of HLA matching could potentially enhance the chance of a successful (OD) pregnancy.

The results of this study suggest that HLA allele frequencies appear randomly in uncomplicated pregnancies, with no deviation from Hardy-Weinberg equilibrium, even in a genetically isolated population. (14) Despite suggested increased parental HLA similarity, the HLA haplotypes within the studied population seem to be sufficiently polymorphic to not require preferential selection on HLA (in)compatibility for successful pregnancies. Nevertheless, the incidence of pregnancy-related hypertensive complications remains unknown for this specific population. The present methodology of retrospective selection of pregnancies with live birth eliminates possible effects of maternal-fetal HLA compatibility on conception. However, we did notice a remarkably high frequency of (recurrent) pregnancy losses in this genetically isolated population, suggesting an advantage of greater disparity between maternal and paternal HLA genotypes for ongoing pregnancy. (47) Noteworthy, the four autosomal-recessive genetic disorders that occur relatively frequently in the studied population are not related to an increased rate of pregnancy losses. Previous studies have reported similar findings, linking recurrent pregnancy loss to an increased degree of parental HLA sharing. (14, 26) However, the data remain inconsistent due to heterogeneous patient populations, varying HLA typing methods, and other contributing factors.

A limitation of this study lies in its sample size. For preeclampsia, p-values and some percentages are not provided due to the small study arm of 21 participants, where a single individual accounts for nearly 5% of the difference, making it difficult to draw strong conclusions from these numbers. Nevertheless, this sample size is relatively large for this specific population and compares favorably to other studies in the field. Furthermore, we selected the composite outcome of hypertensive complications because PIH and preeclampsia share a common pathophysiology, with PIH being part of the diagnostic criteria for preeclampsia or as potential precursor. (2)

Maternal-fetal HLA class II mismatching may be one of the contributing factors in the cascade leading to preeclampsia, serving as an initiating factor or an additional trigger that escalates processes, depending on other factors that determine whether it progresses to clinically symptomatic disease. As discussed, there is a diversity of associations between specific HLA alleles and preeclampsia that varies by population, further underscoring their role in the pathophysiology of the disease. This highlights the need for more in-depth HLA typing; improvements have been made over the years, and it is important to note that each (mis)match is immunogenically distinct. Future studies may also benefit from incorporating non-classical HLA class Ib molecules (e.g. HLA-G, and -E), which are

known to play key roles in immune tolerance and placental development through NK and T cell interactions at the fetal-maternal interface. (48) Additionally, accumulating evidence shows that preeclampsia is a heterogeneous disease, suggesting the necessity for classification into several subtypes, as this heterogeneity dilutes the effects and leads to incomparable cohorts. (49, 50) Furthermore, while PIH may not always serve as a precursor to preeclampsia, thus influencing its impact, we observed similar trends in gestational hypertension as we did in preeclampsia alone.

In conclusion, understanding the underlying mechanisms of maternal-fetal immune interactions is crucial for elucidating the pathways that lead to both healthy and complicated pregnancies. This study's findings on high maternal-fetal HLA-DQB1 mismatches emphasize its potential role in hypertensive complications and preeclampsia, suggesting that mismatching of specific HLA alleles may influence pregnancy outcomes. These insights can significantly contribute to risk assessment for preeclampsia and may help inform future research and clinical approaches.

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APPENDIX

Supplementary Table 1. Analysis of Hardy-Weinberg equilibrium for uncomplicated pregnancies and pregnancies with hypertensive complications.

Uncomplicated (n=125)	Mothers						Children					
Locus	homozygotes		heterozygotes		Odds Ratio	95% C.I.		Chi square test		homozygotes		heterozygotes	
	N	perc.	N	perc.		lower	upper	P	Pc	N	perc.	N	perc.
HLA-A	24	19.2%	101	80.8%	1.00	0.533	1.877	1.000	1.000	24	19.2%	101	80.8%
HLA-B	10	8.0%	115	92.0%	2.46	1.111	5.431	0.026*	0.154	22	17.6%	103	82.4%
HLA-C	19	15.2%	106	84.8%	1.69	0.888	3.199	0.110	0.661	29	23.2%	96	76.8%
HLA-DRB1	10	8.0%	115	92.0%	2.06	0.917	4.633	0.079	0.474	19	15.2%	106	84.8%
HLA-DQB1	18	14.4%	107	85.6%	2.70	1.441	5.044	0.002*	0.011**	39	31.2%	86	68.8%
HLA-DQA1	34	27.2%	91	72.8%	1.35	0.788	2.327	0.272	1.000	42	33.6%	83	66.4%
Hypertensive complications (n=50)	**Mothers**						**Children**						
Locus	homozygotes		heterozygotes		Odds Ratio	95% C.I.		Fisher's exact test (2-tailed)		homozygotes		heterozygotes	
	N	perc.	N	perc.		lower	upper	P	Pc	N	perc.	N	perc.
HLA-A	7	14.0%	43	86.0%	1.73	0.611	4.912	0.436	1.000	11	22.0%	39	78.0%
HLA-B	6	12.0%	44	88.0%	0.81	0.232	2.865	1.000	1.000	5	10.0%	45	90.0%
HLA-C	12	24.0%	38	76.0%	1.00	0.399	2.504	1.000	1.000	12	24.0%	38	76.0%
HLA-DRB1	2	4.0%	48	96.0%	3.27	0.627	17.072	0.269	1.000	6	12.0%	44	88.0%
HLA-DQB1	8	16.0%	42	84.0%	1.48	0.540	4.064	0.611	1.000	11	22.0%	39	78.0%
HLA-DQA1	15	30.0%	35	70.0%	0.91	0.382	2.154	1.000	1.000	14	28.0%	36	72.0%

C.I. = Confidence Interval; Pc = Corrected P-value (Bonferroni); * = significant before correction; ** = significant after correction.

Supplementary Table 2. Comparison of homozygosity between the genetically isolated population and a larger random Dutch population.

Maternal homozygosity in uncomplicated pregnancies								
Locus	Genetically isolated population (n = 125)				Larger random Dutch population (n = 452)			
	Observed	%	Expected	%	Observed	%	Expected	%
HLA-A	24	19.2	25.8	20.6	76	16.8	74.6	16.5
HLA-B	10	8.0	14.1	11.3	35	7.7	39.0	8.6
HLA-C	19	15.2	26.8	21.4	64	14.2	79.1	17.5
HLA-DRB1	10	8.0	16.8	13.5	55	12.2	52.8	11.7
HLA-DQB1	18	14.4	32.6	26.0	115	25.6	107.4	23.9
HLA-DQA1	34	27.2	39.1	31.3	x	x	x	x

Supplementary Table 3. Number of maternal-fetal HLA (mis)matches for pregnancies complicated by preeclampsia.

Pregnancies complicated by preeclampsia (n = 21)								
	Observed (nr. of matches)		Expected (nr. of matches)		Observed (nr. of mis-matches)		Expected (nr. of mis-matches)	
	1	*2*	*1*	*2*	*0*	*1*	*0*	*1*
HLA-A	21	0	18.7	2.3	6	15	7.0	14.0
HLA-B	20	1	19.0	2.0	5	16	5.3	15.7
HLA-C	19	2	16.7	4.3	9	12	9.3	11.7
HLA-DRB1	18	3	17.7	3.3	8	13	6.7	14.3
HLA-DQB1	19	2	15.7	5.3	8	13	10.3	10.7
HLA-DQA1	17	4	17.3	3.7	12	9	12.0	9.0

The observed and expected (mis)matches are depicted as numbers.
No percentages or p-values are presented since the study arm consists of 21 participants. One individual makes up for almost 5% difference.

Supplementary Table 4. Observed and expected-by-chance KIR/HLA-C combinations for uncomplicated pregnancies and pregnancies complicated by preeclampsia

	Uncomplicated (n = 122)$		**Preeclampsia (n = 21)**	
	Observed (%)	**Expected** (%)	**Observed** (%)	**Expected** (%)
Genotype				
KIR AA/HLA C1	23.8	22.1	28.6	33.3
KIR AA/HLA C2	18.9	18.3	28.6	28.6
KIR Bx/HLA C1	58.2	61.5	52.4	47.6
KIR Bx/HLA C2	41.8	54.3	42.9	42.9
Paternally inherited C1/C2 only				
KIR AA/HLA C1	17.9	20.7	19.0	23.8
KIR AA/HLA C2	9.8	10.6	19.0	14.3
KIR Bx/HLA C1	41.5	42	38.1	33.3
KIR Bx/HLA C2	30.9	30.6	23.8	28.6

Due to small sample size, chi-squared analysis was not possible for the pregnancies complicated by preeclampsia. P-values for uncomplicated pregnancies are depicted in table 5.
$ For 2 samples, no C1 or C2 group could be allocated and for 1 sample KIR A/B count not be determined; those were excluded for the KIR/HLA analysis.

Supplementary Table 5. Frequency of activating and inhibiting KIR receptors.

		Uncomplicated (n = 125)	**Preeclampsia (n = 21)**	***P-value#***
Activating receptors	2DS1*	57.3 %	38.1 %	*0.154*
	2DS2	62.9 %	42.9 %	*0.096*
	2DS3	48.4 %	28.6 %	*0.103*
	2DS5	38.7 %	33.3 %	*0.809*
	3DS1*	58.1 %	38.1 %	*0.101*
Inhibiting receptor	2DL1	98.4 %	100 %	*1.000*
KIR B		72.6 %	61.9 %	*0.227*
KIR A		27.4 %	38.1 %	

* strong linkage disequilibrium. # P-values are calculated using the Fisher exact test. $p < 0.05$ is considered statistically significant. Data not shown for KIR2DL2, KIR2DL3, KIR2DL5, KIR3DL1. KIR2DL4 and KIR2DS4 were not included in the analysis, as these receptors are non-functional in 80% of Caucasians.