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A possible role for HLA-G in development of uteroplacental acute atherosis in preeclampsia

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ABSTRACT

HLA-G, a non-classical HLA molecule expressed by extravillous trophoblasts, plays a role in the maternal immune tolerance towards fetal cells. HLA-G expression is regulated by genetic polymorphisms in the 3′ untranslated region (3′UTR). Low levels of HLA-G in the maternal circulation and placental tissue are linked to preeclampsia.

Our objective was to investigate whether variants of the 3′UTR of the HLA-G gene in mother and fetus are associated with acute atherosis, a pregnancy specific arterial lesion of the decidua basalis that is prevalent in preeclampsia.

Paired maternal and fetal DNA samples from 83 normotensive and 83 preeclamptic pregnancies were analyzed. We sequenced the part of the HLA-G 3′UTR containing a 14-bp insertion/deletion region and seven single nucleotide polymorphisms (SNPs). Associations with acute atherosis were tested by logistic regression.

The frequency of heterozygosity for the 14-bp polymorphism (Ins/Del) and the +3142 SNP (C/G) variant in the fetus are associated with acute atherosis in preeclampsia (66.7 % vs. 39.6 %, $p = 0.039$, and 69.0 % vs. 43.4 %, $p = 0.024$). Furthermore, the fetal UTR-3 haplotype, which encompasses the 14-bp deletion and the +3142G variant, is associated with acute atherosis in preeclampsia (15 % vs. 3.8 %, $p = 0.016$).

In conclusion, HLA-G polymorphisms in the fetus are associated with acute atherosis. We hypothesize that these polymorphisms lead to altered HLA-G expression in the decidua basalis, affecting local feto-maternal immune tolerance and development of acute atherosis.

1. Introduction

Preeclampsia, a hypertensive complication in pregnancy, affects at least 3% of pregnancies and confers a high risk of maternal and fetal mortality and morbidity (Ghulmiyyah and Sibai, 2012). Although its pathophysiology has not been completely unraveled, it is generally accepted that development of preeclampsia is secondary to placental dysfunction (Redman and Staff, 2015). There are likely multiple underlying maternal risk factors and pathophysiological pathways at play, involving the genetic background of both the mother and the fetus (Hiby, 2004). These pathways may also be implicated in the development of acute atherosis, a pregnancy-specific lesion of the uteroplacental spiral arteries that is predominantly observed in preeclampsia (Khong, 1991; Staff, 2020).

Typically, acute atherosis comprises intramural lipid-filled foam cells, fibrinoid necrosis, and occasionally a perivascular mononuclear cell infiltrate (Robertson et al., 1976). Acute atherosis is associated with placental pathology, severe preeclampsia, and adverse pregnancy outcomes (Khong, 1991; Stevens et al., 2013; Kim et al., 2015). We hypothesize that acute atherosis is an inflammatory lesion, resulting from

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multiple mechanisms leading to excessive decidual inflammation (Staff et al., 2014). One mechanism potentially responsible for this excessive inflammation may involve reduced feto-maternal immune tolerance in the decidua basalis, a physiological process where HLA-G is thought to play a role.

HLA-G is a non-classical MHC Class I antigen. In the placental bed, its expression is predominantly restricted to extravillous trophoblasts (EVTs) (Kovats, 1990; Hackmon, 2017). It can also be found in other organs (thymus, pancreas, and cornea), immune and epithelial cells, and mesenchymal stem cells (Persson, 2017). Several types of cancer, viral infections, and inflammatory diseases are associated with aberrant HLA-G expression (Persson, 2017).

HLA-G has immune suppressive effects, inhibiting both innate and adaptive immune functions by binding to immune cell receptors (Carosella, 2015). Specifically, HLA-G promotes feto-maternal tolerance by interacting with decidual T-cells, NK-cells, macrophages, and dendritic cells (Djurisic and Hviid, 2014; Rizzo, 2014; Carosella, 2015; Ferreira, 2017). It can be either membrane-bound or soluble (Persson, 2017). During the 1st trimester of pregnancy the concentration of soluble HLA-G in maternal circulation increases five-fold, then declines towards term (Hunt, 2000; Steinborn, 2007; Klitkou, 2015). Relatively low levels of circulating HLA-G are associated with preeclampsia, recurrent miscarriages, and infertility (Persson, 2017). We recently confirmed this finding in early- and late-onset preeclampsia (Jacobsen, 2020). Relatively low levels of HLA-G in the placenta are also associated with preeclampsia (Hara, 1996; Goldman-Wohl, 2000; Yie, 2004, Yie, 2005; Hackmon, 2007; Rizzo, 2009; Tang, 2015).

While classical HLA class I is highly polymorphic, the HLA-G coding region is less polymorphic (Castelli, 2014). Genetic variability in the HLA-G gene is mainly confined to the promoter region and the 3 prime untranslated region (3′UTR) (Castelli, 2014). The 3′UTR contains polymorphisms that post-transcriptionally affect the level of HLA-G expression (Hviid, 2006; Castelli, 2014). Most studied are the 14-bp insertion/deletion region (14-bp InsDel) and the single nucleotide polymorphism (SNP) +3142 C/G. The 14-bp InsDel region may affect mRNA stability, and some studies report an association between the insertion at this genomic region and decreased levels of soluble HLA-G in plasma (Hviid, 2004; Chen, 2008; Martelli-Palomino, 2013). Similarly, presence of a G nucleotide at the +3142 SNP position is associated with reduced HLA-G expression, an association thought to be secondary to enhanced affinity of microRNA molecules that negatively regulate gene expression (Tan, 2007; Castelli, 2009; Rizzo, 2014).

Several studies have shown an association between HLA-G polymorphisms and the occurrence of preeclampsia (O'brien, 2001; Hylenius, 2004; Larsen, 2010; Quach, 2014; De Almeida, 2018) although the results are inconsistent (Iversen, 2008; Pabalan, 2015; Nilsson, 2016). In acute atherosis, the role of HLA-G has not yet been explored. Since acute atherosis occurs predominantly in preeclampsia, understanding the mechanisms behind acute atherosis could provide insight into the underlying pathophysiology of preeclampsia. The goal of the current study was to determine whether the 14-bp InsDel region and seven additional polymorphisms in the 3′UTR of the HLA-G gene are associated with acute atherosis.

2. Materials and methods

2.1. Patient inclusion, collection of biological material, and DNA isolation

Pregnant women were recruited prior to elective caesarian section after informed written consent, as previously described (Johnsen, 2018). We included paired mother and child samples from 166 pregnancies, diagnosed either with preeclampsia (PE, n = 83) or as normotensive (NT, n = 83). Pregnancies in each group were randomly selected. Preeclampsia was defined as new onset hypertension (blood pressure ≥140/90 mmHg) and new onset proteinuria (≥1+ on dipstick, and/or protein/creatinine ratio ≥30 mg/mmol (≥0.3 mg/mg)) at ≥20 weeks' gestation (Roberts, 2003). The majority of preeclamptic pregnancies had early-onset preeclampsia, defined as delivery prior to 34 weeks' gestation (Tranquilli, 2013). Clinical characteristics of the pregnancy groups are shown in Supplemental Table 1. The study was approved by the Regional committee for Medical and Health Research Ethics in South-Eastern Norway, and performed according to the Helsinki Declaration. Maternal and fetal sources of DNA, the DNA extraction protocol, and the decidua basalis collection method are outlined in the Supplemental Methods.

2.2. Acute atherosis evaluation

Acute atherosis was identified based on histological staining of 3-μm thick, formalin-fixed, paraffin-embedded decidua basalis tissue sections, as described in the Supplemental Methods. Acute atherosis was evaluated prior to genetic sequencing (Alnaes-Katjavivi et al., 2016). The rate of acute atherosis was 36 % (30/83) in preeclamptic pregnancies and 10 % (8/83) in normotensive pregnancies, comparable to the rates we previously found in our hospital population (Harsem et al., 2007; Alnaes-Katjavivi et al., 2016).

2.3. DNA sequencing of maternal and fetal HLA-G

We sequenced part of the HLA-G gene, specifically part of exon 8, which is found in the 3′UTR. The selected region contains the 14-bp insertion/deletion polymorphism (rs1704, +2961−2974 ATTTGTTCATGCCT) and seven distinct SNPs (+3003C/T (rs1707), +3010C/G (rs1710), +3027A/C (rs17179101), +3035C/T (rs17179108), +3142C/G (rs1063320), +3187A/G (rs9380142), and +3196C/G (rs1610696)). The forward primer hybridizes at 71 bases before the intron 7/exon 8 border at position +2854 (when the 14-bp insertion is present), and is 699 bp or 713 bp long depending on whether the 14-bp insertion is present or absent. The region was amplified and sequenced by the Leiden Genome Technology Center as previously described (Drabbels, 2020). The results were interpreted using SBT Engine Software (GenDx, Netherlands). Eight distinct HLA-G polymorphism haplotypes were identified based on descriptions by (Castelli, 2010).

2.4. Statistical analyses

Statistical analyses were performed using SPSS version 25.0 (IBM). For clinical characteristics, non-parametric Mann-Whitney U tests (continuous variables) or Pearson chi-squared tests (categorical variables) were used. Allele frequency and genotype frequency of the 14-bp polymorphism and the SNPs were calculated for fetal and maternal samples. Haplotypes were composed based on the eight polymorphisms (Drabbels, 2020). For the seven distinct HLA-G haplotypes, the haplotype frequency was calculated. Associations with acute atherosis were tested using logistic regression, presented as odds ratios (OR) with 95 % confidence intervals (CI). P-values < 0.05 were regarded as significant.

3. Results

3.1. HLA-G 3′UTR polymorphisms are not associated with preeclampsia

Preeclampsia, when compared to normotensive pregnancies, was not associated with any of the HLA-G 3′UTR polymorphisms in terms of allele frequency or genotype frequency, nor with any of the UTR haplotypes studied in the fetus (Supplemental Tables 2–4) or the mother (data not shown).

3.2. The genotype frequencies of the fetal 14-bp polymorphism and +3142 SNP are associated with acute atherosis

The allele frequencies of the fetal (Table 1) and the maternal (data not shown) 14-bp polymorphism and the seven SNPs analyzed were not

Table 1
Allele frequency of fetal HLA-G 3′UTR polymorphisms in acute atherosis (AA).

		Total cohort						Normotensive (NT) group						Preeclampsia (PE) group					
		AA-		AA+		OR	P	NTAA-		NTAA+		OR	P	PEAA-		PEAA+		OR	P
		2n = 256		2n = 76				2n = 150		2n = 16				2n = 106		2n = 60			
		Count	%	Count	%			Count	%	Count	%			Count	%	Count	%		
14-bp	**Del**	165	64.5	49	64.5	1.00	0.997	100	66.7	11	68.8	1.1	0.866	65	61.3	38	63.3	1.09	0.797
	Ins	91	35.5	27	35.5			50	33.3	5	31.3			41	38.7	22	36.7		
+3003	**C**	41	16.0	8	10.5	0.62	0.24	26	17.3	0	0.0	n.a.		15	14.2	8	13.3	0.93	0.884
	T	215	84.0	68	89.5			124	82.7	16	100.0			91	85.8	52	86.7		
+3010	**C**	108	42.2	38	50.0	1.37	0.229	63	42.0	9	56.3	1.78	0.279	45	42.5	29	48.3	1.27	0.464
	G	148	57.8	38	50.0			87	58.0	7	43.8			61	57.5	31	51.7		
+3027	**A**	16	6.3	3	3.9	0.62	0.452	11	7.3	1	6.3	0.84	0.874	5	4.7	2	3.3	0.70	0.672
	C	240	93.8	73	96.1			139	92.7	15	93.8			101	95.3	58	96.7		
+3035	**C**	229	89.5	70	92.1	1.38	0.499	133	88.7	15	93.8	1.92	0.541	96	90.6	55	91.7	1.15	0.812
	T	27	10.5	6	7.9			17	11.3	1	6.3			10	9.4	5	8.3		
+3142	**C**	147	57.4	36	47.4	0.67	0.123	86	57.3	7	43.8	0.58	0.303	61	57.5	29	48.3	0.69	0.253
	G	109	42.6	40	52.6			64	42.7	9	56.3			45	42.5	31	51.7		
+3187	**G**	160	62.5	50	65.8	1.15	0.602	93	62.0	9	56.3	0.79	0.654	67	63.2	41	68.3	1.26	0.506
	A	96	37.5	26	34.2			57	38.0	7	43.8			39	36.8	19	31.7		
+3196	**C**	193	75.4	56	73.7	0.91	0.763	118	78.7	12	75.0	0.81	0.735	75	70.8	44	73.3	1.14	0.723
	G	63	24.6	20	26.3			32	21.3	4	25.0			31	29.2	16	26.7		

The table shows the total patient group, the normotensive group (NT) and the preeclampsia group (PE) separately. Associations of the allele frequencies of the different polymorphisms with acute atherosis (AA) were tested using binary univariate logistic regression (*significant p-value, $p < 0.05$). OR, odds ratio; P, p-value; n.a., not applicable.

associated with acute atherosis.

The genotype frequency of the Ins/Del variant of the 14-bp polymorphism in the fetus was significantly associated with acute atherosis in preeclamptic pregnancies (Table 2). The fetal Ins/Del genotype was present in 66.7 % of PEAA + pregnancies compared to 39.6 % in PEAA- pregnancies (OR 9.52; 95 % CI 1.12–81.35; p-value 0.039).

The genotype frequency of the G/C variant of the fetal +3142 SNP was significantly associated with acute atherosis both in the total cohort and in preeclamptic pregnancies (Table 2). In the total cohort, the fetal G/C variant was present in 67.6 % of AA + pregnancies compared to 50.8 % AA- pregnancies (OR 3.15; 95 %CI 1.12–8.89; p-value 0.030). In preeclamptic pregnancies, the fetal GC variant was present in 69.0 % of PEAA + pregnancies compared to 43.4 % PEAA- pregnancies (OR 4.13; 95 %CI 1.20–14.81; p-value 0.024). The genotype frequencies of the maternal 14-bp polymorphism and the SNPs were not associated with acute atherosis (data not shown).

3.3. The fetal HLA-G UTR-3 haplotype is associated with acute atherosis

Next, we assessed whether HLA-G haplotype was related to clinical outcome. The frequency of the UTR-3 haplotype in the fetus, which is the only UTR haplotype containing the 14-bp deletion variant combined with the +3142G variant, was significantly associated with acute atherosis both in the total cohort and in preeclamptic pregnancies (Table 3). In the total cohort, the UTR-3 haplotype in the fetus was present in 17.1 % of AA + pregnancies compared to 7.0 % of AA- pregnancies (OR 2.73; 95 %CI 1.27–5.87; p-value 0.01). In preeclamptic pregnancies, the UTR-3 haplotype in the fetus was present in 15.0 % of PEAA + pregnancies compared to 3.8 % of PEAA- pregnancies (OR 4.5; 95 %CI 1.32–15.32; p-value 0.016).

Furthermore, we analyzed the diplotype frequency and found that the UTR-1/UTR-3 diplotype in the fetus was significantly associated with acute atherosis both in the total cohort and in normotensive pregnancies (Supplemental Table 5).

UTR haplotype frequencies in the mother were not associated with acute atherosis (data not shown).

3.4. Combined fetal and maternal HLA-G polymorphisms are not significantly associated with acute atherosis

To investigate whether the UTR-3 haplotype was associated with acute atherosis when present in the fetus and the mother concurrently, we created a univariate logistic regression model combining the presence versus absence of UTR-3 haplotype in the mother and the fetus in a categorical variable. The percentage of pregnancies where the fetus and the mother both had the UTR-3 haplotype was higher in pregnancies with acute atherosis in the total cohort and in normotensive and preeclamptic pregnancies (Table 4); however this association was not statistically significant. In the total cohort both the fetus and the mother had the UTR-3 haplotype in 21.1 % of AA + compared to 10.9 % in AA- pregnancies (OR 2.33; 95 % CI 0.88–6.20; p-value 0.089). In preeclampsia both the fetus and the mother had the UTR-3 haplotype in 16.7 % of PEAA + compared to 3.8 % in PEAA- pregnancies (OR 5.25; 95 % CI 0.94–29.44; p-value 0.059).

4. Discussion

In the present study, we investigated a 14-bp InsDel polymorphism and seven SNPs located in the 3′UTR of the HLA-G gene. We found that certain HLA-G polymorphisms in the fetus were associated with decidual acute atherosis. Furthermore, the 14-bp polymorphism and the seven SNPs are in strong linkage disequilibrium and are grouped in 43 different haplotypes based on these linkage associations (Castelli, 2010; Amodio and Gregori, 2020). In order to examine the significance of the combination of these polymorphisms relative to acute atherosis, we studied the most common of these haplotypes (UTR-1 to UTR-8). The remaining haplotypes are present in less than 1% of the population worldwide (Castelli, 2010). Overall, we demonstrated three main findings. First and secondly, acute atherosis was associated with the fetal genotype frequencies of the heterozygous Ins/Del of the 14-bp polymorphism and the heterozygous G/C variant of the +3142 SNP. Our third and most interesting finding was that acute atherosis was associated with the fetal UTR-3 haplotype, the only one of the eight haplotypes studied containing the 14-bp Del variant and the +3142G variant.

The 14-bp deletion is known to enhance mRNA stability, a feature that facilitates HLA-G protein expression (Castelli, 2014), while the insertion is linked to enhanced mRNA degradation and lower HLA-G expression (Castelli, 2009). This is in line with findings from prior studies that have linked the presence of the 14-bp insertion to decreased soluble HLA-G (Persson, 2017). The same goes for studies on membrane-bound HLA-G; the fetal Del/Del genotype is associated with higher HLA-G expression on trophoblasts compared to the Ins/Ins genotype (Djurisic, 2015). In summary, the evidence points towards the 14-bp insertion correlating with decreased HLA-G expression and the deletion with increased HLA-G expression, suggesting that the heterozygous genotype may be linked to intermediate expression of HLA-G. Our findings link the heterozygous 14-bp Ins/del polymorphism in the fetus with acute atherosis in preeclampsia. Unlike us, some studies report an association between the 14-bp Ins/Ins genotype and severe preeclampsia in primiparas (Hylenius, 2004; Larsen, 2010) and early-onset preeclampsia (Zhang, 2012). Other studies, however, report no association with preeclampsia (Iversen, 2008; Vianna, 2007), which is consistent with our findings.

The +3142 SNP was the other polymorphism for which we demonstrate an association with acute atherosis. *In silico* analyses predict that the +3142 G variant is related to decreased HLA-G expression (Tan, 2007). Consistent with this, a clinical study demonstrated that the +3142 CC genotype was associated with elevated sHLA-G levels in recipients of living-donor kidney transplants who had a higher susceptibility for cytomegalovirus infection (Guberina, 2017). Again, from this evidence in the literature one might infer that the heterozygous genotype of the +3142 SNP might be linked to an intermediate expression level of HLA-G. As for the clinical relevance of this polymorphism in pregnancy, we found that the heterozygous G/C variant of the +3142 SNP in the fetus is associated with acute atherosis in preeclampsia, but did not find an association between this SNP and preeclampsia overall. Other studies of the +3142 C/G SNP have shown that the GG genotype is associated with severe preeclampsia (Larsen, 2010).

We consider our finding that the fetal UTR-3 haplotype is associated with acute atherosis in preeclampsia to be the most interesting, as it speaks to the significance of the combination of polymorphisms in the context of pregnancy. We hypothesize that the association is linked to the impact this genetic combination has on HLA-G expression during pregnancy. UTR-3 is the only UTR haplotype of the eight most common haplotypes containing the 14-bp deletion combined with the +3142 G variant. In addition, the UTR-3 haplotype contains six other SNPs that are less studied, but that could still have an impact on HLA-G protein expression. In addition, we found that the frequency of the fetal UTR-1/UTR-3 diplotype was significantly associated with acute atherosis. We consider that this result is driven by the effect of the UTR-3 haplotype as UTR-1 is the most common haplotype in the population worlwide.

HLA-G expression is partly regulated by the polymorphisms in the 3′UTR (Castelli, 2011). These polymorphisms are associated with mRNA stability and protein expression (Persson, 2017). At present it is not clear whether the UTR-3 haplotype is associated with high or low expression of HLA-G. An association between the UTR-3 and low levels of soluble HLA-G was reported outside of pregnancy (Di Cristofaro, 2015). Another study in healthy males reported that UTR-3 was associated with intermediate plasma levels of sHLA-G (Martelli-Palomino, 2013), and yet other studies point to UTR-3 being associated with high levels of HLA-G expression in seminal plasma (Craenmehr, 2019), and in dendritic DC-10 cells (Amodio and Gregori, 2020).

Table 2
Genotype frequency of fetal HLA-G 3'UTR polymorphisms from pregnancies with acute atherosis (AA).

		Total cohort						Normotensive (NT) group						Preeclampsia (PE) group					
		AA- n = 83		AA + n = 83		OR	P	NTAA- n = 75		NTAA + n = 8		OR	P	PEAA- n = 53		PEAA + n = 30		OR	P
		Count	%	Count	%			Count	%	Count	%			Count	%	Count	%		
14-bp	**DelDel**	53	41.4	13	34.2	1.96	0.406	31	41.3	4	50.0	0.77	0.832	22	41.5	9	30.0	4.09	0.209
	InsDel	59	46.1	23	60.5	3.12	0.150	38	50.7	3	37.5	0.47	0.545	21	39.6	20	66.7	9.52	0.039*
	InsIns	16	12.5	2	5.3	Ref.	–	6	8.0	1	12.5	Ref.	–	10	18.9	1	3.3	Ref.	–
+3003	**CC**	2	1.6	0	0.0	n.a.	–	2	2.7	0	0.0	n.a.	–	0	0.0	0	0.0	n.a.	–
	CT	37	28.9	8	21.1	0.64	0.317	22	29.3	0	0.0	n.a.	–	15	28.3	8	26.7	0.92	0.873
	TT	89	69.5	30	78.9	Ref.	–	51	68.0	8	100.0	n.a.	–	38	71.7	22	73.3	Ref.	–
+3010	**CC**	22	17.2	7	18.9	0.89	0.807	11	14.7	2	25.0	1.49	0.658	11	20.8	5	17.2	0.58	0.384
	CG	64	50.0	23	62.2	Ref.	–	41	54.7	5	62.5	Ref.	0.528	23	43.4	18	62.1	Ref.	–
	GG	42	32.8	7	18.9	0.46	0.106	23	30.7	1	12.5	0.36	0.360	19	35.8	6	20.7	0.40	0.108
+3027	**AA**	0	0.0	0	0.0	n.a.	–	0	0.0	0	0.0	n.a.	–	0	0.0	0	0.0	n.a.	–
	AC	16	12.5	3	7.9	0.6	0.438	11	14.7	1	12.5	0.83	0.869	5	9.4	2	6.7	0.69	0.664
	CC	112	87.5	35	92.1	Ref.	–	64	85.3	7	87.5	Ref.	–	48	90.6	28	93.3	Ref.	–
+3035	**CC**	103	80.5	32	86.5	Ref.	–	59	78.7	7	87.5	Ref.	0.873	44	83.0	25	86.2	Ref.	–
	TC	23	18.0	5	13.5	0.7	0.503	15	20.0	1	12.5	0.56	0.603	8	15.1	4	13.8	0.88	0.847
	TT	2	1.6	0	0.0	n.a.	–	1	1.3	0	0.0	n.a.	–	1	1.9	0	0.0	n.a.	–
+3142	**CC**	41	32.0	5	13.5	Ref.	–	22	29.3	1	12.5	Ref.	–	19	35.8	4	13.8	Ref.	–
	GC	65	50.8	25	67.6	3.15	0.030*	42	56.0	5	62.5	2.62	0.393	23	43.4	20	69.0	4.13	0.024*
	GG	22	17.2	7	18.9	2.61	0.135	11	14.7	2	25.0	4.00	0.278	11	20.8	5	17.2	2.16	0.318
+3187	**GG**	58	45.3	22	57.9	Ref.	–	35	46.7	5	62.5	Ref.	0.691	23	43.4	17	56.7	Ref.	–
	AG	19	14.8	2	5.3	3.6	0.102	11	14.7	1	12.5	1.57	0.694	8	15.1	1	3.3	5.91	0.109
	AA	51	39.8	14	36.8	2.61	0.232	29	38.7	2	25.0	0.76	0.828	22	41.5	12	40.0	4.36	0.188
+3196	**CC**	75	58.6	20	54.1	Ref.	–	46	61.3	5	62.5	Ref.	0.560	29	54.7	15	51.7	Ref.	–
	CG	43	33.6	15	40.5	1.31	0.49	26	34.7	2	25.0	0.71	0.692	17	32.1	13	44.8	1.48	0.422
	GG	10	7.8	2	5.4	0.75	0.72	3	4.0	1	12.5	3.07	0.369	7	13.2	1	3.4	0.28	0.249

The table shows the total patient group, the normotensive group (NT) and the preeclampsia group (PE) separately. Associations of the genotype frequencies of the different polymorphisms with acute atherosis (AA) were tested using binary univariate logistic regression (*significant p-value, $p < 0.05$). The most common genetic variant was used as the reference for each variable, except for the 14-bp region and the +3142 SNP, where the reference value was set as the variant which in theory would give the highest level of HLA-G expression (i.e., DelDel for the 14-bp region and CC for the +3142 SNP) based on published studies. OR, odds ratio; P, p-value; n.a., not applicable.

Table 3
Haplotype frequencies of HLA-G 3'UTR polymorphism from pregnancies with acute atherosis (AA).

HLA-G 3'UTR Haplotypes									Total cohort						Normotensive (NT) group (n = 83)						Preeclampsia (PE) group (n = 83)					
									AA-		AA+		OR	P	NTAA-		NTAA+		OR	P	PEAA-		PEAA+		OR	**P**
	14-bp	+3003	+3010	+3027	+3035	+3142	+3187	+3196	2n = 256		2n = 56				2n = 150		2n = 16				2n = 106		2n = 60			
									Count	%	Count	%			Count	%	Count	%			Count	%	Count	%		
UTR-1	Del	T	G	C	C	C	G	C	94	36.7	26	34.2	0.90	0.689	56	37.3	7	43.8	1.31	0.616	38	35.8	19	31.7	0.83	0.586
UTR-2	Ins	T	C	C	C	G	A	G	61	23.8	19	25.0	1.07	0.834	32	21.3	4	25.0	1.23	0.735	29	27.4	15	25.0	0.89	0.741
UTR-3	Del	T	C	C	C	G	A	C	18	7.0	13	17.1	2.73	0.01*	14	9.3	4	25.0	3.24	0.067	4	3.8	9	15.0	4.50	0.016*
UTR-4	Del	C	G	C	C	C	A	C	40	15.6	8	10.5	0.64	0.27	26	17.3	0	0.0	n.a.	n.a.	14	13.2	8	13.3	1.01	0.982
UTR-5	Ins	T	C	C	T	G	A	C	10	3.9	2	2.6	0.67	0.603	6	4.0	0	0.0	n.a.	n.a.	4	3.8	2	3.3	0.88	0.884
UTR-6	Del	T	G	C	C	C	A	C	6	2.3	0	0.0	n.a.	n.a.	2	1.3	0	0.0	n.a.	n.a.	4	3.8	0	0.0	n.a.	n.a.
UTR-7	Ins	T	C	A	T	G	A	C	16	6.3	3	3.9	0.62	0.452	11	7.3	1	6.3	0.84	0.874	5	4.7	2	3.3	0.70	0.672
UTR-8	Ins	T	G	C	C	G	A	C	1	0.4	1	1.3	3.40	0.389	1	0.7	0	0.0	n.a.	n.a.	0	0.0	1	1.7	n.a.	n.a.
UTR-X									10	3.9	4	5.3	1.37	0.607	2	1.3	0	0.0	n.a.	n.a.	8	7.5	4	6.7	0.88	0.833

The 14-bp insertion/deletion and the SNP combinations comprising each haplotype are shown. The table shows the total patient group, the normotensive group (NT) and the preeclampsia group (PE) separately. Associations of the different UTR haplotypes with acute atherosis (AA) were tested using binary univariate logistic regression (*significant p-value, $p < 0.05$). OR, odds ratio; P, p-value; n.a., not applicable.

Table 4
Multivariate logistic regression analysis of fetal and maternal UTR-3 haplotype combinations in acute atherosis (AA).

	Total cohort						Normotensive (NT) group						Preeclampsia (PE) group					
	AA- n = 128		AA+ n = 38		OR	P	NTAA- n = 75		NTAA+ n = 8		OR	P	PEAA- n = 53		PEAA+ n = 30		OR	P
	Count	%	Count	%			Count	%	Count	%			Count	%	Count	%		
Neither have UTR-3	98	76.6	24	63.2	Ref.	0.095	56	74.7	4	50.0	Ref.	0.312	42	79.2	20	66.7	Ref.	0.143
Both have UTR-3	14	10.9	8	21.1	2.33	0.089	12	16.0	3	37.5	3.50	0.130	2	3.8	5	16.7	5.25	0.059
Only fetus has UTR-3	4	3.1	4	10.5	4.08	0.058	2	2.7	1	12.5	7.00	0.143	2	3.8	3	10.0	3.15	0.228
Only mother has UTR-3	12	9.4	2	5.3	0.68	0.629	5	6.7	0	0.0	n.a.	n.a.	7	13.2	2	6.7	0.60	0.546

Association of the UTR-3 haplotype with the occurrence of acute atherosis were tested using multivariate logistic regression. The possible outcomes for mother and fetus were combined in one categorical variable. The reference category consisted of neither the mother nor fetus having the UTR-3 haplotype (*significant p-value, $p < 0.05$). OR, odds ratio; P, p-value; n.a., not applicable.

We recently performed whole HLA-G gene amplification in the same patient population as in the present study and found a strong linkage disequilibrium between the UTR-3 haplotype and the HLA-G*01:04 allele in the DNA coding sequence (Drabbels, 2020). The same association between UTR-3 and the 01:04 allele was related to low levels of serum soluble HLA-G and adverse outcome in lung transplant recipients (Di Cristofaro, 2015).

In the decidual tissue the main source of fetal HLA-G is the EVTs that express membrane-bound and soluble forms of HLA-G during placentation (Hackmon, 2017), both of which interact with receptors on maternal immune cells inhibiting their activation and proliferation, thereby inducing maternal-fetal tolerance (Ferreira, 2017). Reduced placental HLA-G expression is associated with impaired EVT invasion, and could potentially contribute to the impaired placentation characteristic of early-onset PE (Goldman-Wohl, 2000). We hypothesize that altered HLA-G expression affects maternal-fetal tolerance, creating a local pro-inflammatory environment contributing to acute atherosis development.

Another source of HLA-G expression in the decidua is maternal immune cells. However, we found no associations between maternal HLA-G polymorphisms and acute atherosis. This is not surprising as HLA-G is primarily expressed by placental EVTs, and acute atherosis is a focal decidual lesion. However, the percentage of pregnancies where both the mother and the fetus possessed the UTR-3 haplotype were higher in pregnancies with acute atherosis (although this finding was not statistically significant). This is consistent with a prior report showing that the combined feto-maternal genotype (14-bp Ins/Del and +3142 C/G) was associated with maternal sHLA-G levels (Dahl, 2015).

Acute atherosis is a subtype of preeclampsia. Yet, we found no association between the HLA-G 3′UTR polymorphisms analyzed and preeclampsia as a whole. This is in line with several other studies (Mando, 2016; Nilsson, 2016; Pabalan, 2015; Vianna, 2007). However, the literature is inconsistent (Persson, 2017), and none of these studies specifically looked at preeclamptic pregnancies with presence of decidual acute atherosis. We hypothesize that some of these inconsistencies may be driven by the heterogeneity of preeclampsia; different gene polymorphisms may predispose to different subtypes of disease, all resulting in the same clinical syndrome.

The main intrinsic strengths of our study are the use of paired fetal and maternal samples, the decidual tissue collection method (Harsem et al., 2004), the well-defined histological characterization of acute atherosis (Alnaes-Katjavivi et al., 2016), and the comprehensive clinical information.

The main extrinsic strength is that we divided preeclampsia into two phenotypes based on the presence or absence of acute atherosis. No other studies have explored HLA-G polymorphisms relative to this uteroplacental artery lesion. Benton et al. proposed that distinct subtypes of preeclampsia could be identified by combining gene expression and histopathology analyses (Benton, 2018). They identified three preeclampsia subgroups based on placental gene expression patterns, which also showed distinct placental lesions (Benton, 2018). Similarly, our results suggest that studies of genetic polymorphisms in paired fetal-maternal samples combined with histological characterization of decidual lesions could help further delineate preeclampsia subtypes. The importance of combined fetal and maternal immunogenetic factors is further emphasized by our recent study demonstrating that acute atherosis in preeclampsia is associated with the combination of maternal KIR-B and fetal HLA-C2 (Johnsen, 2018).

The main limitation of the present study is the small sample size for assessing genetic associations, especially in subgroups. The associations we found were not statistically significant after adjusting for multiple testing using Bonferroni correction, a conservative method for adjusting for false positive findings as a result of multiple testing. Hence, our findings should be confirmed in a larger cohort.

Our results must furthermore be interpreted with caution as HLA-G expression during pregnancy is modified by several transcriptional and posttranscriptional mechanisms (Amodio and Gregori, 2020), as well as factors in the decidual milieu (Ferreira, 2017). Though further studies are needed to test causality, we speculate that HLA-G polymorphisms, individually or particularly in certain combinations as in the UTR-3 haplotype, alter local decidual immune tolerance and thereby promote a pro-inflammatory environment contributing to the formation of acute atherosis and placental dysfunction. Insight into these mechanisms may help to identify novel biomarkers of preeclampsia subtypes and to develop targeted care for women with preeclampsia.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jri.2021.103284.

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