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The HLA-G 14bp gene polymorphism and decidual HLA-G 14bp gene expression in pre-eclamptic and normal pregnancies

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Abstract

Trophoblast expression of the non-classical MHC, HLA-G, is considered essential for feto-maternal immune tolerance and successful placentation in pregnancy. The HLA-G 14 bp polymorphism in the 3′-untranslated region (UTR) of the HLA-G gene has been reported to be associated with development of pre-eclampsia (PE). In this study, maternal (peripheral blood, $n = 54$) and fetal (cord blood, $n = 57$) HLA-G 14 bp genotypes have been determined by PCR in pre-eclamptic and normal pregnancies. In addition, HLA-G 14 bp gene expression in decidua basalis ($n = 59$) was analyzed by RT-PCR. The pre-eclamptic syndrome was neither associated with the HLA-G 14 bp genotype (maternal or fetal), nor with altered decidual HLA-G 14 bp gene expression. Furthermore, the HLA-G 14 bp mRNA expressed in decidua basalis was of fetal origin and all potential transcripts, as predicted from the fetal HLA-G 14 bp genotype, were expressed. In contrast to previous findings, we found no correlation between the HLA-G 14 bp polymorphism and fetal growth. In conclusion, the fetal HLA-G 14 bp genotype is reflected in the decidual HLA-G mRNA splice form profile, but does not appear to be associated with increased risk for development of PE.
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Keywords: Decidua; Gene polymorphism; HLA-G; Pre-eclampsia; Pregnancy

1. Introduction

Pre-eclampsia (PE) is a pregnancy-associated disorder characterized by poor placentation caused by reduced trophoblast invasion and maladaptation of uteroplacental arteries, but the pathogenesis is not completely understood (Kaufmann et al., 2003). The non-classical MHC class 1b member, HLA-G, is assigned an essential role in pregnancy. HLA-G is mainly expressed by extravillous trophoblasts (EVTs) (Goldman-Wohl et al., 2000; Kovats et al., 1990) in decidual tissue, and inhibits

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maternal uterine natural killer (NK) cells from killing trophoblasts (Rouas-Freiss et al., 1997). This interaction is essential for the feto-maternal immune balance needed for optimal trophoblast invasion during placentation. In pre-eclamptic pregnancies, EVTs show reduced HLA-G expression (Colbern et al., 1994; Goldman-Wohl et al., 2000; Hara et al., 1996; Lim et al., 1997), and a causative role for HLA-G in placental insufficiency has been suggested.

The complexity of HLA-G is revealed by more than 20 different HLA-G alleles (Moscoso et al., 2006) and expression of four membrane-bound (HLA-G1–G4) and three soluble HLA-G isoforms (HLA-G5–G7) (Fujii et al., 1994; Ishitani and Geraghty, 1992; Kirszenbaum et al., 1994; Moreau et al., 1995; Paul et al., 2000). Specific HLA-G alleles have been reported to be associated with PE (Carreiras et al., 2002; O'Brien et al., 2001), and in common for some of the PE-related HLA-G alleles is the presence of a 14 bp insertion in the 3′-untranslated region (UTR) of exon 8 (Hylenius et al., 2004; O'Brien et al., 2001). The HLA-G 14 bp insertion polymorphism is associated with reduced levels of HLA-G mRNA (Hviid et al., 2003; O'Brien et al., 2001; Rousseau et al., 2003) and soluble HLA-G in serum (Rizzo et al., 2005). All together, seven studies from five groups have focused on the specific role for the HLA-G 14 bp polymorphism in PE (Bermingham et al., 2000; Humphrey et al., 1995; Hviid, 2004; Hylenius et al., 2004; Lin et al., 2006; O'Brien et al., 2001; Vianna et al., 2007). The results, however, are contradictory. Whereas it seems established that the maternal HLA-G 14 bp genotype has no influence on occurrence of PE (Bermingham et al., 2000; Humphrey et al., 1995; Hylenius et al., 2004; Lin et al., 2006; Vianna et al., 2007), the influence of the fetal HLA-G 14 bp genotype remains unclear. Both positive (Hylenius et al., 2004; O'Brien et al., 2001) and negative (Bermingham et al., 2000; Humphrey et al., 1995; Lin et al., 2006) association to PE has been reported.

In this study, we have aimed to clarify whether the maternal or fetal HLA-G 14 bp polymorphism is associated with development of PE. In addition, the functional consequence of the HLA-G 14 bp polymorphism in pregnancy was approached by comparing HLA-G 14 bp gene expression in decidua basalis from pre-eclamptic women and normal pregnant women.

2. Materials and methods

2.1. Study groups

Participants were recruited to the study from the Delivery Ward at St. Olavs Hospital, Trondheim, Norway, from 2002 to 2006. Due to the sampling of decidua basalis tissue, only women undergoing caesarean section (CS) were recruited. Pre-eclamptic cases were defined as persistent blood pressure above 140/90 mmHg and proteinuria of more than 0.3 g/24 h or 2+ or higher according to a dipstick test, developing after 20 weeks of pregnancy (Gifford et al., 2000). Cases ($n = 31$) were analyzed both in total and subgrouped into severe/mild PE ($n = 26/5$) (Sibai et al., 2005) or early-onset/late-onset ($n = 26/5$) PE (clinical manifestations before or after the start of the 34th week of gestation). Controls ($n = 29$) were healthy women with normal pregnancies undergoing CS for various reasons considered irrelevant to the aim of this study, i.e. breech presentation, birth anxiety and previous CS. Control women had no history of pregnancies with PE, recurrent spontaneous abortion (RSA) or fetal growth restriction (FGR). In addition, since maternal blood was not collected from the women first enrolled in the study and to increase the statistical power, 14 non-pregnant women with former normal pregnancies were recruited for maternal genotyping. Pregnancies with chromosomal aberrations, fetal and placental structural abnormalities or suspected perinatal infections were not included. Only singletons were included in the study. Informed consent was obtained from all participants, and the study was approved by the Regional Committee for Medical Research Ethics.

2.2. Biological samples

Umbilical cord blood (from 31 cases and 29 controls) was stored as serum, and maternal peripheral blood (from 20 cases and 34 controls) was stored in EDTA (one case and 21 controls, including the non-pregnant controls) or as serum (19 cases and 13 controls). Decidua basalis tissue (from 30 cases and 29 controls) was obtained by vacuum aspiration of the placental bed after the placenta was delivered during CS (Staff et al., 1999), and stored in RNA-later (Ambion, Huntington, UK). For quality control, representative pieces of the collected decidual tissue were fixed in 10% neutral-buffered formalin, paraffin embedded and cut in sections at 4 μm in a motorized microtome. Presence of EVTs were confirmed by immunohistochemical staining with a mAb against cytokeratin 7 (anti-CK7 mAb; clone OV-TL 12/30, DakoCytomation, Glostrup, Denmark) and ChemMate Envision Detection Kit Peroxidase/DAB (DakoCytomation), as previously described (Eide et al., 2007). Only specimens with confirmed presence of EVTs, i.e. decidua basalis, were included for further studies.

2.3. Genotyping of the HLA-G 14 bp gene polymorphism

Genomic DNA was extracted from 200 μl serum using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) or from 200 μl EDTA–blood with the MagAttract DNA Blood Mini M48 Kit (Qiagen) on the BioRobot M48 Workstation (Qiagen). Genotyping of the HLA-G 14 bp polymorphism was performed with HLA-G forward primer 5′-CCTGATGTGTGTGGGTTGTT-3′ and reverse primer 5′-TGAGACAGAGACGGAGACAT-3′. The forward primer was labelled with 6-carboxy fluorescein (6-FAM) in the 5′ (Sigma–Genosys, Haverhill, UK). Forty PCR amplification cycles were done in a Mastercycler®ep (Eppendorf, Hamburg, Germany) and analyzed on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Two samples of each genotype were DNA sequenced and verified as HLA-G specific.

2.4. HLA-G 14 bp gene expression analysis by RT-PCR

Decidua basalis tissue samples (80–110 mg) were disrupted and homogenized in a rotor–stator homogenizer (Ultra Turax T25, IKA, Stanfen, Germany). Total RNA was extracted by RNeasy Midi Kit (Qiagen), and cDNA prepared from 0.4 μg total RNA using the Reverse Transcriptase Core Kit (Eurogentec, Liegè, Belgium). Using the HLA-G RT-PCR primers 5′-GCTCAGATTGAAAAGGAGGGAG-3′ and 5′-GAGACGGAGACATCCCAGCC-3′, 45 cycles of PCR amplification were done in an Applied Biosystems 2720 Thermal Cycler. PCR products were analyzed by gel electrophoresis and UV-detection in a Gel Doc 1000 system (Bio Rad Laboratories, Hercules, CA, USA), and all transcripts from two cases and one control were verified as HLA-G specific by DNA sequencing.

2.5. Statistical analysis

The clinical data were expressed as mean value with corresponding S.D., and Mann–Whitney *U*-test or Fisher's exact test was used for comparisons (Table 1). Maternal and fetal HLA-G 14 bp genotype frequencies were compared to Hardy–Weinberg expectations using χ^2-tests. Allele frequencies, genotype frequencies and gene expression profiles in cases and controls were compared by Fischer's exact test. General linear model was performed to study the relation between the HLA-G 14 bp genotype and fetal growth. For comparison of birth weights, the difference between actual birth weight and expected birth weight for the gestational age and sex was calculated from the birth weight curves of Marsal et al. (1996), and then divided by the S.D. to transform the variable (birth weight) to standard score. The standard score is expressed as units of S.D. and used as a measure for birth weight deviation (BWD). Since birth weight normally increases with parity, fetal growth analysis was adjusted for parity. $P < 0.05$ was considered statistically significant.

Table 1
Clinical characteristics

	PE ($n = 31$)	Controls ($n = 29$)[a]
Maternal age (years)	30 ± 4	30 ± 5
Gestational age (weeks)	32 ± 3[b]	39 ± 1
Systolic BP (mmHg)	158 ± 14[b]	121 ± 15
Diastolic BP (mmHg)	96 ± 10[b]	72 ± 8
Birthweight (g)	1558 ± 636[b]	3499 ± 400
Primipara	21 (68%)[c]	9 (31%)

Values are expressed as mean ± S.D. Cases are compared to controls. PE, pre-eclampsia; BP, blood pressure.
[a] Non-pregnant controls ($n = 14$) are not included.
[b] $P < 0.001$; Mann–Whitney test.
[c] $P < 0.05$; Fisher's exact test.

3. Results

3.1. Patient characteristics

Clinical information on pregnancies in this study is listed in Table 1. As expected from the PE pathogenesis, case pregnancies demonstrated lower gestational age, higher blood pressure and reduced fetal birth weight compared to controls (Table 1). PE was complicated by FGR in 61% of the cases.

3.2. The maternal HLA-G 14 bp gene polymorphism

All maternal blood samples (20 cases and 34 controls) were successfully genotyped for the HLA-G 14 bp polymorphism. The overall frequency of the maternal HLA-G 14 bp insertion allele irrespective of placental disease was 0.38, and is comparable to the frequency of 0.4 reported in Danish studies (Hviid et al., 2002; Hylenius et al., 2004). The maternal HLA-G 14 bp genotype distribution in all cases and controls was 15% +14 bp/+14 bp, 39% −14 bp/−14 bp and 46% −14 bp/+14 bp, and showed expected Hardy–Weinberg equilibrium. Neither the maternal HLA-G 14 bp allele frequency nor the HLA-G 14 bp genotype distribution differed between cases and controls (Table 2). Similar negative findings were obtained when comparing prim-

Table 2
Maternal allele frequencies and genotype distribution of the HLA-G 14 bp gene polymorphism in mothers with and without PE

	PE[a]		PE (primipara)[b]		Controls	
	n	%	*n*	%	*n*	%
Allele						
+14 bp	15	37	10	36	26	38
−14 bp	25	63	18	64	42	62
Total (*n*)	40		28		68	
Genotype						
+14 bp/+14 bp	2	10	2	14	6	18
−14 bp/−14 bp	7	35	6	43	14	41
−14 bp/+14 bp	11	55	6	43	14	41
Total (*n*)	20		14		34	

Case groups are compared to controls.
[a] Alleles: $P = 1.000$; Fisher's exact test. Genotypes: $P = 0.596$; Fisher's exact test.
[b] Alleles: $P = 1.000$; Fisher's exact test. Genotypes: $P = 1.000$; Fisher's exact test.

ipara PE, severe PE or early-onset PE pregnancies to controls (Table 2 and data not shown).

3.3. The fetal HLA-G 14 bp gene polymorphism and PE

Genotyping of the fetal HLA-G 14 bp polymorphism was successful in all but three umbilical cord blood samples, and 29 case and 28 control fetal genotypes were determined (Table 3). The overall frequency of the fetal HLA-G 14 bp insertion allele was 0.44, and in accordance with Danish data (Hviid et al., 2002; Hylenius et al., 2004). The total fetal HLA-G 14 bp genotype distribution in all cases and controls was 17% +14 bp/+14 bp, 30% −14 bp/−14 bp and 53% −14 bp/+14 bp, and showed expected Hardy–Weinberg equilibrium.

Table 3
Fetal allele frequencies and genotype distribution of the HLA-G 14 bp gene polymorphism in pregnancies with and without PE

	PE[a]		PE (primipara)[b]		Controls	
	n	%	*n*	%	*n*	%
Allele						
+14 bp	25	40	16	40	25	45
−14 bp	33	60	24	60	31	55
Total (*n*)	58		40		56	
Genotype						
+14 bp/+14 bp	6	21	3	15	4	14
−14 bp/−14 bp	10	34	7	35	7	25
−14 bp/+14 bp	13	45	10	50	17	61
Total (*n*)	29		20		28	

Case groups are compared to controls.
[a] Alleles: $P = 1.000$; Fisher's exact test. Genotypes: $P = 0.780$; Fisher's exact test.
[b] Alleles: $P = 0.681$; Fisher's exact test. Genotypes: $P = 0.517$; Fisher's exact test.

The fetal HLA-G 14 bp allele frequency and genotype distribution did not differ between cases and controls (Table 3). Others have reported that the fetal HLA-G 14 bp polymorphism is associated to primipara pre-eclamptic pregnancies (Hylenius et al., 2004; O'Brien et al., 2001), but no such association was observed in this study either comparing to all controls (Table 3) or to primipara controls only (data not shown). Similarly, no association was found between fetal HLA-G 14 bp allele frequency or genotype distribution and subgroups of PE (severe or early-onset) (data not shown). The subgroup of primipara severe PE is reported associated with the fetal HLA-G 14 bp genotype (Hylenius et al., 2004), and analysis of this specific group ($n = 16$) gave one +14 bp/+14 bp, seven −14 bp/−14 bp and eight −14 bp/+14 bp fetal genotypes. In conclusion, the fetal HLA-G 14 bp polymorphism did not appear to be related to PE in the Norwegian cohort.

3.4. The fetal HLA-G 14 bp gene polymorphism and fetal growth

Hviid (2004) have reported that the fetal HLA-G 14 bp genotype is associated to birth weight. Birth weight normally increases with parity. The fraction of primiparas in this study varied among the fetal HLA-G 14 bp genotypes: 33% of the fetal +14 bp/+14 bp genotypes, 55% of the fetal −14 bp/−14 bp genotypes and 53% of the fetal heterozygotes were from primipara pregnancies. The difference in parity was adjusted for and no association between fetal growth and fetal HLA-G 14 bp genotype was observed (Table 4). Separate analysis of offspring from cases and controls suggested also that the fetal HLA-G 14 bp genotype and fetal growth are not related (data not shown) and, furthermore, this appeared also to be the case when analysis was restricted to births at ≥38 weeks of gestation as performed in the study by Hviid (2004) data not shown).

3.5. Decidual gene expression of the HLA-G 14 bp polymorphism

The decidua basalis tissue of 30 cases and 29 controls were successfully analyzed by RT-PCR for gene expression from the HLA-G 14 bp region. The 14 bp

Table 4
Fetal HLA-G 14 bp genotype in relation to fetal growth

	Cases and controls	
	n	BWD[a]
Fetal genotype		
+14 bp/+14 bp	10	−0.92 ± 1.07
−14 bp/−14 bp	17	−1.15 ± 1.59
−14 bp/+14 bp	30	−0.77 ± 1.86

Values are expressed as mean ± S.D. Analysis by general linear model adjusted for parity. BWD, birth weight deviation; calculated as the difference between actual birth weight and expected birth weight (adjusted for gestational age and sex) and divided by the S.D. (to transform the variable birth weight to standard score).
[a] Adjusted $P = 0.684$; General linear model, 2 d.f.

insertion allele allows for two potential transcripts, the intact gene and an alternative spliced form lacking 92 bp surrounding the 14 bp insertion (Fujii et al., 1994; Hiby et al., 1999). The RT-PCR analysis revealed expression of one, two or three HLA-G specific transcripts (Fig. 1 and Table 5). The 226 bp PCR product was derived from the HLA-G 14 bp deletion allele, the 240 bp fragment from the HLA-G 14 bp insertion allele, and the short 148 bp fragment identified the splice form of the HLA-G 14 bp insertion allele (−92 bp) (Fig. 1). All three potential HLA-G 14 bp transcripts were detected with individual variations (Fig. 1 and Table 5), and simultaneous expression of all three transcripts was observed in 53% of the deciduas (Table 5). The two splice forms of the HLA-G 14 bp insertion allele were always expressed together (Fig. 1A, lanes 4 and 7; B, lane 4; and Table 5).

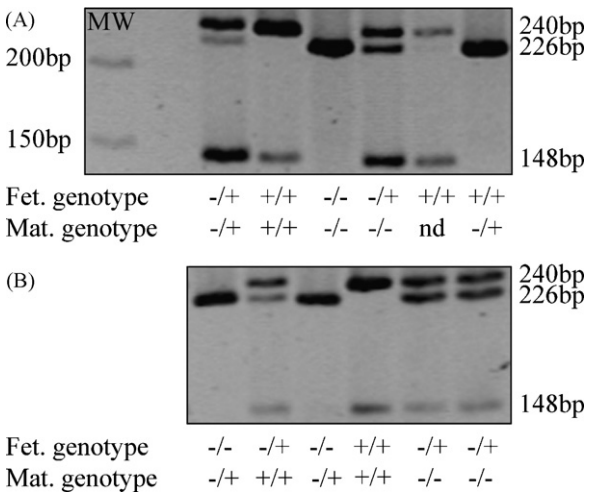


Fig. 1. Decidua basalis gene expression of the HLA-G 14 bp gene polymorphism. RT-PCR with HLA-G specific primers showing HLA-G transcripts (marked 148, 226 and 240 bp) from the gene region including the HLA-G 14 bp gene polymorphism in decidua basalis tissue of cases (A) and controls (B). Data are representative of at least three independent RT-PCR experiments on all cases and controls. Corresponding fetal and maternal HLA-G 14 bp genotypes are indicated. MW represents the molecular weight marker, and nd means no data obtained for specific genotypes.

Table 5
HLA-G 14 bp gene expression in decidua basalis from pregnancies with and without PE

	PE[a]		Controls	
	n	%	*n*	%
mRNA transcripts				
+14 bp/−92 bp	5	17	4	14
−14 bp	11	37	8	28
−14 bp/+14 bp/−92 bp	14	47	17	59
Total (*n*)	30		29	

Cases are compared to controls.
[a] $P = 0.722$; Fisher's exact test.

Decidual gene expression was compared in cases and controls and no difference in the HLA-G 14 bp gene expression profile was detected (Table 5). Separate analysis of decidua basalis tissue from PE subgroups (primipara, severe or early-onset) did not reveal any significant associations to disease for the decidual HLA-G 14 bp gene expression (data not shown).

3.6. HLA-G 14 bp genotype in relation to HLA-G 14 bp gene expression in decidua basalis

Decidual HLA-G 14 bp gene expression was compared to the corresponding maternal and fetal genotypes (exemplified in Fig. 1). For association to the fetal HLA-G 14 bp genotype, 29 cases and 28 controls with both decidua basalis tissue and known fetal genotype were compared. Decidual HLA-G 14 bp mRNA appeared to be linked directly to the fetal genotype (exemplified in Fig. 1). Of the 57 pregnancies compared, 56 deciduas (98.2%) showed an HLA-G 14 bp mRNA profile in agreement with the corresponding fetal HLA-G 14 bp genotype, and only one sample (1.8%) was in disagreement (Fig. 1A, last lane). For relation to the maternal HLA-G 14 bp genotype, 19 cases and 20 controls were compared. Only 15 of the 39 deciduas (38.5%) showed an HLA-G 14 bp gene expression correlating to the maternal HLA-G 14 bp genotype and, in these individuals, maternal and fetal HLA-G 14 bp genotypes were identical (exemplified in Fig. 1A, lanes 3–5; B, lane 4). The single incongruent decidual mRNA (only the −14 bp transcript) did not match either corresponding fetal genotype (+14 bp/+14 bp) or maternal genotype (−14 bp/+14 bp) (Fig. 1A, last lane) and, in lack of obvious biologic explanations, we ascribe this single result

to probable experimental error. In all other individuals, the fetal −14 bp/−14 bp genotype was associated always with one HLA-G 14 bp transcript in decidua basalis and the fetal +14 bp/+14 bp genotype was associated with two transcripts. The heterozygous fetal HLA-G 14 bp genotype (−14 bp/+14 bp) was, without exception, associated with the expression of all three HLA-G 14 bp mRNA transcripts (exemplified in Fig. 1).

4. Discussion

In contrast to that reported previously, we have found no association between the HLA-G 14 bp polymorphism (maternal or fetal) and PE or fetal growth in the present study. The HLA-G 14 bp mRNA expressed in decidua basalis was of fetal origin only and all potential transcripts, as predicted from the genotype, were expressed in all pregnancies.

The finding that the maternal HLA-G 14 bp polymorphism is not related to development of PE is in accordance with previous reports (Bermingham et al., 2000; Humphrey et al., 1995; Hylenius et al., 2004; Lin et al., 2006; Vianna et al., 2007). In contrast, the literature is controversial with regard to the fetal HLA-G 14 bp polymorphism. Whereas three studies report no association between fetal HLA-G 14 bp genotype and PE (Bermingham et al., 2000; Humphrey et al., 1995; Lin et al., 2006), others assert that such a connection exists (Hylenius et al., 2004; O'Brien et al., 2001). The positive association with fetal +14 bp/+14 bp genotype reported by Hylenius et al. was restricted to severe PE in first pregnancies only (Hylenius et al., 2004). We performed a corresponding subgroup-analysis of our data, without observing such an association. The study of O'Brien et al. (2001) was based on only seven PE cases and thus, the statistical power may be questioned. Increased birth weight is reported also for the fetal +14 bp/+14 bp genotype (Hviid, 2004), but we observed no correlation between the HLA-G 14 bp polymorphism and fetal growth, neither in controls nor PE cases either with or without FGR. To explore this point further, our analyses were extended to include pregnancies with FGR only, but again no association with fetal growth was found (data not shown).

In general, it is difficult to compare between studies because of the heterogeneity of PE. Some studies include all PE cases without subgrouping the disease (Lin et al., 2006), whereas others include severe PE only but with different inclusion criteria (Bermingham et al., 2000; Humphrey et al., 1995; Hviid, 2004; Hylenius et al., 2004). And finally, some analyse only mild PE (O'Brien et al., 2001). We have analysed pre-eclamptic cases both in total and subgrouped into primipara, severe or early-onset PE, but an association between the HLA-G 14 bp polymorphism and disease was not observed for any subgroup. Furthermore, to be enrolled as a control in the present study, no history of PE, RSA or FGR pregnancies was a requirement. This implies that our controls may differ from control groups in other studies, defined by a normal index pregnancy only (Hviid, 2004; Hylenius et al., 2004; O'Brien et al., 2001; Vianna et al., 2007).

EVT expression of HLA-G is well established (Kovats et al., 1990), but HLA-G expression has been detected also in other cells (Le Friec et al., 2004; Le Rond et al., 2004; Rebmann et al., 2003) and intrauterine tissue (Blaschitz et al., 1997; Houlihan et al., 1995; Hviid et al., 2003). In decidua basalis, maternal leukocytes represent a significant potential source of HLA-G mRNA (Benirschke et al., 2006; Le Friec et al., 2004; Le Rond et al., 2004; Rebmann et al., 2003). Since EVTs constitute about 20% of cells in decidua basalis (Naicker et al., 2003), decidual HLA-G gene expression may potentially be of both maternal and fetal origin. However, the data obtained in this study indicate that the HLA-G expressed in decidua basalis is of fetal origin only.

Co-expression of both splice forms from the +14 bp/+14 bp genotype is detected also in placenta (Hiby et al., 1999; Rousseau et al., 2003), and co-expression of three HLA-G 14 bp transcripts from the fetal −14 bp/+14 bp genotype confirms the lack of HLA-G imprinting (Hiby et al., 1999; Hviid et al., 1998). Each of the three HLA-G 14 bp transcripts detected in this study may be derived from a combination of the HLA-G1–G6 isoforms since they all contain exon 8 of the HLA-G gene. The splice variants from the HLA-G 14 bp polymorphism and the HLA-G isoforms (HLA-G1–G7) may therefore substantiate expression of seven different HLA-G transcripts from −14 bp/−14 bp genotypes, 13 transcripts from +14 bp/+14 bp genotypes and 19 transcripts from heterozygous genotypes. The HLA-G 14 bp genotype may be linked to selective HLA-G isoform expression (Hviid et al., 2003; O'Brien et al., 2001; Rizzo et al., 2005), and we are currently analyzing decidual HLA-G1–G7 isoform gene expression in pregnancies with or without PE and/or FGR (Tømmerdal et al., in preparation).

In conclusion, as assessed from the present data, the HLA-G 14 bp polymorphism does not appear to be associated with development of PE. However, the lack of association for this specific gene polymorphism does not eliminate a role for HLA-G in pregnancy, and in the pathogenesis of PE, but this remains to be further elucidated.

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