ELSEVIER

Journal of Reproductive Immunology 78 (2008) 158–165

JOURNAL OF REPRODUCTIVE IMMUNOLOGY

www.elsevier.com/locate/jreprimm

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

Ann-Charlotte Iversen [a,*,1], Olav Toai Duc Nguyen [a,1], Linda Føll Tømmerdal [a], Irina Poliakova Eide [a,b], Veslemøy Malm Landsem [c], Nuray Acar [d], Ronny Myhre [c], Helge Klungland [c], Rigmor Austgulen [a]

[a] *Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, Women and Children's Centre, Trondheim, Norway*

[b] *Department of Obstetrics and Gynecology, St. Olav Hospital, Trondheim University Hospital, Trondheim, Norway*

[c] *Department of Laboratory Medicine, Children's and Women's Health, Faculty of Medicine, Norwegian University of Science and Technology, Women and Children's Centre, Trondheim, Norway*

[d] *Department of Histology and Embryology, Faculty of Medicine, Akdeniz University, Antalya, Turkey*

Received 4 December 2007; received in revised form 30 January 2008; accepted 3 March 2008

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.

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

* Corresponding author at: Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, Women and Children's Centre, Olav Kyrres gt11, N-7006 Trondheim, Norway. Tel.: +47 72573305; fax: +47 72574704.

E-mail address: Ann-Charlotte.Iversen@ntnu.no (A.-C. Iversen).

[1] These authors contributed equally to this work.

0165-0378/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jri.2008.03.001

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.