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Short communication

Circulating HLA-G and its association with cardiovascular markers in pregnancy

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ABSTRACT

Human Leukocyte Antigen-G (HLA-G) prevents the activity of immune cells and is decreased in women with preeclampsia. We aimed to investigate the associations between circulating soluble HLA-G (sHLA-G) and 92 cardiovascular disease-related biomarkers from a previously published multiplex study in women with preeclampsia and controls. We found 15 markers significantly associated with circulating sHLA-G in univariate analyses. After multivariable adjusted regression, only proto-oncogene tyrosine-protein kinase Src (SRC) and vascular endothelial growth factor D were significantly associated with sHLA-G. Low SRC, previously observed in the circulation of preeclamptic women, may be regulated by low sHLA-G, and reflect decreased trophoblast differentiation and syncytical formation.

1. Introduction

Human Leukocyte Antigen-G (HLA-G) prevents the activity of immune cells and may protect the fetus from damage by the maternal immune system. It is expressed in various cell types and is implicated in a diverse array of pathological settings, such as autoimmune disease, viral infection, cancer and organ transplantation (Carosella et al., 2015).

The HLA-G molecule binds to several receptors, like the inhibitory immunoglobulin-like transcript 2 receptor (ILT2), ILT4 and killer cell immunoglobulin-like receptor 2DL4, to down-regulate the immune response via both indirect and direct mechanisms (Persson et al., 2019). In pregnancy, HLA-G is expressed on extravillous trophoblast cells at the feto-maternal interface in the placenta, decidua and the uterus. Soluble isoforms of HLA-G (sHLA-G) can also be detected in maternal blood and the levels are highest in the first trimester (Steinborn et al., 2007). Previous research has shown that women with preeclampsia have reduced blood levels of sHLA-G and reduced expression of HLA-G in extravillous trophoblasts of the placenta (Persson et al., 2019).

In the present study, we aimed to investigate the associations between circulating sHLA-G in pregnancy and 92 circulating cardiovascular disease (CVD)-risk biomarkers from a multiplex panel. We selected a cohort of women with preeclampsia and controls, to further investigate the epidemiological association of preeclampsia and future CVD (Lekva et al., 2020).

2. Material and methods

2.1. Subjects

Pregnant women scheduled for elective caesarean section were recruited between 2001 and 2014 to the Oslo Pregnancy Biobank. The Regional Committee for Medical and Health Research Ethics in South-Eastern Norway approved the study. All women signed informed consent. Preeclampsia was defined as new-onset hypertension (blood pressure ≥140/90 mmHg) and proteinuria (≥1+ on dipstick or ≥30 total protein/creatinine ratio) at ≥20 weeks' gestation. Early-onset preeclampsia was defined as delivery prior to 34 weeks' gestation, whereas late-onset preeclampsia as delivery ≥ 34 weeks' gestation. Clinical characteristics of the cases and controls have been previously published (Lekva et al., 2020). In summary, maternal age, parity and

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smoking rates were similar between the groups, whereas body mass index (BMI) and blood pressure at <20 weeks' gestation were significantly higher, and gestational age at sampling was significantly lower in the preeclampsia groups as compared to the control group.

2.2. Multiplex biomarker analysis and soluble HLA-G analysis

EDTA plasma samples from normotensive controls (n = 49), early-onset preeclampsia(n = 37) and late-onset preeclampsia (n = 29) were analyzed using the Proseek multiplex CVD I Olink assay at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala, Sweden, targeting 92 CVD linked biomarkers, as previously published (Lekva et al., 2020). Circulating sHLA-G was measured in duplicate using commercially available antibodies (BioVendor Laboratory Medicine Inc., Brno, Czech Republic), as previously described (Jacobsen et al., 2020).

2.3. Statistics

Statistical analyses were performed using SPSS Statistics 25.0 (IBM). To identify the CVD biomarkers with the strongest associations with circulating sHLA-G, we first conducted univariate analysis for each variable. All variables with $p < 0.05$ were then included in the adjusted multivariable stepwise analysis. A p-value <0.05 was considered significant.

3. Results and discussion

3.1. Circulating soluble HLA-G in normotensive controls and preeclampsia

We found a lower, but not statistically significant (p = 0.268) median circulating sHLA-G in early-onset preeclampsia [median U/mL (25^{th},75^{th}); [125 (93,188)] as compared to normotensive pregnant controls 147 (101, 240)] and late-onset preeclampsia [158 (99, 270)]. This lack of a significant difference is in contrast to our findings in our larger cohort, in which levels of circulating sHLA-G were significantly lower in women with preeclampsia compared to controls (Jacobsen et al., 2020). Several previously published papers have also reported low sHLA-G in the circulation as well as reduced HLA-G expression in the placenta in preeclampsia (Persson et al., 2019). The lacking statistical significance in our cohort may be due to a smaller sample size and the fact that blood was sampled at an earlier gestational age in preeclamptic pregnancies than controls. This could obscure a potential difference between diagnosis groups, as sHLA-G decreases by gestational age during the latter part of pregnancy (Steinborn et al., 2007).

3.2. Circulating HLA-G and its association with CVD markers

We found 15 CVD markers significantly positively associated with sHLA-G in univariate linear regression analysis (Table 1). We found no interactions between any of the 15 markers and diagnosis (group), with sHLA-G as the outcome (not shown). In previously published work (Lekva et al., 2020), we found only tumor necrosis factor receptor superfamily member 5 (CD40), proto-oncogene tyrosine-protein kinase Src (SRC), interleukin 6 (IL6), kallikrein-11 (hK11) and follistatin (FS) significantly different in these 15 markers between the groups (normotensive controls, late-onset preeclampsia and early-onset preeclampsia).

When including these 15 CVD markers in a multivariable analysis, only SRC and endothelial growth factor D (VEGFD) were significantly associated with sHLA-G after adjusting for age and BMI. Regarding the potential mechanisms behind the association between sHLA-G and SRC in our data, we speculate that activation of ILT-4 by sHLA-G results in the recruitment of SH2 containing tyrosine phosphatase (SHP)-1 and -2 (Liang et al., 2008). These phosphatases may, in turn, activate NF-κB and downstream IL-6 production, as well as SRC (Walter et al., 1999). Interestingly, previous research has shown that SRC plays a role in trophoblast differentiation and syncytical formation (Daoud et al., 2006). In summary, low levels of sHLA-G may lead to less SHP-1 and 2, which may result in decreased SRC signaling and potentially reduced trophoblast differentiation. In the present study, SRC is measured in plasma, but circulating protein levels could reflect the expression levels in the placenta or possibly regulate the maternal-fetal interface indirectly. Indeed, a decrease in SRC activation has been found in the placentas of women with preeclampsia, and the downregulation of SRC signaling has been suggested as an explanation for the defective trophoblast differentiation and invasion leading to abnormal development of the placenta in preeclampsia (Irtegun et al., 2017).

VEGFD, the other protein associated with sHLA-G in the multivariable analysis, may promote the growth and remodeling of blood vessels and lymphatics by activating endothelial receptors. Binding and activation of the receptors VEGFR2 or VEGFR3 induces intracellular signaling through a variety of mediators including SRC (Pandey et al., 2018). Thus, reduced VEGFD may lead to lower VEGFR2 and SRC signaling. Cytoplasmic SRC may also phosphorylate VEGFR2 and initiate downstream signaling, and may result in lower angiogenesis when SRC is low. As previously published (Lekva et al., 2020), the circulating maternal levels of VEGFD were similar in preeclamptic pregnancies compared to controls, while the SRC level was lower in preeclamptic women. However, since the VEGFD measured by the Olink assay represents the *total* VEGFD, we do not know how much is bound and how much is free (bioactive). As we lack the mechanistic data, it is difficult to interpret the importance of VEGFD and its connection to sHLA-G, but this should be studied in future research.

Trophoblast development and tumor progression share similar

Table 1
Association of circulating HLA-G and circulating CVD markers in univariate and multivariable models for the included pregnancy cohort (n = 115).

Circulating HLA-G	Univariate		Multivariable adjusted*	
Variables	*Beta*	*P-value*	*Beta*	*P-value*
Maternal age at delivery (years)	−0.02	0.857	−0.06	0.494
Body mass index (BMI) early pregnancy (kg/m^2)	0.08	0.383	0.09	0.342
Systolic blood pressure <20 weeks (mmHg)	−0.01	0.978		
Diastolic blood pressure <20 weeks (mmHg)	0.03	0.775		
Gestational age at delivery and sampling (weeks)	0.15	0.124		
Soluble fms-like tyrosine kinase 1/ placental growth factor (sFLT/PlGF) ratio	−0.14	0.161		
CD40 ligand (CD40 L)	0.29	0.002	−0.17	0.433
Tumor necrosis factor receptor superfamily member 5 (CD40)	0.26	0.006	0.09	0.402
Epidermal growth factor (EGF)	0.27	0.005	−0.14	0.484
Proto-oncogene tyrosine-protein kinase Src (SRC)	**0.34**	**<0.001**	**0.34**	**<0.001**
Interleukin-6 (IL6)	0.19	0.049	0.12	0.243
Platelet-derived growth factor subunit B (PDGF subunitB)	0.20	0.037	−0.08	0.565
C-X-C motif chemokine 1 (CXCL1)	0.27	0.005	0.05	0.661
Heat shock 27 kDa protein (HSP27)	0.30	0.002	0.03	0.840
NF-kappa-B essential modulator (NEMO)	0.26	0.005	−0.14	0.361
Platelet endothelial cell adhesion molecule (PECAM1)	0.25	0.010	0.08	0.469
Kallikrein-11 (hK11)	0.20	0.040	0.14	0.138
C-C motif chemokine 4 (CCL4)	0.26	0.007	0.17	0.061
C-C motif chemokine 3 (CCL3)	0.24	0.011	0.16	0.099
Vascular endothelial growth factor D (VEGFD)	**0.19**	**0.048**	**0.21**	**0.021**
Follistatin (FS)	0.21	0.027	0.14	0.146

*Adjusted for age and BMI. Standardized coefficients Beta are given in the multivariable model for significant and adjustment variables and Beta ln for the other variables.

properties and interestingly the HLA-G/ILT4/VEGF signaling is found increased in tumor progression (Garcia et al., 2020; Zhang et al., 2016). The reduced trophoblast differentiation and invasion in preeclampsia and decreased HLA-G/SRC and potential involvement of VEGF signaling strengthens these similarities.

4. Conclusion

We aimed to investigate the associations between circulating sHLA-G and 92 CVD biomarkers from a previously published Olink study in a cohort of early- and late-onset preeclampsia versus controls. We found 15 markers significantly associated with circulating sHLA-G by univariate regression analysis. In the multivariable adjusted regression analysis with these 15 biomarkers, only SRC and VEGFD were positively associated with sHLA-G. Low SRC, previously observed in the circulation of women with preeclampsia, may be regulated by low sHLA-G, and may reflect decreased trophoblast differentiation and syncytical formation.

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