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Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1) is reduced with preeclampsia and small for gestational aged fetuses

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ABSTRACT

Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1) is an inhibitory receptor expressed on immune cells. We evaluated LAIR1 in placentas from preeclamptic or small for gestational age (SGA) pregnancies, and placental explant model (1 % O_2, IL6 and TNFα, or control). *LAIR1* mRNA was reduced in placentas from preeclamptic ($p < 0.0001$, $n = 78$) and SGA ($p < 0.0001$, $n = 32$) pregnancies. LAIR1 protein expression was reduced in placentas from preeclampsia ($p < 0.0001$, $n = 43$) and SGA ($p = 0.009$, $n = 10$) pregnancies. Hypoxia (1 % O_2) reduced *LAIR1* mRNA expression in placental explants ($p = 0.008$). These findings suggest hypoxia modulates *LAIR1* expression in the placenta.

1. Introduction

Placental dysfunction is associated with several pregnancy complications, including preeclampsia and fetal growth restriction (FGR) [1]. Preeclampsia arises from anti-angiogenic factors released by the placenta, leading to maternal vascular injury, hypertension and multi-organ damage [2,3]. FGR occurs when the fetus fails to achieve its genetically determined growth potential [4,5]. Small for gestational age (SGA), often a proxy for FGR, is defined as estimated fetal birthweight <10th percentile [6]. Both are associated with impaired placental development and function, leading to compromised fetal nutrient and oxygen supply.

Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1) is an inhibitory receptor expressed on various immune cells, including T cells, B cells, and natural killer (NK) cells [7,8]. LAIR1 plays a crucial role in maintaining immune homeostasis by modulating immune cell activation and preventing excessive inflammatory responses in cancer and autoimmune diseases [9]. However, its role in placental function and pregnancy pathologies remains unexplored. Given the importance of immune regulation in placental development and function, the aim of this study was to characterise LAIR1 expression in placentas complicated by preeclampsia and small for gestational age, and in a placental explant model. We hypothesised that LAIR1 would be dysregulated in models of placental dysfunction.

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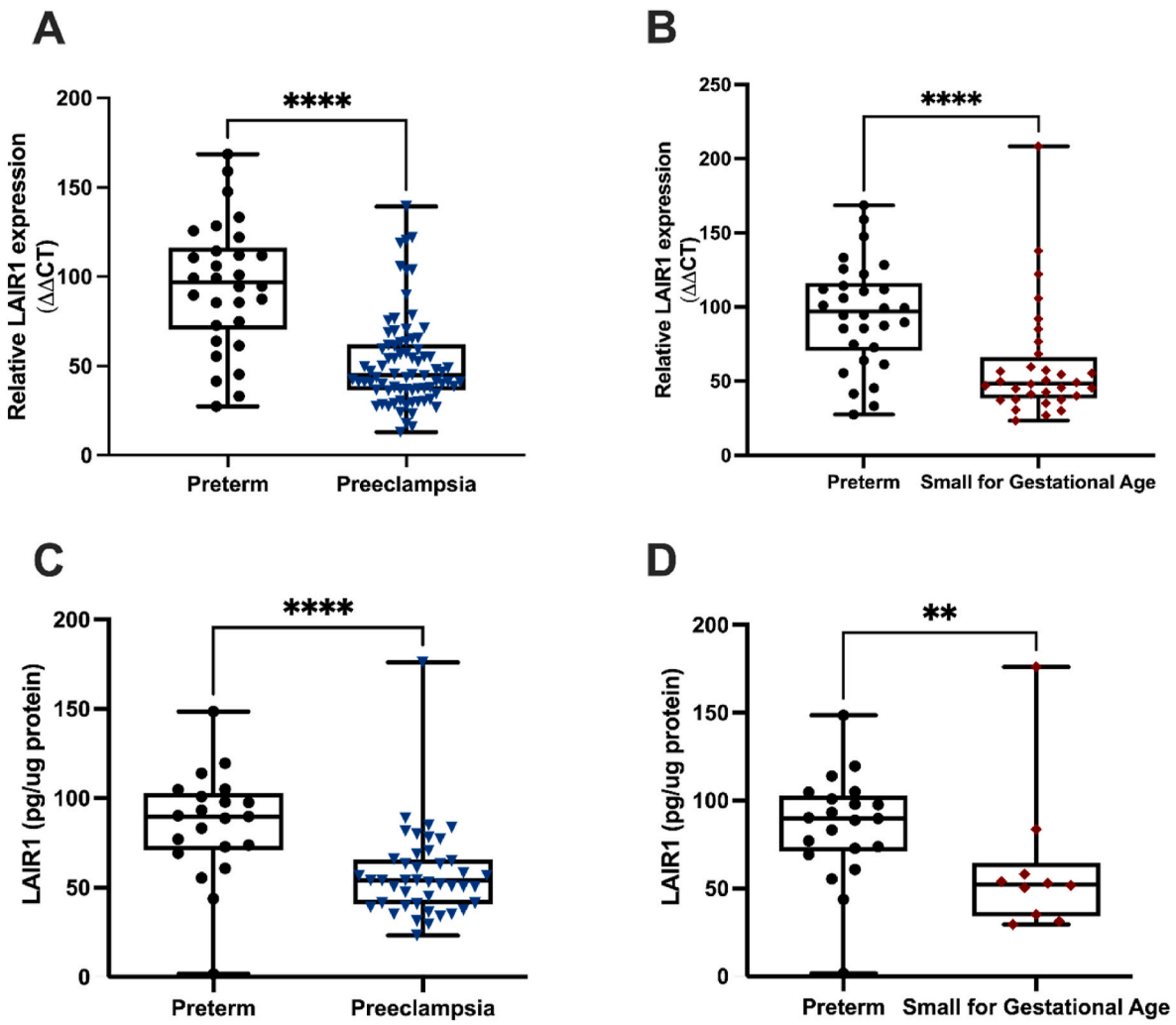

Fig. 1. ***LAIR1*** **mRNA and protein levels are reduced in placentas from women with early onset preeclampsia or small for gestational age. (A)** *LAIR1* mRNA expression was significantly reduced in placentas from women with early onset preeclampsia (blue triangles, n = 78), compared to gestation matched controls (black dots, n = 30). **(B)** *LAIR1* mRNA expression was significantly reduced in placentas from women who delivered an infant small for gestational age (SGA; <10th birthweight centile, red diamonds, n = 32) compared to gestation matched controls (black dots, n = 30). **(C)** LAIR1 protein expression was significantly reduced in placentas from women with early onset preeclampsia (blue triangles, n = 43), compared to gestation matched controls (black dots, n = 21). **(D)** LAIR1 protein expression was significantly reduced in placentas from women who delivered an SGA infant (red diamonds, n = 10) compared to gestation matched controls (black dots, n = 21). Data points represent individual patients. Data are presented as box and whisker plot. $^{**}P < 0.01$, $^{****}P < 0.0001$.

2. Methods

2.1. Placental tissue bank

Human placental tissue lysates were obtained from biobanked samples at Mercy Hospital for Women. Written informed consent was obtained, and all studies were approved by the Mercy Health Human Research ethics committee (R11/34). Tissue was collected from women with established preterm preeclampsia (<34 weeks' gestation; RNA n = 78; protein n = 43); and/or small for gestational age (SGA, <10th birthweight centile; RNA, n = 32; protein n = 10), and preterm controls (RNA, n = 30; protein n = 21). RNA was extracted from placental lysates as previously described [10]. Protein was extracted from placental lysates as previously described [11]. Patient characteristics for RNA and protein analysis are in Supplementary Tables 1 and 2

2.2. Placental explants

Human placental tissue was collected from five women following caesarean section delivery. Placentas were obtained from term (37–40 weeks' gestation) delivery. Placentas were collected from normotensive pregnancies, with a normal birthweight centile (>10th centile according to gestation) was delivered. Placental with evidence of chorioamnionitis, confirmed by placental histopathology were not included. Villous explants were prepared as previously described [12,13]. Following overnight incubation, explants were treated with inflammatory cytokines, 0, 10 ng/mL IL6 or TNFα, for 24 h under 8 % O_2 and 5 % CO_2 at 37 °C (triplicate, n = 5). Explants were cultured in 1 % O_2 (hypoxia) or 8 % O_2 (normoxia, 5 % CO_2, 37 °C) for 48 h (triplicate, n = 5). To assess LAIR1 secretion, protein levels were normalised against wet explant weights. Tissue was collected for RNA and protein extraction.

2.3. LAIR1 in plasma and placenta

RNA was extracted and cDNA was described previously [10]. Taqman fast advanced Master Mix (Applied Biosystems) and specific fluorescein amidite-labelled Taqman Gene expression Assays (Life Technologies) were used to measure *LAIR1* (Hs00253790_m1). qRT-PCR was performed on the QuantStudio5 (Thermo Fisher Scientific) with thermocycling parameters: 95 °C for 20 s, 40 cycles of denaturation for

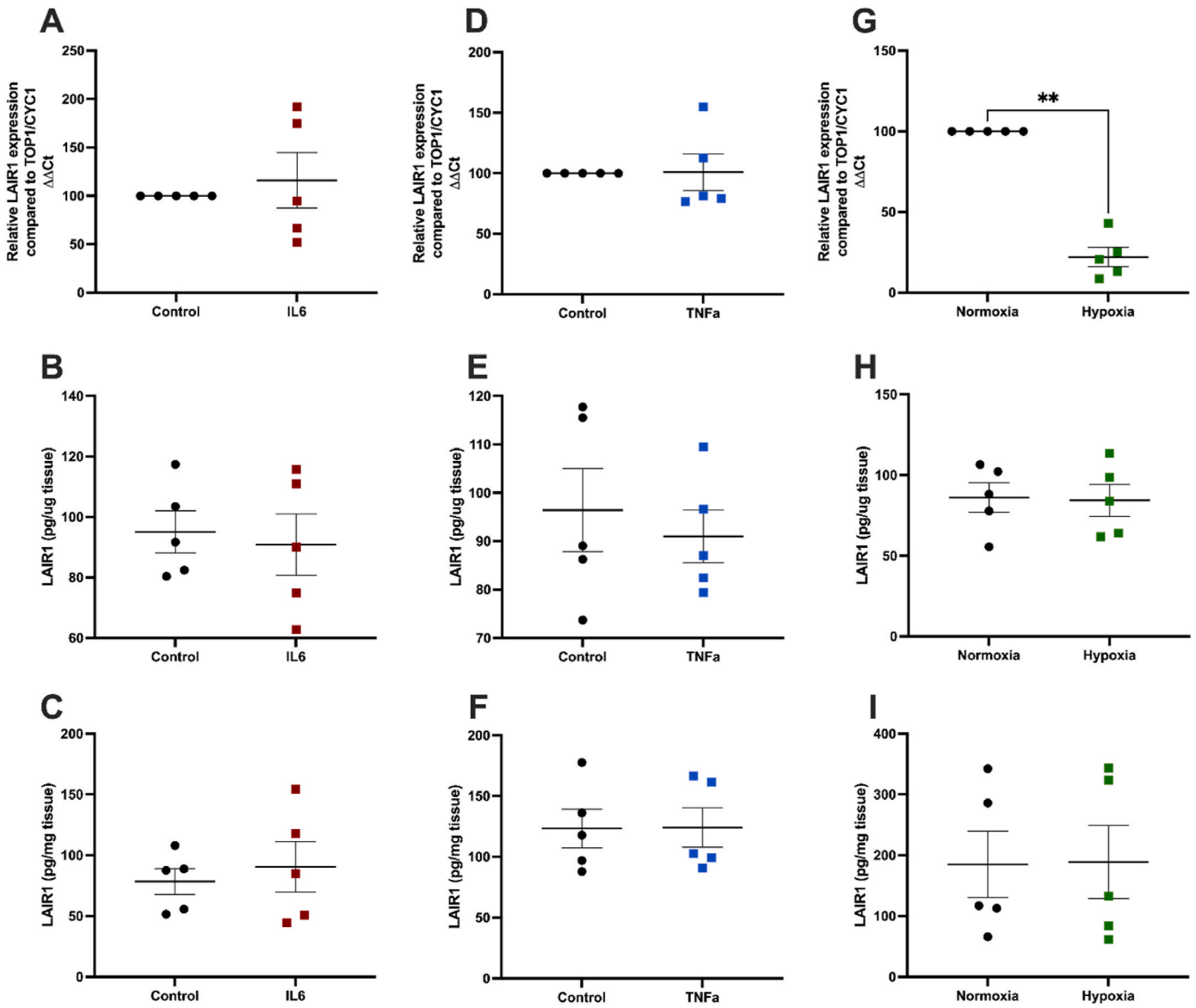

Fig. 2. ***LAIR1*** **mRNA is reduced in placental explants exposed to hypoxia, but not inflammatory cytokines, IL6 or TNFα.** Following IL6 treatment (10 ng/mL), *LAIR1* mRNA expression **(A)** and protein expression in placental lysates **(B)**, and media **(C)** of placental explants were not altered (red squares) compared to controls (0 ng/mL; black dots). Following TNFα treatment (10 ng/mL), *LAIR1* mRNA expression **(D)** and protein expression in placental lysates **(E)**, and media **(F)** of placental explants are not altered (blue squares) when compared to controls (black dots, 0 ng/mL). Following exposure to hypoxia (1 % O_2), *LAIR1* mRNA expression **(G)** is significantly reduced, while protein expression in placental lysates **(H)**, and media **(I)** of placental explants were not significantly altered (green squares) compared to controls (black dots, 8 % O_2). All experiments were done in triplicate, n = 5 patients. Data are presented as mean ± SEM. **P < 0.01.

3 s at 95 °C, and 60 °C for 30 s. No product was detected in the non-template control and gene expression was normalised to the mean of stable housekeepers, *CYC1* (Hs00357717_m1) and *TOP1* (Hs00243257_m1). Samples were run in duplicate, and an average Ct was used. Gene expression was normalised to the Ct mean of each control group and the delta delta Ct ($2^{-\Delta\Delta Ct}$) method was expressed relative to controls.

Levels of LAIR1 in plasma and protein lysates were quantified using the Human LAIR1 DuoSet ELISA kit (DY2664–05, R&D System, Minnesota, USA) according to the manufacturer's instructions. The limit of detection for the assay was between 39.1 and 2,500 pg/mL.

2.4. Statistical analysis

Maternal characteristics were compared for patients with preeclampsia or SGA, compared to controls using a Mann-Whitney test for continuous data, and Chi-square for categorical data. Data was assessed for normal distribution using Anderson-Darling test, D'Agostino and Pearson test, Shapiro-Wilk test, and Kolmogorov-Smirnov test. Mann-Whitney test was used for data containing two groups and unpaired, non-parametric data. *In vitro* experiments were performed in triplicate and repeated five times. $p < 0.05$ was considered significant. Statistical analyses were performed using GraphPad Prism 10.2.3 (GraphPad Software, LLC).

3. Results and discussion

LAIR1 mRNA and protein levels were measured in placentas from preeclampsia and SGA pregnancies. *LAIR1* mRNA was significantly reduced in placenta from women with preeclampsia (Fig. 1A, $p < 0.0001$, n = 78) and SGA (Fig. 1B, $p < 0.0001$, n = 32), compared to controls (n = 30). LAIR1 protein was significantly reduced in placenta

from preeclamptic (Fig. 1C, $p < 0.0001$, $n = 43$) and SGA (Fig. 1D, $p = 0.009$, $n = 10$) pregnancies, compared to controls ($n = 21$).

LAIR1, a member of the immunoglobulin superfamily, is predominately expressed in peripheral blood mononuclear leukocytes, including natural killer (NK) cells. It serves as an inhibitory receptor on NK and human T cells [14] and is reduced in many autoimmune conditions [15]. Our study is the first to demonstrate altered LAIR1 expression in placentas complicated by preeclampsia or SGA. We were unable to detect LAIR1 protein expression using immunohistochemistry (data not shown). Given the placenta interacts with immune cells, the observed changes in LAIR1 may stem from alterations in the maternal immune system.

Given the role of inflammation and hypoxia in placental dysfunction, we assessed the impact of inflammatory cytokines IL6 and TNFα on LAIR1 expression using a placental explant model. Neither IL6 nor TNFα altered *LAIR1* mRNA (IL6; Fig. 2A, TNFα; Fig. 2D) or protein expression in lysates (IL6; Fig. 2B, TNFα; Fig. 2E) or media (IL6; Fig. 2C, TNFα; Fig. 2F). Hypoxic conditions (1 % O2) reduced *LAIR1* mRNA (Fig. 2G, $p = 0.008$), but not protein expression (Fig. 2H and I). The 48-h exposure to hypoxia in the explant model may have been sufficient to alter *LAIR1* mRNA expression but not long enough for these changes to impact protein levels. This may be due to the stability and turnover rates of the LAIR1 protein, although more work is required to understand the role of altered LAIR1 driven by hypoxia.

Overall we identified reduced LAIR1 mRNA and protein in the placenta of patients with preeclampsia and SGA. Our *in vitro* studies demonstrate hypoxia may contribute to reduced LAIR1. Future research may benefit from exploring other cell types within the placenta that influence reduced LAIR1 in preeclampsia and SGA pregnancies, such as leukocytes.

Ethics approval

The studies involving human participants were reviewed and approved by the Mercy Health Human Research Ethics Committee (R11/34). The patients/participants provided their written informed consent to participate in this study.

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CRediT authorship contribution statement

Lucy A. Bartho: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Susan P. Walker:** Writing – review & editing, Resources. **Ping Cannon:** Writing – review & editing, Validation, Data curation. **Tuong-Vi Nguyen:** Writing – review & editing, Validation, Data curation. **Anna Nguyen:** Writing – review & editing, Data curation. **Stefan M. Botha:** Writing – review & editing, Conceptualization. **Natalie J. Hannan:** Writing – review & editing, Project administration, Data curation. **Stephen Tong:** Writing – review & editing, Project administration, Funding acquisition. **Tu'uhevaha J. Kaitu'u-Lino:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2024.08.018.

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