The Influence of Diabetes in Pregnancy on Maternal and Fetal LRG1 Levels, and Implications for Pathological Neovascularisation

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Background

- Leucine-rich alpha-2-glycoprotein 1 (LRG1) mediates TGFβ signalling and is linked with aberrant neovascularisation in retinal pathologies.
- It has been hypothesized that complications of diabetes during pregnancy may be due to placental abnormalities
- Pathological neovascularisation is present in the placenta of offspring born to mothers with diabetes during pregnancy.
- A proposed mechanism of this pathological neovascularisation is maternal diabetes induced elevation of LRG1

Aim

To examine LRG1 expression in pregnancies affected by diabetes by:
1. Detecting LRG1 in placental tissue
2. Assessing if LRG1 expression was elevated in the placenta and cord blood of offspring born to mothers with diabetes.

Materials & Methods

Recruitment

Patients were consented at diabetic clinic, prior to elective Caesarian section or induction of labour.

Sample Collection

Maternal blood samples were collected before delivery, and cord blood and placental samples were collected after delivery of the placenta. Serum was isolated from the blood samples and stored at -80ºC.

Serum Analysis

Serum samples were analysed with IBL LRG1 ELISA.

Placental Analysis

Western Blot

Placental samples were homogenized using a tissue lyser (Thermo Scientific). Extracted protein was quantified using BCA assay prior to Western blot. LRG1 was identified using Anti-LRG1 monoclonal antibody (Abcam). GAPDH was utilised as a loading control. The results were analysed using Image J densitometry software.

Immunohistochemistry

Placental samples were formalin fixed and paraffin embedded into wax blocks. Tissue sections were probed for LRG1 expression using an anti-LRG1 monoclonal antibody (Abcam). Slides were digitised and positive pixels were quantified using automated image analysis (Aperio). Sections were also stained with Haematoxylin and eosin.

Population

- All participants had singleton otherwise uncomplicated pregnancies at term.
- Patient groups included control without diabetes, then patients with gestational-diabetes on diet, gestational-diabetes on insulin, and pre-existing diabetes on insulin.

Patient Group Name	WB n= 14	IHC n= 15	ELISA n= 42
Control without diabetes	n = 6	n = 7	n = 10
Gestational-diabetes on diet	n = 4	n =4	Maternal= 11 Cord= 9
Gestational-diabetes on insulin	n = 3	n = 3	n = 10
Pre-existing diabetes on insulin	n =1	n = 1	Maternal= 11 Cord= 7

Table 1. Population grouped on diabetic status.

Results

Western Blot

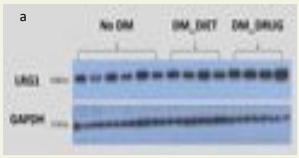
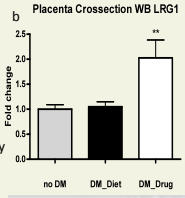


Figure 1 (a) LRG1 Western blot (top), GAPDH loading control Western blot (bottom). 1 (b) LRG1 Western blot densitometry results; noDM (n=6), DM_Diet (n=4), DM_Drug (n=4) (p=0.006), One way ANOVA) 1 (c) LRG1 protein expression was detected across the placenta; Fetal side (F) Maternal side (M). C/S (cross section)

LRG1 protein expression was detected in placental tissue. A significant increase in LRG1 was detected by Western Blot in placental tissue from pregnancies that required insulin compared to pregnancies complicated by gestational diabetes controlled by diet alone or in the control group. Analysis of individual areas from the placenta revealed increased levels of LRG on the maternal side of the placenta.



Immunohistochemistry

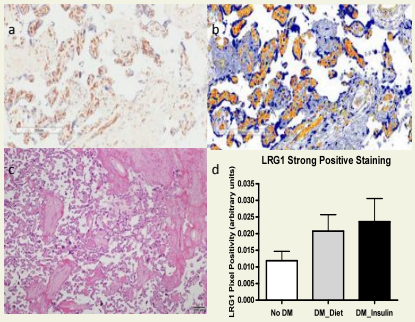
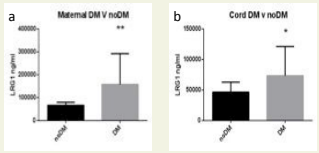


Figure 2. (a)Placenta section stained for LRG1, (b) Markup of (a) LRG1 IHC. Yellow and red colour demonstrate positive brown staining for LRG1. (c)Placenta stained with haematoxylin and eosin. (d) Strong positive LRG1 Immunohistochemistry results.

The immunohistochemistry (IHC) detected LRG1 in the placenta and its general localisation in the tissue. A non-significant increase in the strong positive pixel count for LRG1 was detected in the placental tissue of mothers with diabetes compared to mothers without diabetes.

ELISA

LRG1 was detected in maternal and cord serum samples. LRG1 was significantly increased in diabetic maternal serum compared with non diabetic control ($p<0.0047$). Similarly, LRG1 was increased in diabetic cord serum versus non diabetic cord serum ($p= 0.0221$). LRG1 levels were also found to be significantly increased ($p= 0.002$) in maternal serum compared to cord serum when diabetic status was disregarded.



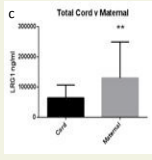


Figure 2. (a) Maternal serum affected by diabetes vs control, unpaired t test, (b) Cord serum affected by diabetes vs control unpaired t test, (c) Total cord serum vs maternal serum, paired t-test.

Discussion and Conclusions

Discussion: This study has shown for the first time that LRG1 protein is detectable in placental tissue, and LRG1 expression levels are also elevated in placentas from pregnancies that require insulin. Maternal and cord levels of circulating LRG1 were significantly increased in pregnancies complicated by diabetes compared to control. Irrespective of patient type, LRG1 levels were significantly higher in the maternal circulation compared to the fetal circulation.

Ongoing work: Further work is also required to determine the pathological significance of elevated LRG1 in placental tissue of offspring born to mothers with diabetes. We plan to assess the vessel density in placental samples and its association with LRG1 as a possible mechanism of neovascularisation.

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