# Loss of Jag1 in endothelial cells (ECs) during early pregnancy at E7.5

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## INTRODUCTION

Murine implantation triggers decidual angiogenesis, which involve development of a rich network of capillaries in the uterine decidua that serves to support embryonic growth and development prior to placentation. The decidual vascular network includes maternal spiral arteries (SpA) and decidual capillaries. Vascular mural cells, specifically pericytes and vascular smooth muscles cells, are recruited in decidual angiogenesis to bolster vessel stabilization. By mid-gestation, the local environmental changes resulting from decidualization lead to structural remodeling with dilation and enlargement of maternal SpAs.

VEGF signaling plays an essential role in vascular development and has been shown, in mice and non-human primates, to be important in decidual angiogenesis. The VEGF and Notch signaling pathways are interconnected. Notch signaling is involved in placental vascular development in mice, however, the role of Notch signaling in murine decidual angiogenesis has not yet been fully elucidated.

We have previously shown that Notch signaling is active in endothelial cells (EC) of capillaries and SpAs and Notch ligands Delta like-4 (Dll4) and Jagged1 (Jag1) are co-expressed in ECs of SpAs. Herein, we seek to determine the role of Jag1/Notch signaling in the formation and maintenance of the maternal vasculature in early mouse pregnancy. We created a mouse model of *Jag1* deletion in maternal ECs and determined the impact on the decidual vasculature and progression of pregnancy. In order to evaluate decidual vasculature prior to placentation, we evaluated mouse implantation sites (IS) at embryonic day (E) 7.5, a stage of pregnancy when decidual angiogenesis is complete and uterine vascular density is maximal.

## METHODS

**Mouse model**

- Endothelial cell (EC)-specific *Cdh5-Cre$^{ERT2}$* transgenic mice were bred to *Jag1$^{flox/flox}$* mice to delete *Jag1* in ECs.
- *Cdh5-Cre$^{ERT2}$;Jag1$^{fl/fl}$ (Jag1ΔEC)* mutant and *Cdh5-Cre* control and *Cdh5-Cre;ROSA26 tdTomato* females were generated and tamoxifen (TMX) was given by oral gavage to induce Cre-recombination.
- The impact of loss of EC *Jag1* on ovarian function is not known. Progesterone (P4) pellets (30mg, XR) were placed at E4.5 to overcome any loss of ovarian function in *Jag1 ΔEC* mice.
- Uteri were harvested at E7.5

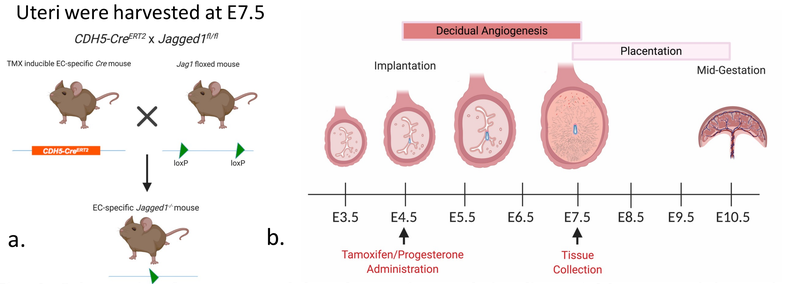


**Fig. 1. Schematic of mouse model and experimental timeline.** a) Mouse models used to produce EC-specific deletion of *Jag1*. b) Schematic of the murine gestation highlighting important processes. TMX and P4 were administered at E4.5 and tissue collected at E7.5.

**qRT-PCR**

- Total RNA was isolated from control and *Jag1ΔEC* mutant whole implantation sites (IS) and analyzed for expression of Notch targets with qRT-PCR. Primers were used to amplify *Hey2*, *Nrarp,* and *Jag1*. Relative expression levels were quantified using the $2^{-\Delta\Delta CT}$ method and are expressed as fold change normalized to $\beta$-actin.

**Immunofluorescence**

- Sections were stained for antibodies specific to detect Jag1 or Dll4, EC marker, CD31, or mural cell markers, NG2, PDGFRβ and SMA. Images were captured with the Keyence BZ-X710 Automated Microscope. Vasculature was analyzed and quantified with ImageJ software.

## RESULTS

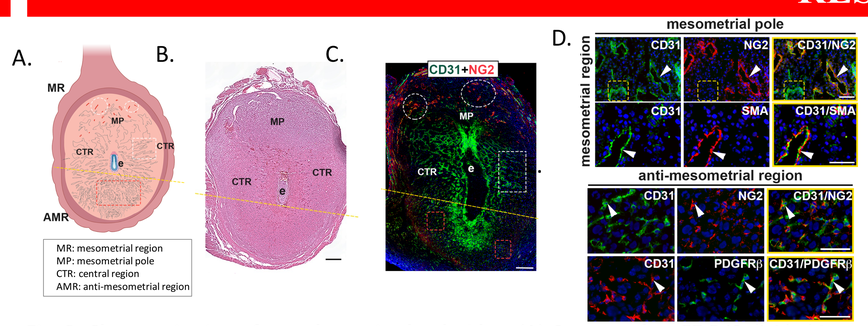


**Fig. 2. Characterization of vasculature in the decidua.** (A) Cartoon of an E7.5 IS divided into regions. SpAs (white ellipses), capillaries (white rectangles) in the CTR, capillaries (red rectangles) in the AMR and the embryo (e). (B) H&E of an IS at E7.5. IS is divided into AMR and MR, MR then into MP and CTR. (C) An E7.5 IS stained for CD31 and NG2. (D) In the MP, SpAs are CD31$^+$ ECs covered by NG2$^+$, and SMA$^+$ mural cells (white arrowheads) and capillaries are ECs not associated with NG2, or SMA (yellow rectangles). (E) In the AMR, capillaries are CD31$^+$ ECs closely associated with NG2$^+$/PDGFRβ$^+$ cells (white arrowheads). Merged images outlined in yellow. Scale bars = 50 um.

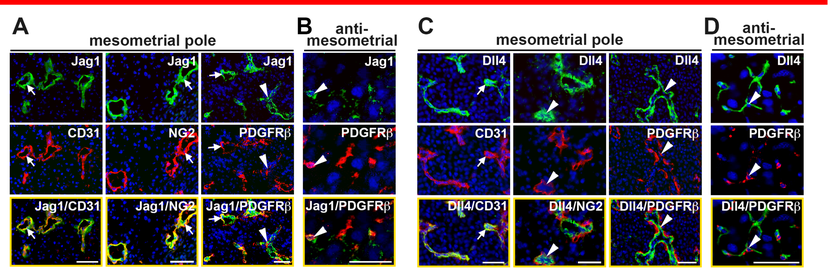


**Fig. 3. Expression of Notch ligands, Jag1 and Dll4, in the decidual vasculature.** (A) In MP SpAs, Jag1 is expressed in CD31$^+$ ECs and in NG2$^+$ and PDGFRβ$^+$ mural cells. Some Jag1$^+$ ECs are closely associated with PDGFRβ$^+$/Jag1$^-$ mural cells. (B) In the AMR, Jag1 is sparse and is expressed in CD31$^+$ ECs and PDGFRβ$^+$ mural cells. Jag1$^+$ cells are closely associated with PDGFRβ$^+$ mural cells. (C) In MP SpAs, Dll4 is expressed in CD31$^+$ ECs. NG2$^+$ and PDGFRβ$^+$ mural cells are closely associated with Dll4$^+$ cells. (D) In the AMR, Dll4 is expressed in CD31$^+$ Ecs. Dll4$^+$ cells are associated with PDGFRβ$^+$ mural cells. Scale bars = 50 um

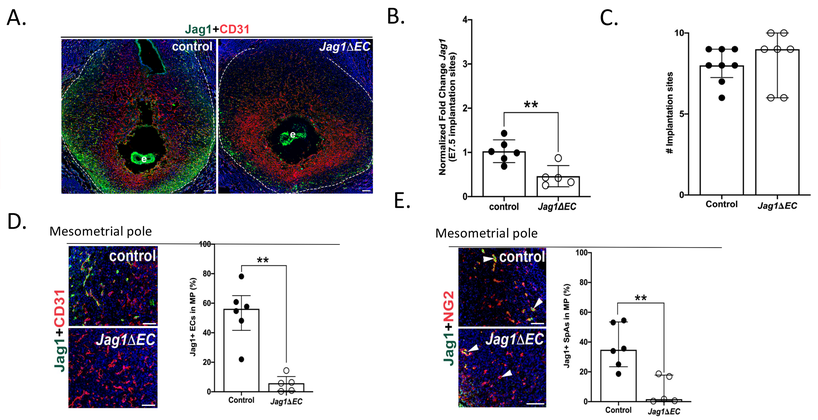


**Fig. 4 TMX-induced Cre recombination decreases expression of Jag1 in *Jag1ΔEC* mutants.** (A) Control and *Jag1ΔEC* pregnancies stained for Jag1 and CD31. Jag1 is reduced in decidua, but not embryo of *Jag1ΔEC* mutants. (B) *Jag1* expression is decreased in *Jag1ΔEC* (n=5) vs controls (n=6). (C) Number of IS at E7.5 is similar between controls (n=8) and *Jag1ΔEC* groups (n=7). (D) Expression of Jag1 in all CD31$^+$ ECs is reduced in *Jag1ΔEC* mutants (n=6) vs controls (n=5). (E) Expression of Jag1 in the ECs of the NG2$^+$ SpAs is reduced in *Jag1ΔEC* mutants (n=5) vs controls (n=6). Scale bars = 100 um. Data shown as median + IQR; *$p < 0.05$,** $p < 0.01$.

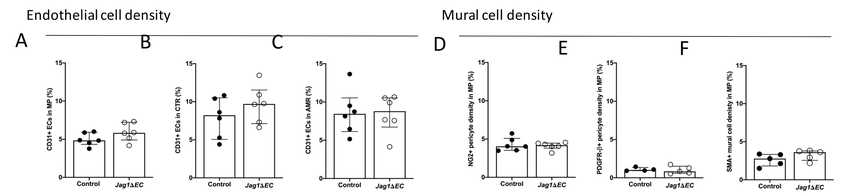


**Fig 6. EC specific loss of *Jag1* does not impact endothelial and mural cell density.** (A-C) Expression of CD31$^+$ was used to determine blood vessel density in the IS. (A) Density of CD31$^+$ ECs was similar in control vs *Jag1ΔEC* pregnancies in the MP (A), CTR (B) and in the AMR (C). (D-F) IS stained for mural cell markers, NG2, PDGFRβ or SMA were assessed for mural cell content at the MP. Expression of NG2 (D), PDGFRβ (E), and SMA (F) is similar in control vs *Jag1 ΔEC* pregnancies in the MP. Data shown as median + IQR.

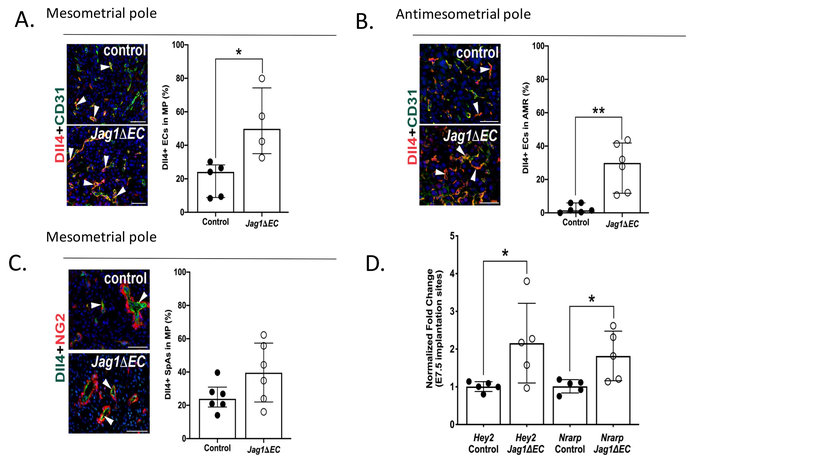


**Fig 5. EC specific loss of *Jag1* increases expression of Dll4 and Notch signaling activity in IS at E7.5.** Expression of Dll4 in capillaries and SpAs in control and *Jag1ΔEC* pregnancies was determined by staining for Dll4 and CD31 or NG2. (A) Dll4 expression is increased in CD31$^+$ ECs at the MP of *Jag1ΔEC* mutants (n=4) vs controls (n=5). (B) Dll4 expression is increased in CD31$^+$ capillary ECs in AMR of *Jag1ΔEC* mutants (n=6) vs controls (n=6). (C) Expression of Dll4 in the ECs of the NG2$^+$ SpAs is unchanged in *Jag1ΔEC* mutants (n=6) vs controls (n=6). (D) qRT-PCR determination of Notch effector gene expression in IS from control (n=5) and *Jag1ΔEC* (n=5) pregnancies. *Nrarp* and *Hey2 are* significantly increased in *Jag1ΔEC* mutants vs controls. Data shown are median +IQR. *$p < 0.05$, **$p < 0.01$

## CONCLUSIONS

Herein we show that EC-specific loss of Jag1 in early pregnancy significantly increases Dll4 expression in CD31$^+$ ECs in the MR and AMR, two areas of the decidua with newly formed decidual vessels. Increased Dll4 was associated with increased Notch signaling. Loss of Dll4 inhibition when Jag1 is deleted in ECs has been previously described in retinal angiogenesis. Our findings are consistent with the retinal model and contribute to understanding Notch signaling in decidual angiogenesis. While we saw no vascular changes at E7.5, future studies at later gestational ages are needed to determine how loss of EC-Jag1 impacts the newly formed capillaries and SpA remodeling.