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PARITY INDUCES ABBERANT RELATIONSHIPS BETWEEN BONE MORPHOGENIC PROTEIN 1 AND EXTRACELLULAR MATRIX SYNTHESIS PROTEINS IN A MOUSE MODEL OF FEMALE PELVIC FLOOR DISORDERS

Hypothesis / aims of study

Basic science and clinical studies have shown underlying pathophysiological changes in connective tissue homeostasis in women with pelvic organ prolapse (POP) compared to those without POP. Additionally, women with POP have decreased elastic fiber organization, altered elastic fiber metabolism, and altered expression of elastin synthesis machinery genes (eg. lysyl oxidase like 1 [*Loxl1*], fibulin 5 [*Fbln5*]) [1]. Lysyl oxidases are involved in the final steps required for collagen and elastin cross-linking. Similar to women, parity is the leading risk factor for POP in the *Loxl1* knockout (*Loxl1* KO) mouse model, and the pathophysiology of POP in these mice may mimic that of women [2]. We aimed to determine differences and relationships in the gene expression of collagen 1 (*Col1*), collagen 3 (*Col3*), fibulin 5 (*Fbln5*), and bone morphogenic protein 1 (*BMP1*) in female *Loxl1* KO and wild-type (WT) mice.

Study design, materials and methods

Loxl1 KO mice (n=54) and WT mice (n=53) were set up in single breeding pairs at 8 wks of age. The pairs were allowed to cohabitate for 2 weeks at which time the males were removed. The females were euthanized at 20d gestation or 4hr, 48hr, 7d, 15d, 25d, 7wk, or 12wk postpartum. Additionally, a group of nulliparous *Loxl1* KO (n=21) and WT mice (n=15) were aged to 11wk, 18wk, or 23wk at which time they were euthanized. The 11wk group was matched in age with the 20d gestation to 25d time point, and the 18wk and 23wk nulliparous groups were matched in age for the 7wk and 12wk postpartum timepoints, respectively. At each time point, the vaginas were harvested and qRT-PCR was used to analyze gene expression of *Col1*, *Col3*, *Fbln5*, and *BMP1* relative to 18s. For analysis of correlations between the genes, the timepoints were categorized into the following groups: day 20 gestation, early postpartum (4hr, 48hr, 7d, 15d, and 25d), and late postpartum (7wk and 12 wk). Pearson's Product Moment test was used to assess correlations between the genes in the two groups. Two-way ANOVA with Bonferroni correction was used to evaluate statistical significance in gene expression between the two groups. $P<0.05$ indicated a significant difference.

Results

There was significantly lower *Col1* and *Col3* expression at 7 days postpartum in KO compared to WT mice. *BMP1* expression was significantly lower at 4 hours postpartum in KO compared to WT mice. There were no significant differences in expression of *Fbln5* between the groups. Analysis for correlations revealed multiple discordances involving relationships between *Col1*, *Col3*, and *Fbln5* to *BMP1* in parous animals*;* however, these discordances were not present in the nulliparous groups (Figure 1). There were positive correlations between *Col1*, *Col3*, and *Fbln5 to BMP1* at 20 days gestation in WT, but there were no correlations in KO animals. In the early postpartum timepoints, there were negative correlations between *Col1* and *Col3* to *BMP1*, but the correlations observed in WT animals were all positive. In the late postpartum period, there was no discordance in correlations between *Col1 and Col3* to *BMP1*, but the discordance in correlation between *Fbln5* and *BMP1* persisted. Although there were also discordances between *Col1* and *Col3* to *Fbln5*, these discordances were present in both parous and nulliparous animals.

Interpretation of results

This data shows a significant abnormality in the regulation of *BMP1* and its relationships to *Col1*, *Col3*, and *Fbln5* in mice that are prone to developing POP. In addition to being required for the activation of lysyl oxidase, *BMP1* is a metalloprotease that plays a key role in extracellular matrix homeostasis by converting precursors into mature proteins necessary for matrix synthesis [3]. In this study, relationships between *BMP1* and *Col1*, *Col3*, and *Fbln5* were different between KO and WT groups only in parous animals. During the early postpartum period, the pelvic tissue remodelling occurs to recover the tissues to a near pre-pregnancy state. During this time, there were negative correlations observed between Col1 and Col3 to Bmp1 suggesting that regulation of *BMP1* (and perhaps elastic fiber remodelling) is not coordinated with collagen remodelling. Furthermore, the dependence on parity is significant given that, like women, nulliparous *Loxl1* KO mice rarely develop POP. The clinical implications of these findings point to an underlying connective tissue deficit that is unmasked by the events of pregnancy and parturition.

Concluding message

BMP1 and its relationships to extracellular matrix synthesis proteins are significantly altered in parous but not nulliparous mice that are prone to developing POP. These findings provide a potential novel target for the development of therapeutics aimed at preventing POP in high-risk women by addressing aberrant connective tissue homeostasis in the pelvic tissues following pregnancy and parturition.

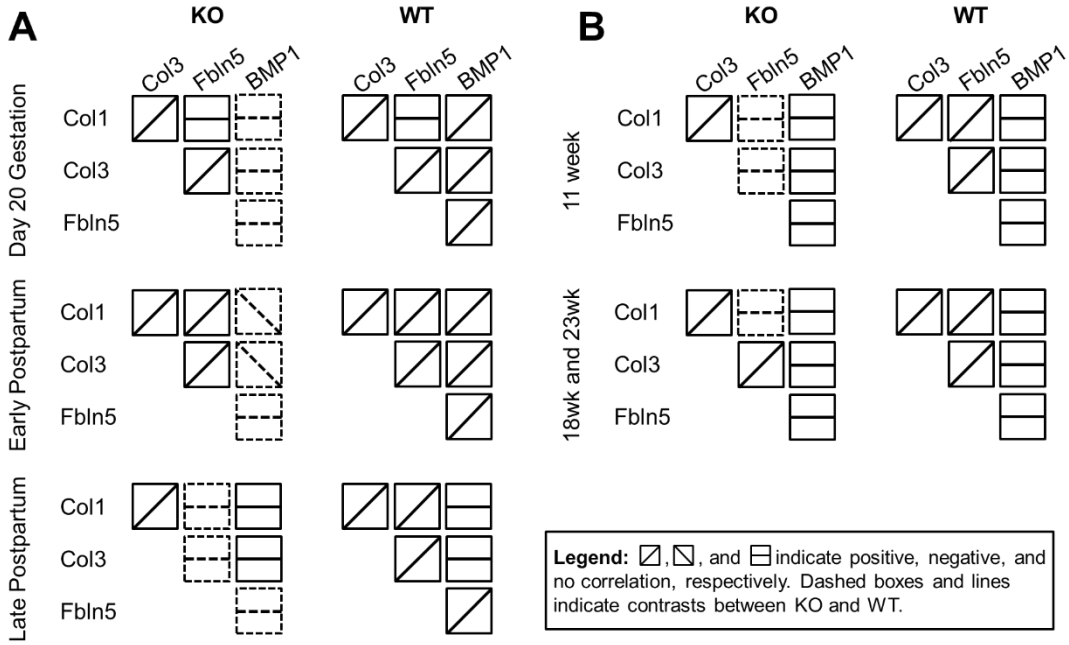

Figure 1. Analysis of Correlations Between *Bmp1*, *Col1*, *Col3*, and *Fbln5*.

References

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Disclosures

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