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Transcriptional Regulation of Connective Tissue Metabolism Genes in Women with Pelvic Organ Prolapse

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Abstract

Objective—To compare differences in expressions and relationships between key genes involved in extracellular matrix metabolism and tissue cellularity in women with and without pelvic organ prolapse (POP)

Methods—A total of 80 biopsies (anterior cuff, posterior cuff, and/or leading edge) were obtained from 30 women: n=10 premenopausal without POP (controls), n=10 premenopausal with POP, and n=10 postmenopausal with POP. Quantitative reverse-transcriptase polymerase chain reaction was used to assess gene expression of BMP1, COL1, COL3, RXFP1, MMP2, TIMP2, and TIMP3. H&E staining was used to assess cellularity of the connective tissue layer. Kruskal-Wallis, Mann-Whitney, Pearson's correlation, or linear regression analyses were used as appropriate.

Results—BMP1 expression was significantly upregulated in patients with POP compared to controls. BMP1 expression was correlated with COL1 expression in all groups, but only correlated with TIMP3 expression in controls. Similarly, COL3 expression was correlated with RXFP1 expression in women with POP but not in controls. The degree of dependence (slope of the regression line) between COL1 and COL3 expression was significantly elevated in premenopausal women with POP compared to both other groups. The slopes between COL1-COL3, COL3-MMP2, COL1-RXFP1, and COL3-RXFP1 expression were significantly lower in postmenopausal

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women compared to premenopausal women with POP. No differences were found in overall tissue cellularity.

Conclusions—BMP1 expression may play a significant role in the pathophysiology of POP. The finding that BMP1 expression was correlated with COL1 expression in all groups suggests a conserved association between BMP1 and collagen synthesis in the vaginal wall. The elevated slope between COL1-COL3 expressions may be associated with early (premenopausal) development of POP. The expression of RXFP1 in postmenopausal women and its altered inter-gene regulation, suggests a role for RXFP1 in connective tissue metabolism outside of pregnancy.

Keywords

pelvic organ prolapse; bone-morphogenetic protein 1; relaxin family peptide receptor 1; collagen metabolism; tissue cellularity

INTRODUCTION

Pelvic organ prolapse (POP) is defined as the descent of the anterior, posterior, and/or apical vaginal compartment(s) with protrusion of one or more of the pelvic organs¹. These pathological changes, which result from loss of structural support to the pelvic organs, can greatly reduce a woman's quality of life^{2,3}. Over 12% of all women will require surgery for POP by the age of 80, and this demand is expected to rise significantly by 2030^{4,5}. Despite the high incidence of POP, little is known regarding its pathophysiology. Therefore, current treatment options primarily involve surgical reconstruction, which does not address the underlying mechanisms of disease^{6,7}.

The pelvic organs are held in proper anatomical position via a complex system of supportive structures that includes both skeletal muscles and connective tissues. Childbirth trauma, e.g. to the levator ani, is associated with POP; however, a significant proportion of women develop POP with no identifiable anatomical injuries⁸. Furthermore, Cesarean section does not completely eliminate the risk of POP^{9–11}. Therefore, childbirth trauma does not provide a complete explanation for the pathophysiology of POP, and genetics likely plays an important role, suggesting that aberrant connective tissue metabolism may be important for the development of POP^{12,13}. This is further highlighted by the higher rates of POP in women with underlying connective tissue disorders such as Marfan and Ehlers-Danlos syndrome¹⁴.

The pelvic organs are primarily supported by collagen and elastic fibers within the pelvic connective tissues. These fibers are maintained by equilibrium between synthesis and degradation^{3,15–17}. During pregnancy and parturition, both collagen and elastic fibers undergo significant remodeling to accommodate gestation and in preparation for childbirth^{18–20}. This tissue remodeling is triggered by pregnancy hormones, particularly, relaxin which has been shown to induce dramatic collagen and elastic fiber remodeling^{21,22}.

Throughout the reproductive cycle and in steady-state, connective tissue metabolism is regulated by a variety of genes including matrix metalloproteinases (MMPs), their tissue inhibitors (TIMPs), and genes involved in collagen and elastin synthesis¹⁵. All MMPs can

degrade one or more components of the extracellular matrix (ECM) and their expression can be induced by various growth factors, cytokines, and by cell-to-ECM interactions^{23,24}. To date, four TIMPs have been identified, and with the exception of TIMP1, which does not bind MMP14, TIMPs have been shown to inhibit all other MMPs²⁵.

Collagens in pelvic connective tissues are composed primarily of collagen III (82%) and collagen I (13%)¹⁷. The major components of these proteins are encoded by the collagen type I alpha 1 (COL1a1) and collagen type III alpha 1 (COL3a1) genes. Unlike collagens, elastic fibers are normally a stable component of the ECM and turn over primarily during pregnancy and parturition²⁶. The synthesis of elastic fibers requires tropoelastin (the monomeric form of elastin) and several cross-linking enzymes including lysyl oxidases and fibulins²⁷. Lysyl oxidases, which cross-link both collagens and elastin, require activation by bone morphogenetic protein 1 (BMP1), a matrix metalloproteinase that also cleaves the C-terminal propeptide of procollagen chains²⁸.

Cellular proliferation of pelvic connective tissue fibroblasts also appears to be involved in the pathophysiology of POP^{29,30}. Cardinal ligament fibroblasts obtained from women with POP have a significantly slower growth rate compared to those obtained from women without POP. Moreover, 17 β estradiol suppresses proliferation of fibroblasts, but the inhibitory effect appears to be more prominent in fibroblasts from women with POP²⁹. In contrast, in vivo studies have suggested an increase in proliferation of fibroblasts and decreased levels of p53 and p21 (proteins known to arrest cell cycle at G1) in women with POP compared to women without POP³⁰.

In this study, we hypothesized that 1) the expression of and relationships between genes involved in connective tissue metabolism is altered in women with POP compared to women without POP; and 2) connective tissue cellularity is increased in the vaginal subepithelia of women with POP compared to women without POP. To this end, we aimed to investigate the gene expression of BMP1, COL1a1, COL3a1, relaxin family peptide receptor 1 (RXFP1), MMP2, TIMP2, and TIMP3, as well as tissue cellularity in premenopausal and postmenopausal women. Given the complex interactions between these genes, we also aimed to identify genes whose expressions are correlated and determine differences in these correlative relationships between groups, yielding mechanistic insight into the pathophysiology of POP.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of the Cleveland Clinic and was a planned secondary analysis of specimens obtained from a previously described group of women with and without POP³¹. Women with symptomatic stage II or greater POP who elected to undergo surgical repair were included in the POP group. This group was further divided into two sub-groups based on age and menopause status: premenopausal women less than 40 years (Premenopausal POP group) and postmenopausal women older than 40 years who were not taking oral or vaginal estrogen replacement therapy (Postmenopausal POP group). Menopause was defined as >1 year cessation of menstruation, and there was no overlap with regard to menopause status between pre- and postmenopausal groups.

As previous authors utilizing similar study designs have noted, the most common benign indication for hysterectomy in postmenopausal women is POP, which makes obtaining “healthy” tissues from postmenopausal women relatively difficult³². Furthermore, it has been suggested that premenopausal tissues may represent an ideal reference for normal pelvic support³². Therefore, we identified patients for our control group by searching through the operating room schedule for patients under age 40 who were undergoing hysterectomy for benign reasons. Each potential subject underwent a focused history and physical examination including detailed pelvic examination by a Gynecologist. In order to be included in the study, the patient had to deny bulge symptoms and the examination had to confirm that no vaginal compartment was within a centimeter of the hymen while straining (POP-Q stage 0 or I). Women with clinical or occult incontinence were allowed in the study. Women who had a history of prior prolapse surgery, malignant disease, or who were less than 18 years of age were excluded. Age and BMI data were collected through review of the medical record, and data pertaining to obstetric history (i.e. maximum newborn weight, mode of delivery, >2 degree obstetric laceration) were collected by patient recall.

After informed consent, a total of 80 biopsies were obtained from 30 women (n=10, each group) who met the inclusion criteria. Women with POP underwent vaginal biopsies from three sites (leading edge, anterior and posterior cuffs) at the time of prolapse repair (n=60 biopsies total). Subjects without POP underwent biopsies at the anterior and posterior cuffs at the time of hysterectomy (n=20 biopsies total). Surgeons were instructed to obtain full-thickness vaginal wall biopsies of approximately 1cm². Each specimen was embedded in OCT media, snap frozen with liquid nitrogen, and stored at -80°C.

RNA Isolation and cDNA Generation

Ten micron thick shavings from frozen tissue blocks were obtained and total RNA was isolated using the RNAqueous-4PCR kit (Cat. AM1914, Life Technologies, NY) according to the manufacturer’s protocol. Following DNase treatment and inactivation, the RNA concentration of each sample was determined using a NanoDrop ND-1000 Spectrophotometer (ThermoScientific, Wilmington, DE). The mean 260/280 ratio was 2.02 ± 0.09 which is considered acceptable for RNA analysis. Reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Cat. 4374966, Life Technologies, NY) in a 20µl reaction volume with 250 ng of total RNA, random primers, dNTP mix, 20 units of RNase inhibitor, and 1 unit of MultiScribe reverse transcriptase at 25°C for 10 minutes, 37°C for 120 minutes, and 85°C for 5 minutes.

Quantitative reverse-transcriptase PCR

Quantitative reverse-transcriptase PCR (qRT-PCR) was used to assess gene expression. Approximately 30 ng of input cDNA was used in each 25 µl amplification reaction using TaqMan Gene Expression Master Mix (Cat. 4369016, Life Technologies, Grand Island, NY) and TaqMan primer-probes (Table 1). Each reaction was carried out using the ABI 7500 Real-time PCR system (Life Technologies, Grand Island, NY). The standard curve method was used to determine the relative amount of each target gene.

Histological Analysis

Hematoxylin and eosin (H&E) staining was used to ensure biopsies were full-thickness (including lamina propria and muscularis) and to assess connective tissue cellularity. For cellularity analysis, images were obtained from three randomly selected fields within the connective tissue layer (lamina propria and muscularis) using a bright-field microscope equipped with a CCD camera. Each image was imported into Image J (US National Institutes of Health, Bethesda, MD) and the multi-point function was used to manually count the number of cells within each field. The number of cells was divided by the total area of each field ($87,000\mu\text{m}^2$), and the mean values of the three fields were used for analyses.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism Version 6.05 (GraphPad Software Inc, La Jolla, CA). The Kruskal-Wallis test followed by Dunn's post hoc test or the Mann-Whitney test were used, as appropriate, to compare the different study groups and biopsy sites. Pearson's correlations analysis was performed between genes within each group to identify genes that are co-expressed (i.e. linearly associated). To determine whether the degree of dependence between a gene-gene pair in one group was significantly different from that of another group, linear regression analysis was performed separately between the groups and a two sample t-test followed by Bonferroni correction for multiple comparisons was used to test the slopes of the regression lines for significant difference. Correlations between cellularity of the stromal layer and genes were assessed similarly. Categorical values in demographics were compared using the Chi-square test or Fisher's exact test, as appropriate. The Robust regression and Outlier removal method (ROUT) was used to identify potential outliers for relative gene expression comparisons and linear regression analysis³³. In all cases, $p < 0.05$ was used to determine statistically significant differences between groups. Data is presented as mean $\pm$ standard error (SE), slope $\pm$ SE, or median with interquartile range.

RESULTS

Thirty subjects were included in this study. Postmenopausal women were significantly older than women in both the premenopausal POP and control groups ($p < 0.001$), and women in the premenopausal POP group had significantly lower BMI than those in the control group ($p = 0.048$). There were no significant differences between groups with regard to maximum newborn weight, mode of delivery, or history of obstetrical tears (Table 2).

Comparison of relative gene expression levels between groups was used to determine genes that are upregulated or downregulated between the 3 groups (Figure 1). To prevent bias due to biopsy sites, comparisons against controls were performed using data from only the anterior cuff and posterior cuff biopsies obtained from pre- and postmenopausal POP groups. Overall, BMP1 was significantly upregulated in both postmenopausal (2.46 ± 0.33 vs 1.11 ± 0.16 , $p = 0.012$) and premenopausal women with POP (2.33 ± 0.35 vs 1.11 ± 0.16 , $p = 0.025$) compared to controls. Furthermore, pairwise analysis between individual biopsy sites showed significantly upregulated BMP1 in the anterior cuffs of premenopausal (2.23 ± 0.56 vs 1.01 ± 0.07 , $p = 0.032$) and postmenopausal women (2.36 ± 0.29 vs 1.01 ± 0.07 ,

p=0.002) with POP compared to controls. No other significant differences in relative gene expression levels were found between the two POP groups compared to controls.

Pairwise analysis of individual biopsy sites between groups revealed that specimens obtained from the leading edge in patients in the premenopausal POP group had significantly higher expression of MMP2 compared to leading edge specimens from the postmenopausal POP group (1.18 ± 0.22 vs 0.61 ± 0.13 , $p=0.0435$). No significant differences were found between posterior cuff and anterior cuff biopsies in any group. Interestingly, COL1, TIMP2, and TIMP3 were all significantly downregulated in the leading edge compared to the posterior cuff in postmenopausal POP women.

Pearson's correlation analysis was performed to determine co-expressed genes (Figure 2 and Supplement 1). BMP1 expression was correlated with COL1 expression in all groups (Control, Correlation Coefficient [CC]: 0.58, $p=0.03$; Premenopausal, CC: 0.403, $p=0.037$; Postmenopausal, CC: 0.44, $p=0.017$). However, BMP1 and TIMP3 expression were correlated only in the control group (CC: 0.564, $p=0.036$). Additionally, COL1 expression was correlated with TIMP3 expression in the POP groups (Premenopausal, CC: 0.684, $p<0.001$; Postmenopausal, CC: 0.466, $p=0.012$), but not in the control group (CC: 0.308, $p=0.245$). Similarly, TIMP2 and TIMP3 expression were correlated in the POP groups (Premenopausal, CC: 0.640, $p<0.001$; Postmenopausal, CC: 0.553, $p=0.002$), but not in the control group (CC: 0.462, $p=0.072$). The relationship between COL3 and RXFP1 showed a similar pattern (Premenopausal, CC: 0.609, $p=0.002$; Postmenopausal, CC: 0.458, $p=0.014$; Control, CC: 0.399, $p=0.113$).

When correlations between any gene-gene pair were significant in more than one study group, linear regression analyses followed by a two-sample t-test for comparison between the two groups was performed. The magnitude of correlation (i.e. slope of the regression line) between COL3 and MMP2 was significantly smaller in postmenopausal women with POP compared to both the premenopausal POP group and controls ($p=0.012$ and $p=0.003$, respectively). Similarly, the slopes of regression lines between COL1-COL3, COL1-MMP2, COL1-RXFP1, COL3-MMP2, COL3-RXFP1, MMP2-RXFP1 were significantly decreased in postmenopausal women compared to premenopausal women (Figure 2 and Table 3).

With regard to cellularity of the vaginal subepithelium, we found no significant difference between groups or biopsy sites (Figure 3). The mean cellularity for the anterior and posterior cuff biopsies were 2058 ± 608 , 2018 ± 687 , and 2009 ± 526 cells/mm² for the control, premenopausal POP, and postmenopausal POP groups, respectively. No significant correlations to gene expression levels were found except with regard to BMP1 and RXFP1. BMP1 was positively correlated to tissue cellularity in the premenopausal POP group (CC=0.47, $p=0.049$) and RXFP1 was positively correlated to tissue cellularity in the postmenopausal POP group (CC=0.397, $p=0.036$).

DISCUSSION

Most studies investigating the role of connective tissue metabolism in the pathophysiology of POP have focused on alterations at the protein level. However, we chose to focus our

investigation at the transcriptional level since many connective tissue metabolism genes, especially MMPs and TIMPs, are primarily regulated at this level. Previously published work on this subject has demonstrated conflicting findings, perhaps due to differences in biopsy sites³⁴ (Table 4). However, our study is the first to assess the correlational relationships between these genes, which we believe are helpful in elucidating the pathophysiological pathways involved^{35,36}.

Of the previously published studies that investigated gene expression levels in women with POP compared to controls, only one assessed BMP1³⁷. In their study, the authors found a 3-fold reduction in BMP1 gene expression in women with POP compared to controls. It is important to note that the reported mRNA sequence used in the study (GenBank: NM_006132) has been permanently removed from the GenBank database as it has been found to be a target of nonsense-mediated mRNA decay (NMD) and thus not translated into protein^{38–40}. NMD pathways exist in all eukaryotic cells and serve to degrade mRNA transcripts that contain premature stop codons, which if translated could lead to accumulation of harmful truncated proteins^{39–41}. Therefore, since the reported mRNA sequence used in the study has been identified as a target for NMD, the targeted transcripts may have been subjected to degradation, which adds significant uncertainty to the interpretation of relative gene expression^{41,42}.

Furthermore, the analysis of BMP1 protein levels was conflicting in this prior paper³⁷. The authors found downregulated protein levels of the 130, 92.5, and 82.5 kDa isoforms of BMP1 and no differences in the 70 kDa isoform in postmenopausal women with POP (n=7) compared to controls (n=4). However, they found upregulated protein levels of the 130 kDa isoform and no differences in the 92.5, 82.5, and 70 kDa isoforms in premenopausal women with POP (n=14) compared to controls (n=7).

In our study, we found that BMP1 gene expression was over 2-fold higher in women with POP (n=20) compared to controls (n=10), regardless of menopause status. Additionally, we found that BMP1 was positively correlated with COL1 in all study groups (Figure 2) although COL1 expression was not significantly different between the groups. This suggests that the increase in BMP1 expression in women with POP indicates a state of increased tissue remodeling. Interestingly, BMP1 expression was correlated with TIMP3 expression only in the control group, which suggests decreased MMP inhibition along with increased tissue remodeling in POP patients.

The finding that BMP1 was correlated to connective tissue cellularity in the premenopausal POP group implicates a role for apoptotic pathways in the development of POP in this group of patients. We also found that RXFP1, which other studies have found to be involved in prostate cancer growth⁴³, was positively correlated with tissue cellularity. Indeed, several studies have found alterations in apoptosis-related mechanisms in women with POP compared to controls^{44–46}. Wen et al. found that women with POP have increased cellular sensitivity to apoptosis in vaginal tissues, as determined by ratios between proapoptotic and anti-apoptotic gene expression levels⁴⁴. Similarly, Bai et al found decreased protein expression of the tumor suppressor genes, p53 and p21, in POP patients compared to controls⁴⁶. With regard to tissue cellularity, Takacs et al conducted a histological study

comparing women with (n=6) and without (n=6) POP⁴⁵. In their study, the authors counted only smooth muscle cells identified by alpha-actin staining, and found that women with POP have a decreased number of smooth muscle cells compared to controls (1166 ± 727 vs. 2370 ± 467 , $p < 0.01$)⁴⁵. In contrast, we found no differences in total connective tissue cellularity in women with POP compared to those without (2013 ± 505 vs 2058 ± 444 , $p = 0.87$). Collectively, this indicates that although total tissue cellularity may be the same, the proportion of cells staining for smooth muscle α -actin may be decreased in women with POP compared to controls.

Despite utilizing the standard curve method for qRT-PCR analysis, which is more sensitive to small changes in gene expression levels than the comparative CT method, we found no differences in expression levels of MMP2, TIMP2, and TIMP3. Similarly, Chen et al. utilized an absolute quantification method, and found no differences in the expression levels of these genes⁴⁷. With regards to TIMP2 and TIMP3, our study is the third study to find no differences in the vaginal wall of patients with POP compared to controls^{47, 48}. Despite their different methodologies, the unanimity between these studies suggests more conclusively that the overall expression levels of TIMP2 and TIMP3 are not altered in the vaginal wall of women with POP. However, a role for these genes in the pathophysiology of POP cannot be ruled out. We found that COL1 and TIMP2 were correlated with TIMP3 in both pre and postmenopausal women with POP but not in the control group, suggesting alterations in the regulation of connective tissue modeling in patients with POP (Figure 2).

Our findings with regards to COL1 and COL3, contradict those of Mosier et al, who found increased levels of both genes in postmenopausal women with POP compared to age-comparable controls⁴⁹. This discrepancy could be due to differences in the control groups. In their study, the control group consisted of women undergoing radical cystectomy for invasive bladder cancer, whereas our control group consisted of women undergoing hysterectomy for benign indications. Although we found no differences in overall expression levels of COL1 and COL3, we found a significantly larger slope (i.e. closer correlation in expression) between COL1 and COL3 expression in premenopausal women with POP compared to both controls and postmenopausal women with POP (Table 3), suggesting that abnormal regulation of COL1 and COL3 may contribute to the early predisposition to POP in premenopausal women.

We also found significant differences in the relationships between these genes and RXFP1, since the slope of the regression line between RXFP1 and both COL1 and COL3 in postmenopausal women with POP was significantly less than premenopausal women with POP. Furthermore, the slopes between COL3-MMP2 and MMP2-RXFP1 were both significantly lower in postmenopausal women compared to premenopausal women with POP. Relaxin (ligand for RXFP1) is predominantly produced by the corpus luteum and placenta during pregnancy; however, it is also produced by the breast and prostate and is detectable in serum outside of pregnancy⁵⁰. Although we found no differences in overall RXFP1 expression levels, which corroborate results from previous reports comparing uterosacral ligament biopsies⁵¹, our findings of altered inter-gene regulation and the fact that it is expressed in postmenopausal women with POP suggests a role for RXFP1 in connective tissue metabolism outside of pregnancy.

A limitation of our study is that women in the control group did not undergo the same standardized POP-Q examination as women in the POP groups. Therefore, this group likely included some women with stage I POP, which could be indicative of a pathophysiological process in progress. Moreover, the majority of patients in the POP groups had stage II or III prolapse, with only one patient with stage IV prolapse. This may have dampened our results given that if we had included more patients with stage III and IV prolapse, we may have seen greater differences. Nonetheless, we still found significant differences between controls and both premenopausal and postmenopausal women with POP.

Another limitation of this study is that protein expression levels were not assessed. However, our aim was to assess regulation of connective tissue metabolism downstream of translation in order to identify differences in gene expression as well as genes that are co-expressed. Gene expression correlational analyses are routinely performed throughout the basic sciences and co-expressed genes are of biological interest because they are likely controlled by the same transcriptionally regulatory pathway and are functionally related^{35,36,52}. Given our results regarding increased BMP1 mRNA levels in women with POP compared to those without POP, comprehensive analyses of the various isoforms of this protein should be an area of future research.

In future work protein expression could be correlated with severity of symptoms. Since the aim of the current study was to assess expression and correlation of multiple genes, it was not powered to also correlate expression to severity of symptoms. Nonetheless, the results of our study could provide rationale for future studies since a strength of our study is the utilization of robust correlational analyses in addition to assessment of gene expression levels. Using this method, we were able to more comprehensively characterize the transcriptional regulation of various connective tissue metabolism genes that may be associated with POP. Overall, our results help elucidate potential molecular pathways involved in the pathophysiology of POP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Relative Gene Expression Levels of BMP1 (A), COL1 (B), COL3 (C), RXFP1 (D), MMP2 (E), TIMP2 (F), and TIMP3 (G). Data is presented as mean $\pm$ standard error of the mean. Abbreviations: AC, anterior cuff; PC, posterior cuff; LE, leading edge; a, indicates significant difference compared to control.

Figure 2.

Correlation matrices and linear regression analysis. Correlation matrices with scatter plots and regression lines are presented in panels A, B, and C. Shaded circles (red) indicate outliers, which were automatically excluded from linear regression analyses. Shaded boxes indicate a significant Pearson's correlation coefficient ($p < 0.05$). Comparisons of regression lines (slopes) are presented in panels D, E, and F. Regression lines were only compared when a significant correlation existed for the specific gene-gene pair in all three study groups. Blank boxes represent regression lines that were not compared due to lack of correlation.

Figure 3.

Cellularity analysis (A) and correlations with gene expression (B). Shaded boxes indicate a significant Pearson's correlation coefficient ($p < 0.05$).

Table 1

Primer Probes

Gene Name	Gene Symbol	Assay ID
Bone morphogenetic protein 1	BMP1	Hs00241807_m1
Relaxin/insulin-like family peptide receptor 1	RXFP1	Hs01073141_m1
Collagen, type I, alpha 1	COL1a1	Hs00164004_m1
Collagen, type III, alpha 1	COL3a1	Hs00943809_m1
Matrix metalloproteinase 2	MMP2	Hs01548727_m1
TIMP metalloproteinase inhibitor 2	TIMP2	Hs00234278_m1
TIMP metalloproteinase inhibitor 3	TIMP3	Hs00165949_m1
18s ribosomal RNA	18s	4319413E

Note: All primer probes purchased from Life Technologies, Inc.

Table 2

Demographics

	Control	Premenopausal POP	Menopausal POP	p-value
Sample size	10	10	10	
Age, years	47.0 (39.3, 51.5)	36.5 (34.8, 37.5)	58 (54.5, 61.8) <i>ab</i>	<0.0001
BMI	27.7 (26.0, 38.0)	22.4 (21.1, 30.4) <i>a</i>	26.1 (22.9, 28.2)	0.050
Parity	2 (1, 3)	3 (1.8, 4)	2.5 (1.8, 3.3)	0.596
Max Newborn Weight, g	3520 (3150, 4055)	3770 (3150, 4203)	3700 (3498, 4288)	0.769
Delivery history				
Cesarean only (n)	4	1	1	NS
Vaginal delivery (n)	4	7	7	
Operative delivery (n)	1	1	2	
Data not available	1	1	0	
Obsetrical tear > 2nd degree laceration (n)				
Yes	9	7	9	NS
No	0	2	1	
Data not available	1	1	0	
POPQ Stage (n)				
1	N/A	0	0	0.003
2		10	3	
3		0	6	
4		0	1	

Note: Continuous variables presented as median (inter-quartile range)

NS, not significant; NA, not applicable;

^a p<0.05 compared to control;

^b p<0.05 compared to premenopausal

Table 3

Inter-Gene Regression Slopes

	Control	Premenopausal	Postmenopausal
COL1 vs. COL3	0.49 ±0.06	0.70 ±0.05 ^A	0.42 ±0.04 ^B
COL1 vs. MMP2	0.75 ±0.2	0.82 ±0.19	0.35 ±0.06 ^B
COL1 vs. RXFP1	0.41 ±0.16	0.47 ±0.13	0.13 ±0.04 ^B
COL3 vs. MMP2	1.53 ±0.26	1.26 ±0.21	0.60 ±0.08 ^{A,B}
COL3 vs. RXFP1	0.53 ±0.32	0.71 ±0.16	0.18 ±0.07 ^B
MMP2 vs. RXFP1	0.49 ±0.08	0.46 ±0.06	0.24 ±0.07 ^B
TIMP2 vs. RXFP1	0.47 ±0.07	0.28 ±0.06	0.21 ±0.08 ^A

Note: Data presented as slope ± standard error

^A
p<0.05 compared to control

^B
p<0.05 compared to premenopausal

Review of the Literature

Table 4

Study	Tissue	Reference Gene	Gene Expression in POP vs Control							
			BMP1	COL1	COL3	RXFP1	MMP2	TIMP2	TIMP3	
Chen et al. 2002	VW	---					NS	NS	NS	
Mosier et al. 2010	VW	18s		↑	↑					
Bortolini et al. 2011	VW	ACTB, TBP, SDHA	↓							
Shynlova et al. 2013	VW	SDHA, YWHAZ, ACTB						NS	NS	
Alarab et al. 2014	VW	β-actin, TBP, YWHAZ						↓	↓	
Soderberg et al. 2009	PUL	18s		NS	NS					
Connell et al. 2008	USL	β-actin		↓	↓	↑				
Moon et al. 2011	USL	α-tubulin				↑				
Liang et al. 2012	USL	GAPDH				↑		↑		
Dietrich et al. 2012	USL	B2M, 18s, TBP				NS				
Yilmaz et al. 2014	USL	β-actin				↑				

Abbreviations: VW, vaginal wall; PUL, pubo-urethral ligament; USL, uterosacral ligament; NS, no significance

Note: The absolute quantification method used by Chen et al did not require a reference gene.