85

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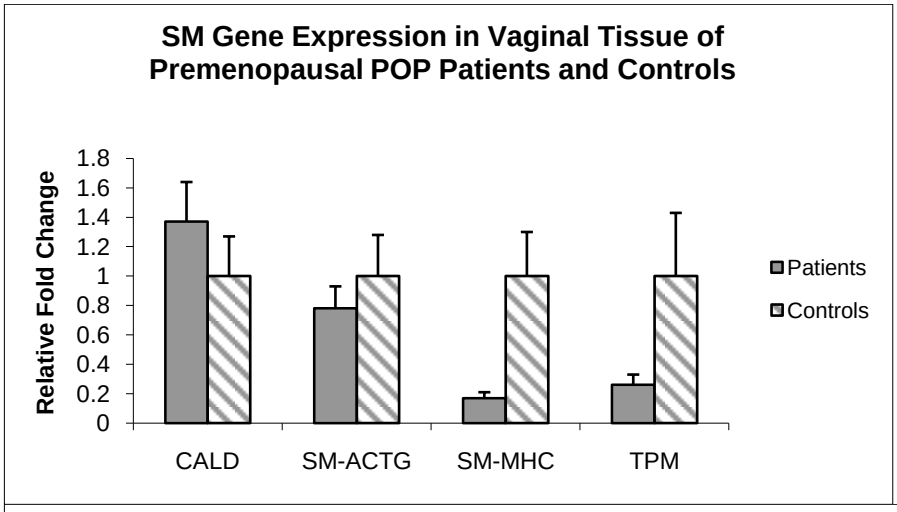
EXPRESSION OF GENES REGULATING THE SMOOTH MUSCLE CONTRACTION IN THE VAGINAL TISSUE OF WOMEN WITH AND WITHOUT PELVIC ORGAN PROLAPSE

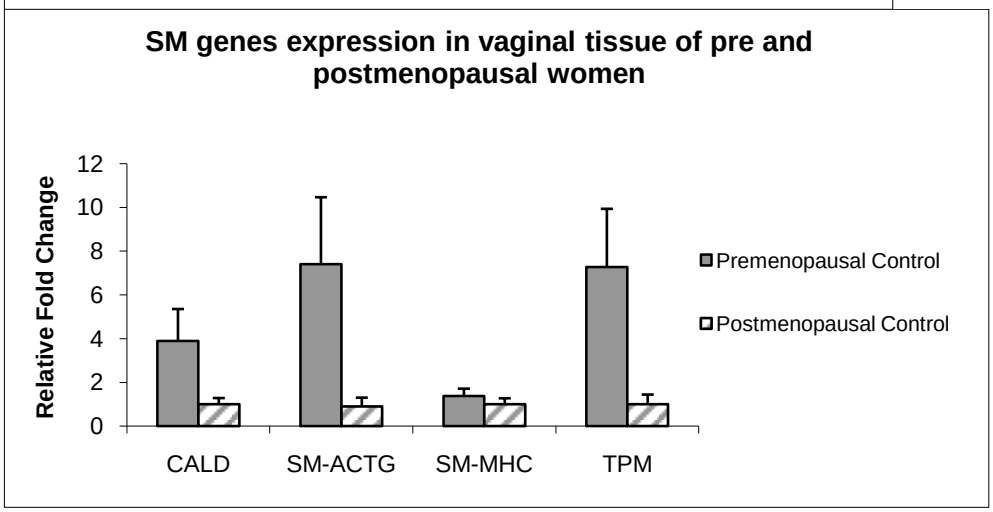
Hypothesis /Aims of Study: Pelvic Organ Prolapse (POP) is a result of mechanical failure in the pelvic floor tissue that supports the abdominal and pelvic organs. The pelvic floor support is provided mainly by the interaction between the pelvic muscles (Levator Ani Muscle) and connective tissue of the urethra, rectum and vaginal wall. The smooth muscle (SM) fibers derived from the vaginal wall attach to the Levator Ani Muscle complex also playing an integral role in the pelvic floor support [1]. Dysfunction of the SM may affect the attachment of the lateral vagina to the pelvic side wall. Some evidences suggest that the SM of the vaginal wall is altered in women with POP [2]. The reason for the decrease in SM fraction of women with POP may be as simple as aging and menopause or complex as a genetic disorder involving SM gene and protein expression. In fact, many studies affirm a synergistic and deleterious interaction between hypoestrogenism status after the menopause and normative ageing in the whole urogenital tract [3]. Smooth muscle content is found diminished in vaginal tissues of postmenopausal women [2]. However, more studies are needed to clarify the relationship between vaginal SM, estrogen and age. In this study, we hypothesize that expression of genes that regulates the vaginal smooth muscle contraction are (1) altered in women with POP and (2) altered in women after the menopause. We aim to study the gene expression of Smooth Muscle-Myosin Heavy Chain (SM-MHC), Caldesmon (CALD1), Tropomyosin (TPM1) and Smooth Muscle Gamma-Actin (SM-ACTG2) in vaginal tissue of (1) premenopausal women with advanced POP compared to premenopausal controls and (2) premenopausal compared to postmenopausal healthy women.

Study Design, Material and Methods: Caucasian women undergoing total hysterectomy for benign conditions were recruited and divided in 4 groups: premenopausal women with advanced POP and controls, and postmenopausal women with POP and control. We considered stage ≥ 3 by POP-Q classification as advanced POP and stage 0 as controls. Exclusion criteria included: hormonal replacement therapy, steroids therapy, pelvic organ prolapse or stress urinary incontinence history, previous pelvic surgery and history of connective tissue diseases. During the surgical procedure, 1 cm^2 of full thickness vaginal tissue was obtained from the surgical cuff. The samples were immediately frozen in liquid nitrogen and storage at -80^oC until analysis. Total RNA was extracted using TRIZOL. Real time PCR was performed to quantify mRNA levels of SM-MHC, TPM1, CALD1 and SM-ACTG2. We would need 15 patients in each group to have 80% power to detect differences in these genes using independent groups for testing. Mann-Whitney test ($p<0.05$) was used for statistical analysis.

Results: 37 premenopausal (23 patients and 14 controls) and 17 postmenopausal women (12 POP and 5 controls) were enrolled. The four genes of interest are expressed in vaginal tissue. In premenopausal group, we could observe that SM-MHC ($p<0.05$), TPM1 ($p<0.05$) and SM-ACTG2 genes were down-regulated in POP patients compared to controls, while CALD1 expression was up-regulated in the POP patients group. SM-MHC and TPM1 were decreased in 6-fold and 4-fold change, respectively, in the POP patients group. We observed that the expression of the four genes: SM-MHC, TPM1, CALD1 and SM-ACTG2 were decreased in postmenopausal compared to premenopausal healthy women. The gene expression of TPM1, CALD1 and SM-ACTG2 were down-regulated after menopause in 7-fold, 4-fold and 7-fold change, being those differences statistically significant ($p<0.05$).

Interpretation of Results: Actin and myosin are the major contractile proteins involved in the generation of tone by smooth muscle. The SM contractile machinery is also composed by the group of actin-binding proteins including tropomyosin and caldesmon. The observation that expression of SM-MHC, TPM1 and SM-ACTG2 genes is decreased in patients with POP suggests that SM fibers of the prolapsed vaginal wall may not exhibit normal contractilily. The upregulation of CALD1 gene expression in POP women also contributes to the impaired SM contraction in the vagina once this protein acts as an inhibitor of the maintenance of contractile force. Age and menopause are known to influence the components of the pelvic floor tissue. Our observation that the expression of the SM contractile machinery genes are decreased after the menopause reaffirms the age-hormonal effect. Those alterations may indicate the decreased contractile ability of the vaginal wall, which may predispose postmenopausal women to the development or aggravation of pelvic floor dysfunction.
Concluding Message: Patients with severe POP showed altered expression of genes regulating the smooth muscle contraction in the vaginal wall tissue, which may contribute to the physiopathology of POP in women. Our preliminary results show that age-related hormonal status may influence the expression of the SM contractile machinery genes in the vagina of women. This information will help us to understand the physiopathology of some of the pelvic floor dysfunctions affecting women after the menopause.





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Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**Research Ethics Board - Mount Sinai Hospital - Toronto**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**