BJUI
BJU INTERNATIONAL

Pelvic floor disorders: linking genetic risk factors to biochemical changes

Lysanne Campeau, Ilya Gorbachinsky*, Gopal H. Badlani* and Karl Erik Andersson

*Institute for Regenerative Medicine, Wake Forest University, and *Department of Urology, Wake Forest University Baptist Medical Center, Winston-Salem, NC, USA*

Accepted for publication 11 March 2011

Pelvic floor disorders (PFDs) such as stress urinary incontinence (SUI) and pelvic organ prolapse (POP) may share a common pathophysiological process related to pelvic floor tissue laxity and loss of support. We reviewed recent literature on observed biochemical changes in women with SUI and POP, linking them to genetic predisposition. We found that studies of pelvic tissues showed differences between control subjects and women with POP and SUI in collagen and elastin structure at a molecular and fibrillar level. Studies were heterogeneous but showed a trend towards decreased collagen and elastin content. The contribution of matrix metalloproteinases to increased collagenolysis can be related to genetic polymorphisms present in higher frequency in women with PFD. Extracellular matrix (ECM) protein turnover plays a role in the development of POP and SUI, but much remains to be understood of this complex dynamic interplay of enzymes, proteins and molecules. Genotyping of candidate genes participating in ECM formation will elucidate the missing link between the manifestation of the disease and the biochemical changes observed systematically, in addition to those in the pelvic floor.

What's known on the subject? and What does the study add?
Pelvic floor disorders are associated with altered biochemical composition and structure of pelvic tissue. Epidemiological studies have postulated a genetic contribution to pelvic floor disorders.

Certain biochemical changes can be explained by candidate genes and polymorphism involved in the expression of ECM-related proteins.

KEYWORDS

pelvic floor disorder, pelvic organ prolapse, stress urinary incontinence, collagen, elastin, matrix metalloproteinase

INTRODUCTION

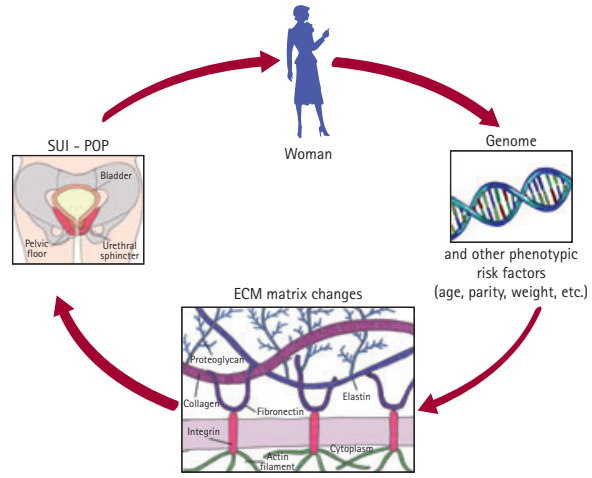
Pelvic floor disorders (PFDs) can greatly affect the health-related quality of life of women. Although not life-threatening, PFDs can be manifested in many different ways such as stress urinary incontinence (SUI), anal incontinence, chronic pain syndromes and pelvic organ prolapse (POP). POP is defined by the ICS as the descent of one or more parts of the vaginal wall. This condition has a direct impact on urinary continence status and is clinically associated with SUI in >72% of cases [1]. SUI is defined by the ICS as leakage resulting from increased intra-abdominal pressure. A nationwide cross-sectional analysis found that 24% of women >20 years old suffered from at least one PFD, with 16% affected with SUI and 3% having POP [2]. The proportion of women with one condition increased with age and parity, but SUI is the general manifestation of multiple contributing factors, including nerve damage and sphincteric injury, that are not directly linked to POP. This may partly explain the difference in prevalence of the two disorders. A recent prevalence study by Buckley and Lapitan [3] revealed that SUI is a disease of all ages, but with a peak prevalence of 8–70% in people 40–60 years old.

It has been well described that women suffering from Ehlers–Danlos syndrome and Marfan's disease have a higher risk of POP and SUI, suggesting that connective tissue disorder is an etiological factor [4]. Other connective tissue deficiencies such as hernias may share common pathophysiological mechanisms with POP and SUI. In a group of 60 women with advanced prolapse, the total prevalence of hiatal and inguinal hernias was significantly higher than in a control group of 60 women with mild or no prolapse (31.6% vs 5%, $P < 0.001$) [5]. A population-based, cross-sectional study of 5,489 Stockholm women found an odds ratio (OR) of 1.8 for a positive association with symptomatic prolapse in women with a history of conditions suggestive of deficient connective tissue (varicose veins/hernia/haemorrhoids) [6].

One of the causes of SUI may be linked to biochemical processes in the pelvic floor that are also present in POP and that initiate laxity and loss of support. The present review will focus on observed biochemical changes in women with SUI and POP and a link to genetic predisposition (Fig. 1).

Using the PubMed, MEDLINE and MeSH databases, we performed a critical review

FIG. 1.
Linking genetic risk factors to biochemical changes in PFDs.



of English-language publications that contained the following keywords: pelvic organ prolapse, stress urinary incontinence, polymorphism, genetic, collagen, elastin and extracellular matrix. A total of 155 articles were considered in the process but, based on subject relevance and scientific content, only 65 were included in the present review.

FAMILIAL TENDENCIES

The reason that certain women will develop PFDs at a young age has been attributed mainly to risk factors such as increased parity or obesity. A retrospective study by Kim *et al.* [7] found that age >70 years, parity >3, and menopause were all independent risk factors for POP; however, family history also plays a role in predisposing women to these disorders. Altman *et al.* [8] used the Swedish Twin Registry to determine the contribution of genetic and environmental factors in the development of SUI and POP. They found that genetic effects account for 41% and 43% of the variation in liability for SUI and POP respectively. They also revealed that environmental factors contributed to the same extent as genetic heritability to the development of both SUI and POP [8]. In a cohort study, McLennan *et al.* [9] showed that the risk of POP increased 1.4 times in women with a family history of prolapse and/or hernia after adjusting for vaginal deliveries, hysterectomy and incontinence. Buchsbaum *et al.* [10] studied the concordance of the presence of incontinence and POP in parous women and their nulliparous sisters. They did not find any difference in the rate, severity or type of UI between the groups. They identified a high concordance in continence status between sisters. The same authors found a high concordance for POP between parous women and their nulliparous sisters, but women who had a vaginal delivery were found to have more advanced prolapse in 88% of cases [11]. The UCLA group reported an autosomal dominant transmission as the most likely mode of inheritance, based on a collection of families with a high incidence of prolapse [12].

Several publications suggest a familial component to overall cases of POP and SUI. The heritability of these diseases appears to be a well accepted fact in the scientific community, although the data is so far based on case-control studies. The Swedish Twin Registry Study [8] provides a higher level of evidence (level 2) that SUI and POP are heritable conditions, but more studies of this calibre, looking at each condition separately, are needed to ascertain a familial link.

EXTRACELLULAR MATRIX IN PFD

COLLAGEN ARCHITECTURE AND CONTENT

The female pelvic floor is an anatomically complex structure made of smooth and skeletal muscles, ligaments and fascia. The ligaments and fascia are primarily composed of extracellular matrix (ECM). The latter is made of a ground substance of proteoglycans and glycoproteins with collagen type III and I, along with elastin, which confers the extraordinary compliance and elasticity required for vaginal childbirth. This ECM is in constant rearrangement with synthesis and breakdown of collagen through a process of remodelling. Collagen is a triple helix structure made of α polypeptide chains stabilized with hydrogen bonds. There are 28 types of collagen described in the body, but the most abundant in the pelvic floor are types I and III. The arrangement of the different types of collagen into fibrils of variable sizes confers strength or laxity depending on the ratio. Type I collagen is considered the strongest, being highly prevalent in fascia, ligaments and fibrous tissues. Type III is also found in the same tissues, but tends to be located on the surface of the fibril. A larger contribution of type III to a fibre will reduce its diameter and mechanical strength [13]. Several studies have taken a closer look at tissue properties through the use of human pelvic floor biopsies of women with either POP or SUI. Salman *et al.* [14] showed, using light microscopy, that samples of cardinal ligament from 8/10 women with uterine prolapse had loosely arranged connective tissue fibres and less dense ECM compared with three women in the control group. Electron microscopy revealed more orderly, densely packed and smaller collagen fibres in the control group compared with the POP group (52.5 vs 61.2 nm; $P < 0.001$). Another study performed immunohistochemical analysis of the anterior vaginal wall of 23 women with anterior vaginal wall prolapse and 15 women with normal genital support. They found that women with POP had statistically significantly less dense staining of collagen type III than women in the control group after controlling for age, weight, parity, urodynamic stress incontinence and menopause [15]. Connell *et al.* [16] studied the function of a homeobox gene, *HOXA11*, involved in the development of the lower uterine segment and cervix. They found a decreased expression of collagen type I and III, which correlated with a decrease in *HOXA11* expression in uterosacral ligaments of women with POP using immunohistochemistry and real-time PCR.

Diminished collagen levels have been found in multiple pelvic tissues of women with SUI, including the endopelvic fasciae [17], anterior vaginal walls [18], and round ligaments [19]. Several of these investigators [17,19] also showed a similar finding within skin cells, suggesting that the observation within the pelvic tissues was not only a consequence of SUI, but probably

represented an underlying systemic process of low collagen state. Although some have suggested that an alteration in the collagen type I to type III ratio may alter the pelvic connective tissues, both *ex vivo* [17] and *in vivo* [20] data from populations with SUI fail to support this view. Weakened pelvic floor support may result from lower collagen content, but also from alterations within the distribution of collagen fibres. Trabucco *et al.* [21] noted a much more disordered collagen fibre distribution within periurethral specimens of women with SUI compared with unaffected controls. Alteration in proteoglycan content can affect fibrillogenesis and therefore cause disorganized collagen fibre distribution. The small leucine-rich proteoglycans (SLRP) decorin and fibromodulin have been found to be more prevalent in premenopausal SUI periurethral tissues [21,22]. It is possible that excessive amounts of these SLRPs are interfering in the formation, maintenance, or even destruction of the ECM superstructure [21]. By contrast, Söderberg *et al.* observed a lower amount of mRNA of decorin and lumican, both SLRPs, using real-time reverse-transcriptase PCR in women with POP [23].

Recent data support older publications that found decreased total collagen in women with PFDs. A consensus has not yet been reached owing to the diverse, yet evolving, methods of detection, biopsy type and assessed outcomes. The investigated patient population has so far been heterogeneous and too small to allow satisfying conclusions on the different collagen components to be drawn. Further studies should examine a consistent and standardized biopsy area with a quantitative and reliable method in groups of women with possibly fewer confounding factors such as age and parity.

COLLAGEN SYNTHESIS

Collagen is mainly synthesized by fibroblast and other connective tissue cells. The α chains are synthesized in the endoplasmic reticulum, released in the cytoplasm where the lysine and proline of the α chains are hydroxylated to create bonds of the triple helix. This helix, called procollagen, is secreted extracellularly and assembles itself into collagen fibrils and fibres. Studies on collagen content and fibrillar arrangement have yielded divergent results in the literature, but PFDs may be a problem of decreased collagen synthesis or increased collagen breakdown. Edwall *et al.* [24] looked at markers of collagen synthesis and breakdown in 24 women with uterovaginal prolapse and in 24 control subjects. They collected suburethral tissue and analysed serum and tissue concentrations of procollagen type I carboxyterminal propeptide (PICP), the collagen Type I carboxyterminal telopeptide (ICTP) and procollagen type III aminoterminal propeptide (PIIINP) by radioimmunoassay. PICP is a marker of collagen type I synthesis and ICTP is a marker of collagen type I breakdown. PIIINP is a novel marker of collagen type III synthesis. Both tissue PICP and PIIICP were significantly increased in women with prolapse which, the authors explain, reflects an increased synthesis after collagen breakdown.

Biopsies from the uterosacral ligaments were analysed for hydroxyproline content and were found to be significantly lower in women with POP and SUI than in controls. Serum levels of factors related to collagen synthesis (TGF-β, leptin) and serum levels of PINP and PIIINP were not different between groups [25]. Our studies from fibroblast cultures derived from skin and endopelvic fasciae of women with SUI and continent controls have failed to show a difference in levels of collagen synthesis [17].

Collagen synthesis can be assessed using fibroblast cultures or by using specific markers. The latter are fairly recent and may prove to be very useful in examining the dynamic process of synthesis and breakdown in the future. It is difficult at this point to establish if women with PFD have an increased synthesis attributable to overactive degradation, or if they have disorganized or deficient synthesis leading to tissue laxity.

COLLAGEN BREAKDOWN

Collagen breakdown is regulated by matrix metalloproteinases (MMPs). These enzymes are secreted as pro-enzymes and require activation. There are 23 different types identified as being involved in different tissue types. The interstitial and neutrophil collagenases (MMP-1, MMP-8, MMP-13) cleave fibrillar collagen, while the denatured peptides are degraded by the gelatinases (MMP-2 and MMP-9). These enzymes' actions are further regulated by tissue-derived inhibitors of metalloproteinases (TIMPs) that bind to them and regulate their activity.

Jackson *et al.* [26] studied the vaginal-epithelial tissue of premenopausal women with POP and found increased MMP-2 and MMP-9 activity indicating increased turnover. Connell *et al.* [16] showed a twofold increased expression of MMP-2 in women with POP compared with normal controls. Two other studies confirmed increased activity of pro-MMP-2 and MMP-2 activity by immunohistochemistry in a similar group of women [27,28]. Chen *et al.* [18] found an 80% higher level of MMP-1 expression in anterior vaginal wall biopsies of women with SUI and POP compared with continent controls. These investigators further noted a concurrent decrease in the expression of TIMP-1 in the same cohort [18] as well as in another study involving periurethral tissues of women with SUI [29]. They hypothesize that diminished inhibition of MMP-1 through lower TIMP-1 levels in these women facilitates elevated MMP-1 activity.

Helical peptide α1, a collagen breakdown product, is excreted in higher amounts in the urine of women with SUI [30], suggesting the role of an overactive degradation mechanism. Edwall *et al.* [31] also showed that women with SUI had a lower level of serum PICP and tissue ICTP than matched controls for age, parity and body mass index.

Since Jackson *et al.* [26] described the increased MMP activity in women with POP, there has been increasing interest in examining the relationship between collagen degradation and PFDs. There have been consistent results showing the impact of higher MMP activity in the development of SUI and POP, although studies have used different methodologies. The contribution of TIMP to this process remains to be established.

ELASTIN

Elastin is another component of ECM and is responsible for the elasticity and recoil of tissue across all organs of the body. Altered elastin metabolism is responsible for diseases such as emphysema and aortic

aneurysm. Tissue strength relies on cross-linkages between elastin and collagen fibres formed by the enzyme lysyl oxidase (LOX). Elastin is synthesized and broken down in the female genital tract and allows for tremendous accommodation and recoil after parturition. An analysis of uterosacral ligaments in women with POP found that elastic fibres were undetectable on immunofluorescent staining or sometimes fragmented in women with POP [32]. Another case-control study showed that elastin fibres were smaller and less expressed in vaginal tissue of women with POP [33]. A lower elastin content and decreased mRNA expression of LOX was found in uterosacral ligaments of women with POP compared with normal controls [34]. The same authors investigated the cause of decreased expression of LOX by detecting methylation sites in the *LOX* gene promoter in women with and without prolapse. They identified 66 methylated CpG sites in the group of patients with prolapse, and only one methylated CpG site in the non-prolapse control group [35]. Decreased expression of LOX was confirmed in a different study assessing mRNA levels and immunohistochemistry of vaginal tissue in women with advanced POP [36].

Zong *et al.* [37] used Western immunoblotting to measure the amount of tropo-elastin, the precursor of elastin, in the vaginal tissue of 87 patients. Of these, 67 had POP and 20 served as controls. They also measured mature elastin content using a desmosine cross-link radioimmunoassay (crosslinks/total protein). This group found an increased amount of tropo-elastin (by 431%, $P < 0.001$), and of desmosine (by 55%, $P = 0.019$) in women with POP compared with controls. In order to correlate this finding to elastin degradation in the same subjects, they measured the amount of MMP-2 and MMP-9 by zymography, and showed a higher level of MMP-9 in women with POP, but a lower level of MMP-2. They suggested that their results may reflect a higher rate of remodelling in the vaginal tissue of women with POP attributable to mechanical stretch.

An interesting study by Chen *et al.* [38] used real-time PCR and Western immunoblotting to study the levels of α-1 antitrypsin, neutrophil elastase (NE), and lysyl oxidase-like protein 1 in vaginal tissue from the anterior and posterior vaginal wall of women with POP and from controls. They found that expression of several enzymes varied within the same individual depending on the biopsy site. This emphasizes the challenge of using a standardized approach to studying tissue in women with prolapse.

Elastin content is also important in SUI, as lower and more degraded elastin complexes have been noted in the periurethral tissues of women with 'hypotonic' SUI [39]. Damage to elastin fibres can stimulate not only its own remodelling, but that of adjacent collagen fibres as well [40]. Certain MMPs are capable of degrading elastin, while NE can promote collagenolysis [40]. Women with SUI have been shown to have a threefold increase in systemic elastase activity [41,42], possibly contributing to collagen degradation. Chen *et al.* [40] further demonstrated higher NE activity and lower expression of active α-1-anti-trypsin, an elastase inhibitor, in periurethral tissues of women with secretory-phase SUI. This imbalance between elastase proteolytic activity and its inhibitor relates back to MMP-1 and TIMP-1 activity in the collagen metabolism, as incontinent women may have higher elastin degradation attributable to lower elastase inhibition [40]. Lower levels of fibrillin-1, an essential elastin scaffold, have been shown in periurethral specimens of women with SUI, which may lead to impaired deposition of elastin [43]. Overall, a highly active elastin remodelling state may result in weakened ultrastructure, ultimately causing SUI.

The data on elastin content and metabolism is quite diverse, but shows a trend for a decreased level of elastin in pelvic tissues using methods such as immunohistochemistry, Western immunoblotting and real-time PCR. Future studies should analyse markers of synthesis and breakdown, and include inhibitors of elastase in a consistent homogenous group of patients.

HORMONAL CONTRIBUTION

Many of the aforementioned studies report variable findings depending on the patients' menopausal state. In the Women's Health Initiative study in which 24,347 women were randomized to menopausal hormonal therapy or placebo, active treatment increased the risk of SUI by 1.87 [44]. Other studies have linked the use of oestrogen replacement therapy and an increased risk of overall urinary incontinence in pre- [45] and post-menopausal [46] women, but results were not statistically significant for SUI alone.

This suggests a vital role for the sex hormone milieu in the pathogenesis of SUI or POP in these women. Steroidal hormones exert their effect on tissue through an interaction with specific intracellular receptors. Hormone receptor affinity may be at the root of the differences between women with PFDs and normal controls. Progesterone receptors have been found to be more expressed in women with POP than in women without [47]. Several single nucleotide polymorphisms (SNPs) are present in the progesterone receptor gene that can alter its expression. A specific genotype was significantly associated with the risk of having POP in a multivariate analysis [48]. Similarly, the oestrogen receptor β gene also contains multiple SNPs affecting its expression. A case-control study of 69 women with POP and 141 control subjects found that a specific haplotype for the oestrogen receptor β gene was associated with an increased risk of POP [49].

Studies have shown lower serum oestradiol (E2) levels in premenopausal women with SUI, with [50] and without concurrent POP [51], compared with control subjects. The impact of oestrogen on tissue may be related to its systemic or local levels, or altered sensitivity from a decreased amount of receptors noted in genitourinary tissues [50,52]. Recently, Edwall *et al.* [53] showed that E2 stimulated collagen turnover in suburethral specimens of continent pre- and post-menopausal women, but failed to do so in the SUI cohort [53]. They speculate that this may be secondary to decreased sensitivity to E2 in the latter group.

Chen *et al.* [29] compared cultured fibroblasts from a matched pair of premenopausal women with SUI and continent women and noted that the cells from the latter group responded to increasing oestrogen concentrations by amplifying TIMP-1 expression, while the cells from women with SUI did not. This suggests that premenopausal women with SUI may be less sensitive to oestrogen, resulting in impaired TIMP-1 expression and subsequent overactive MMP-1. Negative correlations

between serum testosterone levels and markers of collagen breakdown have been noted in women with SUI, indicating that androgen may hamper collagen degradation [54]. This association can possibly be from testosterone's action on MMP-1 activity, as testosterone was shown to inhibit the endometrial cell line MMP-1 production in a dose-dependent manner [55].

Sex hormones may exert their effect through pathways other than ECM metabolism. Several investigators have shown an increase in periurethral vasculature in post-menopausal women treated with exogenous oestrogens [56,57], subjectively improving SUI in one study [56]. The higher periurethral blood flow can improve both urethral coaptation and periurethral stability and thereby increase maximum urethral pressure [58].

Many case-control studies have shown variable results of markers of ECM metabolism when comparing pre- and post-menopausal women. As age has been clearly shown to affect the prevalence and progression of both SUI and POP [7], it is intuitive to believe that declining sex hormone levels observed with ageing may contribute to biochemical changes observed within tissues. The current status of the literature suggests a hormonal impact on PFD, although the weak level of evidence emphasizes the necessity for future research endeavours in this field to elucidate these complex relationships.

GENETIC CONTRIBUTION TO PFD

Familial predisposition may be related to genetic mutations or polymorphisms. These variations are present throughout the genome and affect the transcription of mRNA coding for a wide variety of proteins responsible for ECM metabolism. Polymorphisms have been identified in genes of ECM component proteins, proteolytic enzymes, regulatory proteins and receptors. These mutations may therefore down-regulate the synthesis of collagen and elastin or up-regulate their breakdown.

Collagen type III synthesis is controlled by the transcription of the gene *COL3A1* which is involved in its α 1 chain production. A case-control study by Kluivers *et al.* [59] of 202 patients with POP and 102 control subjects investigated the association between the presence of SNPs and POP. They identified six different SNPs in the subjects. They found that the OR for the probability of POP in a woman with a homozygous 2209G > A polymorphism was 5.0 (95% CI, 1.4–17.1). Two recent studies have looked at polymorphisms in the *COL1A1* gene, responsible for collagen type I transcription in women with POP, but have failed to show a correlation between the SNPs and the presence of the condition [60,61].

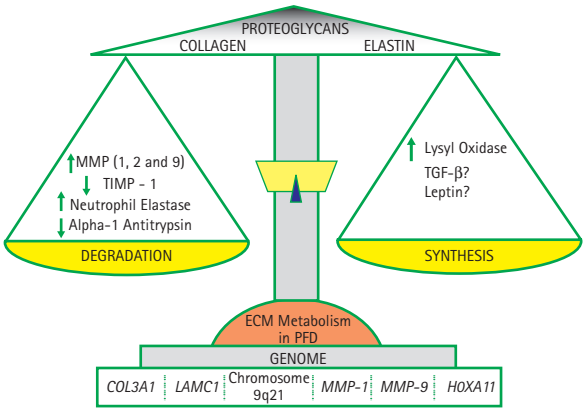


FIG. 2.
Overview of factors involved in ECM metabolism.

Candidate gene studies have identified other possible proteins related to PFD through linkage analysis. Using genome-wide linkage results, Allen-Brady *et al.* [62] showed that chromosome 9q21 contains a predisposition gene to PFD. Some of them have a known protein function and are highly expressed in muscle. Genotyping was also performed in a family where female relatives would present with POP at an unusually young age. Of the candidate genes, the sequencing of *LAMC1* revealed a SNP that may play a role in the phenotypic presentation of POP [12].

Increased activity of MMP-9 can also promote collagen breakdown through an up-regulation of its genetic transcription. Chen *et al.* [63] studied three polymorphisms in the *MMP-9* gene in 92 women with POP, and152 women without POP. On a multivariate analysis, they identified that the *MMP-9* rs17576 genotype AG, and *MMP-9* rs17576 genotype GG were significantly associated with POP, with an OR of 5.79 and 7.04, respectively. Altered genetic control of the *MMP-1* gene may further contribute to the MMP-1/TIMP-1 balance. The *MMP-1* promoter has a two guanine allele at position -1607 known to increase its transcription, and we have found that the variant is more frequently found in women with POP [64]. Our observation that this polymorphism is strongly linked to POP is consistent with previous biochemical studies that have linked increased MMP-1 transcription [18] and enzyme activity [30,65] within the pelvic floor of women with POP. The Ets transcription factor binding site in the GG promoter variant may up-regulate the transcription of MMP-1 and lead to the increased collagenolysis observed in women with PFD.

It is becoming increasingly evident that genetic variations affect the predisposition of women to develop PFD. Several well designed studies have recently identified genomic differences responsible for turning 'on' or turning 'off' gene expression between women with POP and SUI and normal controls. Genome linkage analyses provide a high level of evidence of a genetic contribution by locating specific candidate genes (*LAMC-1*, chromosome 9q21) associated with PFD. Several case-control studies, narrowing their search to specific genes, have correlated the presence of SNPs in women with POP or SUI. They are present in genes coding collagen type III, MMP-1 and MMP-9. As discussed in the hormonal contribution section, these polymorphisms can also affect the expression of steroid receptors and therefore alter the sensitivity to sexual hormones. A clearer understanding of the transcription effect caused by the cross-talk and interactions between these different mutations will require further research in this field.

CONCLUSIONS AND FUTURE DIRECTIONS

Current information supports the hypothesis that ECM protein turnover plays a role in a common pathophysiology of both POP and SUI (Fig. 2). Studies examining collagen and

TABLE 1 Biochemical and genetic findings demonstrated in patients with pelvic floor disorders

	POP group	SUI group	SUI/POP group
Collagen	• Lower density of collagen fibres in pelvic tissue [14,15] • Higher MMP-2 activity in pelvic tissue [16,26–28] • Higher MMP-9 activity in pelvic tissue [26,37] • Positive association with SNP in *COL3A1* [59] • Positive association with SNP in *MMP-9* [63] • Positive association with SNP in *MMP-1* [64]	• Lower amounts of collagen content in pelvic tissue [17–19] • Higher amount of excreted helical peptide α1 in urine [30]	• Higher MMP-1 activity in anterior vaginal wall [18] • Lower TIMP-1 activity in anterior vaginal wall [18] • Lower content of hydroxyproline in USL [25]
Elastin	• Lower amount of elastin in pelvic tissue [32–34] • Smaller elastin fibres in vaginal tissue [33] • Lower expression level of LOX in pelvic tissue [34,36] • Higher amount of tropo-elastin and desmosine in vaginal tissue [37]	• Lower amounts and more degraded elastin in periurethral tissues [39] • Higher NE activity in periurethral tissues [40] • Lower expression of active α-1-anti-trypsin in periurethral tissues [40] • Increased systemic elastase activity [41,42]	

LOX, Lysly oxidase; MMP, Matrix metalloproteinase; NE, Neutrophil elastase; POP, Pelvic organ prolapse; SNP, Single nucleotide polymorphism; SUI, Stress urinary incontinence; TIMP, Tissue-derived inhibitor of metalloproteinases; USL, Uterosacral ligaments.

elastin expression are very heterogeneous, but show a trend towards decreased levels in the pelvic floor of women with PFD (Table 1 [14–19,25–28,32–37,39–42,63,64]). Though it is not yet understood whether this results from impaired synthesis or increased degradation, evidence favours the latter. MMPs and their genetic up-regulation are probably responsible for the predisposition of certain groups of women. Several candidate genes and polymorphisms involved in the expression of ECM-related proteins have been described as contributing to the pathogenesis of the two disorders. Current literature also suggests that sex hormones may alter ECM metabolism by having variable interactions with their corresponding receptors.

A clearer comprehension of the pathophysiology responsible for PFD is clinically relevant on different levels. First, identifying the patient population at risk through screening of known polymorphism can lead to preventive strategies and the avoidance of contributing risk factors. Second, it may allow the development of interventional therapies where we can locally modify ECM maturation and turnover in patients suffering from these conditions. POP and SUI may, however, develop through different pathways not yet explored. Future research should then focus on understanding what processes control ECM remodelling and ageing using specific and standardized measurement methods, and tracing them back to genetic transcription.

ACKNOWLEDGEMENTS

We would like to acknowledge Leslie Kushner and Jan Rhozinski for their efforts in our understanding of the role of ECM and the genetic link in the development of SUI and POP.

CONFLICT OF INTEREST

Gopal H. Badlani is in a Research Trial with Pfizer and Allergans.

REFERENCES

1 **Lowder J, Frankman E, Ghetti C *et al*.** Lower urinary tract symptoms in women with pelvic organ prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 2010; **21**: 665–72
2 **Nygaard I, Barber M, Burgio K *et al*.** Prevalence of symptomatic pelvic floor disorders in US women. *JAMA* 2008; **300**: 1311–6
3 **Buckley B, Lapitan MCM.** Prevalence of urinary incontinence in men, women, and children–current evidence: findings of the Fourth International Consultation on Incontinence. *Urology* 2010; **76**: 265–70
4 **Carley M, Schaffer J.** Urinary incontinence and pelvic organ prolapse in women with Marfan or Ehlers Danlos syndrome. *Am J Obstet Gynecol* 2000; **182**: 1021–3
5 **Segev YAR, Feiner B, Lissak A, Lavie O, Abramov Y.** Are women with pelvic organ prolapse at a higher risk of developing hernias? Int Urogyneco. *J Pelvic Floor Dysfunct* 2009; **20**: 1451–3
6 **Miedel A, Tegerstedt G, Mæhle-Schmidt M, Nyrén O, Hammarström M.** Nonobstetric risk factors for symptomatic pelvic organ prolapse. *Obstet Gynecol* 2009; **113**: 1089–97
7 **Kim CM, Jeon MJ, Chung DJ, Kim SK, Kim JW, Bai SW.** Risk factors for pelvic organ prolapse. *Int J Gynaecol Obstet* 2007; **98**: 248–51
8 **Altman D, Forsman M, Falconer C, Lichtenstein P.** Genetic influence on stress urinary incontinence and pelvic organ prolapse. *Eur Urol* 2008; **54**: 918–22
9 **McLennan M, Harris J, Kariuki B, Meyer S.** Family history as a risk factor for pelvic organ prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 2008; **19**: 1063–9
10 **Buchsbaum G, Duecy E, Kerr L, Huang L-S, Guzick D.** Urinary incontinence in nulliparous women and their parous sisters. *Obstet Gynecol* 2005; **106**: 1253–8
11 **Buchsbaum G, Duecy E, Kerr L, Huang L-S, Perevich M, Guzick D.** Pelvic organ prolapse in nulliparous women and their parous sisters. *Obstet Gynecol* 2006; **108**: 1388–93

12 **Nikolova G, Lee H, Berkovitz S *et al*.** Sequence variant in the laminin gamma1 (*LAMC1*) gene associated with familial pelvic organ prolapse. *Hum Genet* 2007; **120**: 847–56

13 **Fleischmajer R, Perlish J, Burgeson R, Shaikh-Bahai F, Timpl R.** Type I and type III collagen interactions during fibrillogenesis. *Ann N Y Acad Sci* 1990; **580**: 161–75

14 **Salman M, Ozyuncu O, Sargon M, Kucukali T, Durukan T.** Light and electron microscopic evaluation of cardinal ligaments in women with or without uterine prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 2010; **21**: 235–9

15 **Lin S-Y, Tee Y-T, Ng S-C, Chang H, Lin P, Chen G-D.** Changes in the extracellular matrix in the anterior vagina of women with or without prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 2007; **18**: 43–8

16 **Connell K, Guess M, Chen H, Andikyan V, Bercik R, Taylor H.** HOXA11 is critical for development and maintenance of uterosacral ligaments and deficient in pelvic prolapse. *J Clin Invest* 2008; **118**: 1050–5

17 **Chen Y, DeSautel M, Anderson A, Badlani G, Kushner L.** Collagen synthesis is not altered in women with stress urinary incontinence. *Neurourol Urodyn* 2004; **23**: 367–73

18 **Chen BH, Wen Y, Li H, Polan ML.** Collagen metabolism and turnover in women with stress urinary incontinence and pelvic prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 2002; **13**: 80–7; discussion 87

19 **Ulmsten U, Ekman G, Giertz G, Malmstrom A.** Different biochemical composition of connective tissue in continent and stress incontinent women. *Acta Obstet Gynecol Scand* 1987; **66**: 455–7

20 **Bakas PG, Liapis AE, Zervolea I, Voutsinas G, Kletsas D, Creatsas G.** mRNA assessment for procollagen production in women with genuine stress urinary incontinence. *Int Urogynecol J Pelvic Floor Dysfunct* 2004; **15**: 429–31; discussion 431

21 **Trabucco E, Soderberg M, Cobellis L *et al*.** Role of proteoglycans in the organization of periurethral connective tissue in women with stress urinary incontinence. *Maturitas* 2007; **58**: 395–405

22 **Song Y, Hong X, Yu Y, Lin Y.** Changes of collagen type III and decorin in paraurethral connective tissue from women with stress urinary incontinence and prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 2007; **18**: 1459–63

23 **Söderberg M, Byström B, Kalamajski S, Malmström A, Ekman-Ordeberg G.** Gene expressions of small leucine-rich repeat proteoglycans and fibulin-5 are decreased in pelvic organ prolapse. *Mol Hum Reprod* 2009; **15**: 251–7

24 **Edwall L, Carlström K, Jonasson AF.** Markers of collagen synthesis and degradation in urogenital tissue and serum from women with and without uterovaginal prolapse. *Mol Hum Reprod* 2008; **14**: 193–7

25 **Suzme R, Yalcin O, Gurdol F, Gungor F, Bilir A.** Connective tissue alterations in women with pelvic organ prolapse and urinary incontinence. *Acta Obstet Gynecol Scand* 2007; **86**: 882–8

26 **Jackson S, Avery N, Tarlton J, Eckford S, Abrams P, Bailey A.** Changes in metabolism of collagen in genitourinary prolapse. *Lancet* 1996; **347**: 1658–61

27 **Gabriel B, Watermann D, Hancke K *et al*.** Increased expression of matrix metalloproteinase 2 in uterosacral ligaments is associated with pelvic organ prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 2006; **17**: 478–82

28 **Phillips C, Anthony F, Benyon C, Monga A.** Collagen metabolism in the uterosacral ligaments and vaginal skin of women with uterine prolapse. *BJOG* 2006; **113**: 39–46

29 **Chen B, Wen Y, Wang H, Polan ML.** Differences in estrogen modulation of tissue inhibitor of matrix metalloproteinase-1 and matrix metalloproteinase-1 expression in cultured fibroblasts from continent and incontinent women. *Am J Obstet Gynecol* 2003; **189**: 59–65

30 **Kushner L, Mathrubutham M, Burney T, Greenwald R, Badlani G.** Excretion of collagen derived peptides is increased in women with stress urinary incontinence. *Neurourol Urodyn* 2004; **23**: 198–203

31 **Edwall L, Carlstrm K, Jonasson A.** Markers of collagen synthesis and degradation in urogenital tissue from women with and without stress urinary incontinence. *Neurourol Urodyn* 2005; **24**: 319–24

32 **Goepel C.** Differential elastin and tenascin immunolabeling in the uterosacral ligaments in postmenopausal women with and without pelvic organ prolapse. *Acta Histochem* 2008; **110**: 204–9

33 **Karam J, Vazquez D, Lin V, Zimmern P.** Elastin expression and elastic fibre width in the anterior vaginal wall of postmenopausal women with and without prolapse. *BJU Int* 2007; **100**: 346–50

34 **Klutke J, Ji Q, Campeau J *et al*.** Decreased endopelvic fascia elastin content in uterine prolapse. *Acta Obstet Gynecol Scand* 2008; **87**: 111–5

35 **Klutke J, Stanczyk F, Ji Q, Campeau J, Klutke C.** Suppression of lysyl oxidase gene expression by methylation in pelvic organ prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 2010; **21**: 869–72

36 **Alarab MBM, Drutz H, Lye S, Shynlova O.** LOX family enzymes expression in vaginal tissue of premenopausal women with severe pelvic organ prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 2010; **21**: 1397–404

37 **Zong W, Stein S, Starcher B, Meyn L, Moalli P.** Alteration of vaginal elastin metabolism in women with pelvic organ prolapse. *Obstet Gynecol* 2010; **115**: 953–61

38 **Man W, Ho J, Wen Y, Sokol E, Polan M, Chen B.** Is lysyl oxidase-like protein-1, alpha-1 antitrypsin, and neutrophil elastase site specific in pelvic organ prolapse? *Int Urogynecol J Pelvic Floor Dysfunct* 2009; **20**: 1423–9

39 **Goepel C, Thomssen C.** Changes in the extracellular matrix in periurethral tissue of women with stress urinary incontinence. *Acta Histochem* 2006; **108**: 441–5

40 **Chen B, Wen Y, Yu X, Polan ML.** The role of neutrophil elastase in elastin metabolism of pelvic tissues from women with stress urinary incontinence. *Neurourol Urodyn* 2007; **26**: 274–9

41 **Mathrubutham M, Aybek Z, Fogarty J *et al*.** Plasma Elastase Regulation in Stress Urinary Incontinence. *Neurourology Urodynamics* 1999; **18**: 281–2

42 **Mathrubutham M, Rao S.** Single microassay for matrix degrading enzymes. *Front Biosci* 2001; **6**: A13–6

43 **Soderberg MW, Bystrom B, Hammarstrom M, Malmstrom A, Ekman-Ordeberg G.** Decreased gene expression of fibrillin-1 in stress urinary

incontinence. *Neurourol Urodyn* 2010; **29**: 476–81
44 **Hendrix S, Cochrane B, Nygaard I *et al*.** Effects of estrogen with and without progestin on urinary incontinence. *JAMA* 2005; **293**: 935–48
45 **Townsend MK, Curhan GC, Resnick NM, Grodstein F.** Oral contraceptive use and incident urinary incontinence in premenopausal women. *J Urol* 2009; **181**: 2170–5
46 **Townsend MK, Curhan GC, Resnick NM, Grodstein F.** Postmenopausal hormone therapy and incident urinary incontinence in middle-aged women. *Am J Obstet Gynecol* 2009; **200**: 86 e1–5
47 **Ewies A, Thompson J, Al-azzawi F.** Changes in gonadal steroid receptors in the cardinal ligaments of prolapsed uteri: immunohistomorphometric data. *Hum Reprod* 2004; **19**: 1622–8
48 **Chen H-Y, Chung Y-W, Lin W-Y, Chen W-C, Tsai F-J, Tsai C-H.** Progesterone receptor polymorphism is associated with pelvic organ prolapse risk. *Acta Obstet Gynecol Scand* 2009; **88**: 835–8
49 **Chen H-Y, Wan L, Chung Y-W, Chen W-C, Tsai F-J, Tsai C-H.** Estrogen receptor beta gene haplotype is associated with pelvic organ prolapse. *Eur J Obstet Gynecol Reprod Biol* 2008; **138**: 105–9
50 **Xie Z, Shi H, Zhou C, Dong M, Hong L, Jin H.** Alterations of estrogen receptor-alpha and -beta in the anterior vaginal wall of women with urinary incontinence. *Eur J Obstet Gynecol Reprod Biol* 2007; **134**: 254–8
51 **Lang JH, Zhu L, Sun ZJ, Chen J.** Estrogen levels and estrogen receptors in patients with stress urinary incontinence and pelvic organ prolapse. *Int J Gynaecol Obstet* 2003; **80**: 35–9
52 **Bai SW, Jung YW, Kwon HS *et al*.** The role of estrogen receptor, progesterone receptor and p53 in development of stress urinary incontinence. *Yonsei Med J* 2004; **45**: 885–90
53 **Edwall L, Carlstrom K, Jonasson AF.** Different estrogen sensitivity of urogenital tissue from women with and without stress urinary incontinence. *Neurourol Urodyn* 2009; **28**: 516–20
54 **Edwall L, Carlström K, Jonasson A.** Endocrine status and markers of collagen synthesis and degradation in serum and urogenital tissue from women with and without stress urinary incontinence. *Neurourol Urodyn* 2007; **26**: 410–5
55 **Ishikawa T, Harada T, Kubota T, Aso T.** Testosterone inhibits matrix metalloproteinase-1 production in human endometrial stromal cells in vitro. *Reproduction* 2007; **133**: 1233–9
56 **Long CY, Liu CM, Hsu SC, Chen YH, Wu CH, Tsai EM.** A randomized comparative study of the effects of oral and topical estrogen therapy on the lower urinary tract of hysterectomized postmenopausal women. *Fertil Steril* 2006; **85**: 155–60
57 **Kobata SA, Girao MJ, Baracat EC *et al*.** Estrogen therapy influence on periurethral vessels in postmenopausal incontinent women using Dopplervelocimetry analysis. *Maturitas* 2008; **61**: 243–7
58 **Klutke J, Bergman A.** Hormonal influence on the urinary tract. *Urol Clin North Am* 1995; **22**: 629–39
59 **Kluivers K, Dijkstra J, Hendriks JCM, Lince S, Vierhout M, van Kempen LCL.** COL3A1 2209G>A is a predictor of pelvic organ prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 2009; **20**: 1113–8
60 **Rodrigues AM, Girão MJ, da Silva ID, Sartori MG, Martins Kde F, Castro Rde A.** COL1A1 Sp1-binding site polymorphism as a risk factor for genital prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 2008; **19**: 1471–5
61 **Cho H, Jung H, Kim S, Choi J, Cho N, Bai S.** Polymorphism of a COLIA1 gene Sp1 binding site in Korean women with pelvic organ prolapse. *Yonsei Med J* 2009; **50**: 564–8
62 **Allen-Brady K, Norton P, Farnham J, Teerlink C, Cannon-Albright L.** Significant linkage evidence for a predisposition gene for pelvic floor disorders on chromosome 9q21. *Am J Hum Genet* 2009; **84**: 678–82
63 **Chen H-Y, Lin W-Y, Chen Y-H, Chen W-C, Tsai F-J, Tsai C-H.** Matrix metalloproteinase-9 polymorphism and risk of pelvic organ prolapse in Taiwanese women. *Eur J Obstet Gynecol Reprod Biol* 2010; **149**: 222–4
64 **McKenzie P, Rohozinski J, Badlani G.** Genetic influences on stress urinary incontinence. *Current Opinion Urology* 2010; **20**: 291–5
65 **Badlani GH, Davila GW, Michel MC, de la Rosette JJMCH, eds.** Continence: Current Concepts and Treatment Strategies. London (UK): Springer, 2009

Correspondence: Gopal H. Badlani, 140 Charlois Boulevard, Wake Forest Baptist University, Department of Urology, Winston-Salem, NC 27103, USA.
e-mail: gbadlani@wfubmc.edu

Abbreviations: **PFD**, pelvic floor disorder; **SUI**, stress urinary incontinence; **POP**, pelvic organ prolapse; **OR**, odds ratio; **ECM**, extracellular matrix; **SLRP**, small leucine-rich proteoglycan; **PICP**, procollagen type I carboxyterminal propeptide; **ICTP**, type I collagen carboxyterminal telopeptide; **PIIINP**, procollagen type III aminoterminal propeptide; **MMP**, matrix metalloproteinase; **TIMP**, tissue-derived inhibitors of metalloproteinase; **LOX**, lysyl oxidase; **NE**, neutrophil elastase; **SNP**, single nucleotide polymorphism; **E2**, oestradiol.