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Do extracellular matrix protein expressions change with cyclic reproductive hormones in pelvic connective tissue from women with stress urinary incontinence?

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BACKGROUND: To evaluate differential expression of transforming growth factor (TGF-β1), latent transforming factor-binding proteins (LTBP-1, LTBP-2) and elastin microfibril components (fibrillin-1 and fibrillin-2) in vaginal tissue from women with stress urinary incontinence (SUI). METHODS: In this case–control study, vaginal tissue from women in both phases of the menstrual cycle was obtained. Messenger RNA (mRNA) expressions of LTBP-1, LTBP-2, fibrillin-1, fibrillin-2 and TGF-β1 were determined by relative real-time quantification PCR. Tissue localization was analysed by immunohistochemistry, and semiquantitative protein expression was evaluated by Western blot analysis. RESULTS: Vaginal wall fibroblasts synthesized all proteins tested. LTBP-1, LTBP-2 and TGF-β1 co-localized with elastin microfibrils, fibrillin-1 and fibrillin-2 in the extracellular matrix. LTBP-1 mRNA and protein expressions were higher in control versus women affected with SUI in the proliferative phase ($P = 0.04$), while in the secretory phase, mRNA expression in cases was higher ($P = 0.04$). Fibrillin-1 mRNA was higher in women affected by SUI versus controls in both phases, but no statistical differences in fibrillin-1 protein expression were observed between the two groups in either phase. LTBP-2 and TGF-β1 mRNA expressions showed the same trends as LTBP-1. CONCLUSION: LTBP-1, LTBP-2, TGF-β1, fibrillin-1, and fibrillin-2 expressions are hormonally regulated in vaginal wall fibroblasts and differ in women affected by SUI when compared to controls. These data suggest a mechanism to regulate TGF-β1 activity in pelvic connective tissue.

Key words: elastin/fibrillin/latent transforming factor-binding proteins/stress urinary incontinence/transforming growth factor-β

Introduction

The female lower urinary system is supported by pelvic muscles, ligaments and the bony pelvis. The mechanical integrity of these tissues is critical to the continence mechanism as well as to the pelvic floor function. It is well known that vaginal delivery may cause neurologic damage to the pelvic floor as well as cause direct injury to the muscles and connective tissue. These injuries can then result in stress urinary incontinence (SUI) or pelvic floor dysfunction. Genetic differences in connective tissue metabolism may also place women at risk of developing pelvic floor dysfunction. Abnormal connective tissue turnover—specifically, collagen and elastin metabolism—appears to be involved in the aetiology of SUI (Chen *et al*., 2002, 2005).

The extracellular matrix (ECM) is a complex network of numerous macromolecules, which fulfils many mechanical, chemical and biological functions. ECM in pelvic supportive tissues consists mainly of collagens and elastic fibres. Collagens and elastin fibres confer tissue strength and elasticity, respectively, whereas structural glycoproteins create tissue cohesiveness. Latent transforming factor-binding proteins (LTBPs) are components of the ECM that have the dual role of being structural components of ECM microfibrils and transforming growth factor (TGF)-β-binding proteins (Sinha *et al*., 2002). LTBPs form a group of high-molecular weight proteins that are structurally related to the elastin microfibrils or fibrillins. To date, two isoforms of fibrillin (fibrillin-1 and fibrillin-2) (Sakai *et al*., 1986; Zhang *et al*., 1994; Dallas *et al*., 2000), four isoforms of LTBP (LTBP-1 to LTBP-4) (Kanzaki *et al*., 1990; Moren *et al*., 1994; Gibson *et al*., 1995, 1997; Saharinen *et al*., 1998) and numerous splice variants have been cloned. Together they form the fibrillin/LTBP superfamily. TGF-β stimulates ECM biosynthesis and acts as a growth-promoting factor in certain cell types and as a growth inhibitor in many normal and transformed cells (Ignotz and Massague, 1986; Roberts *et al*., 1986). LTBPs bind the small latent TGF-β complex, consisting of TGF-β and the latency-associated peptide, through a covalent disulphide bond, to form a large latent complex

(Miyazono *et al*., 1988; Wakefield *et al*., 1988). LTBPs may be involved in the assembly, secretion and targeting of TGF-β to sites at which it is stored and/or activated. Thus, these proteins play important roles in controlling and directing the activity of TGF-β.

The fibrillin/LTBP family and TGF-β1 are implicated in connective tissue disorders such as arterial injury (Sinha *et al*., 2002), varicose veins (Bujan *et al*., 2003) and capsular opacification (Saika *et al*., 2001). Mutations of fibrillin-1 (Ades *et al*., 1996) or LTBP-2 genes have been documented in Marfan's syndrome (Mathews and Godfrey, 1997), and mutation of fibrillin-2 has been documented in human congenital contractural arachnodactyly. Currently, there is no information on whether these are important in pelvic connective tissue metabolism. Since the fibrillin/LTBP family and TGF-β are implicated in regulating elastin and collagen synthesis, we examined the expression of LTBP-1, LTBP-2 and fibrillin-1, fibrillin-2 in relation to TGF-β1 in vaginal wall tissue from premenopausal women with SUI compared with asymptomatic controls. We also studied the effect of *in vivo* reproductive hormones on the expression of these proteins.

Subjects and methods

Patient selection and tissue collection

The Institutional Review Board of the Stanford University School of Medicine approved this study. The patients were selected, and periurethral vaginal wall biopsies were collected, as previously reported by our group (Chen *et al*., 2004). Roughly 200 mg of tissue sample was collected from each participant. After harvest, mucosa was removed by scraping with a scalpel before processing. Women with a history of endometriosis, gynaecologic malignancies, pelvic inflammatory conditions, connective tissue disorders, emphysema, prior pelvic surgery, advanced pelvic organ prolapse (greater than stage II by the pelvic organ prolapse quantification system) and menopausal symptoms were excluded. We selected age-matched women affected by SUI cases and asymptomatic controls from both the proliferative and secretory phases of the menstrual cycle for this study. The phase of cycle was confirmed by endometrial histology. A total of 36 participants were recruited: 11 in the proliferative phase and 8 in the secretory phase for the case group; and 9 in the proliferative phase and 8 in the secretory phase for the control group.

Relative real-time quantification PCR

RNA was extracted from the tissue sample with the RNA-STAT-60 reagent (Tel-Test, Friendswood, TX, USA). Complementary DNA was generated from total RNA, as previously described (Chen *et al*., 2004). The primers for PCR were designed by OLIGO Software (Table I). Real-time quantification PCR was performed on the Mx4000 Mutiplex Quantitative PCR System with Mx4000 software (Stratagene, La Jolla, CA, USA). QuantiTect SYBR Green PCR Kit (Qiagen, Valencia, CA, USA) was used to perform PCR. The signal detected was the binding of the fluorophore to double-stranded DNA. The amplifications were done following 15 min hot start at 95°C in a three-step protocol with 30 s denaturation (94°C), 1 min annealing (60°C) and extension at 72°C for 30 s. Forty cycles were performed. The dissociation curve was run following a 1 min incubation at 95°C for DNA melt. It started with 30 s incubation at 55°C. Then, it was followed by 40 successive 30 s plateaus, during which the temperature was increased by 1°C for each plateau. All PCR reactions were performed in triplicate. The products were sequenced to ensure that the correct gene sequence was amplified.

Relative quantification of gene expression was performed using the standard curve method. For the accurate normalization of real-time quantification, two reference genes, glyceraldehyde-3-phosphatedehydrogenase (GAPDH) and hypoxanthine phosphoribosyl transferase 1 (HPRT1), were used as endogenous references. The standard curves for both endogenous references and target genes were prepared from the same vaginal tissue samples. For each experiment sample, the amounts of target and endogenous references were determined from the appropriate standard curves. The target amount was then divided by the endogenous reference (GAPDH or HPRT1) to obtain a normalized target value. Each of the experimental normalized sample values was divided by one normalized control sample value (calibrator sample) to generate the relative expression levels. Our results confirmed that both GAPDH and HPRT1 reference genes were consistently expressed in tissues of both the case and control groups. Here, we only show the normalized target amount with HPRT1.

Western blot analysis

Protein extraction was performed, as previously described (Chen *et al*., 2004). One hundred micrograms of total protein from each patient were separated by 8% (LTBP and TGF-β1) or 5% (fibrillin-1) sodium dodecyl sulphate polyacrylamide gel electrophoresis under non-reducing conditions and blotted onto nitrocellulose membranes (Pierce, Rockford, IL, USA) in an electrophoretic transfer cell (Bio-Rad, Hercules, CA, USA). Blots were blocked with 5% non-fat milk at 4°C overnight. After blocking, the membrane was washed three times in PBS-T [phosphate-buffered saline (PBS), pH

Table 1. Characteristics of primers

Gene	Oligonucleotide	5′ to 3′ sequence	Accession number
GAPDH	Sense	CTCAACGACCACTTTGTCAAGCTCA	BC004109
	Anti-sense	GGTCTTACTCCTTGGAGGCCATGTG	
HPRT1	Sense	TGACACTGGCAAAACAAYGCA	NM_000194
	Anti-sense	GGTCCTTTTCACCAGCAAGCT	
LTBP-1	Sense	GCGATGAGTTGAACAACCGGATGTC	NM_206943
	Anti-sense	TCAAGGCGGTATTCATCGGAGTGC	
LTBP-2	Sense	AAGGGGAAAAGGGAGGGTTGCATAA	NM_000428
	Anti-sense	GGGGGATGGTAAGTTCTCCGATGGT	
TGF-β1	Sense	TGAACCGGCCTTTCCTGCTTCTCATGG	NM_000660
	Anti-sense	GCGGAAGTCAATGTAGAGCTGCCGC	
Fibrillin-1	Sense	TGACTGGCCCACACGTGCATAG	NM_000138
	Anti-sense	TGACATTGACCCCTTGTTGACAGGA	
Fibrillin-2	Sense	CACGACGTGAATGAGTGCTCGTCC	NM_001999
	Anti-sense	TGCCCCTTGTTAAATCCCATTCCTG	

7.4 and 0.1% Triton]. The membrane was incubated with antibody against LTBP-1 at 1 : 1000 [Ab39, rabbit polyclonal immunoglobulin G (IgG), a gift from Dr Carl-Henrik Heldin, Sweden], rabbit anti-human TGF-β1, goat anti-human LTBP-2 at 1 : 100 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse anti-human fibrillin-1 at 2 μg/ml (Laboratory Vision, Fremont, CA, USA) or rabbit anti-human fibrillin-2 (Elastin Products Company, Owensville, MO, USA) overnight at 4°C, followed by three washes in PBS-T. The membrane was then incubated in 1 : 5000 dilution of donkey anti-rabbit IgG, sheep anti-mouse or mouse anti-goat IgG (Amersham Pharmacia Biotech, Piscataway, NJ, USA) conjugated to horseradish peroxidase for 1 h at room temperature, followed by three washes in PBS-T. Blots were developed by chemiluminescence. The optical density was determined by GEL-DOC 2000 (Bio-Rad). GAPDH was used as loading control. Semiquantification of respective protein expression was determined by the ratio of LTBPs, TGF-β1 or fibrillins to GAPDH on the same blot.

TGF-β1 enzyme-linked immunosorbent assay

Total TGF-β1 was measured using the R & D System enzyme-linked immunosorbent assay (ELISA) (Minneapolis, MN, USA). Assays were performed on aliquots of the homogenates prepared for Western blot. The assay detects the immunoreactive TGF-β1 form by activating samples with 1N HCl as per the manufacturer's instruction. The range of the assay is 31.2-2000 pg/ml. After the assay, the final concentration of TGF-β1 in the samples was calculated and expressed as pg TGF-β1 per mg protein.

Immunohistochemistry

Immunohistochemical staining for LTBP-1, LTBP-2, TGF-β1 and fibrillin-1, fibrillin-2 was performed on fixed embedded tissue using the avidin-biotin-peroxidase (ABC) method to confirm the presence and distribution of these proteins in vaginal cuff specimens. Paraffin-embedded specimens were cut into 5 μm sections, dewaxed in xylene and rehydrated through graded ethanol solutions. After washing with TBS-T (Tris–HCl–tween buffer), endogenous peroxidases were blocked with 3% H_2O_2 in TBS-T, and non-specific binding was blocked with 1% bovine serum albumin, 5% normal secondary antibody host serum in TBS-T at room temperature for 1 h. After rinsing with TBS-T, the slides were incubated with rabbit anti-LTBP-1 (1 of 200), goat anti-LTBP-2 (1 of 20), rabbit anti-TGF-β1 (1 of 50), mouse anti-fibrillin-1 (2 μg/ml) or rabbit anti-fibrillin-2 (1 of 50) primary antibody overnight at 4°C. Deletion of the primary antibody was used as a negative control. Negative controls were performed with normal goat, horse or rabbit serum, as appropriate, in place of each one of the primary antibodies, to exclude secondary antibody cross-reactivity. After rinsing with TBS-T, slides were incubated with a secondary antibody: goat anti-rabbit biotin conjugate, horse anti-mouse biotin conjugate or rabbit anti-goat biotin conjugate (1 of 50, Vector Laboratories, Burlingame, CA, USA). The slides were then incubated with Vectastatin ABC Kit (Vector Laboratories) reagent for 30 min at room temperature. Immunoreactive products were visualized by incubating slides with the substrate solution in 0.1 M Tris–HCl buffer, blocking alkaline phosphatase activity with levamisole. Slides were counter-stained with 25% of haematoxylin.

Immunofluorescence staining

Immunofluorescence staining of fibroblasts from the vaginal wall tissues was performed, as previously described (Chen *et al*., 2005). Briefly, fibroblast cells from vaginal cuffs were cultured in a four-well chamber slide. The cells were fixed with 4% paraformaldehyde and treated with 5% Triton. After washing with TBS-T and blocking with 5% normal secondary antibody host serum, the slides were incubated with rabbit anti-LTBP-1 (1 of 200), goat anti-LTBP-2 (1 of 20), rabbit anti-TGF-β1 (1 of 50), mouse anti-fibrillin-1 (2 μg/ml) or rabbit anti-fibrillin-2 (1 of 20), and pre-diluted mouse anti-human vimentin (Chemicon International, Temecula, CA, USA) primary antibody at 4°C overnight. Deletion of the primary antibody was used as a negative control. After washing, the slides were incubated with goat anti-mouse IgG tetramethylrhodamine isothiocyanate (TRITC) 1 of 50 and goat anti-rabbit IgG fluorescein isothiocyanate (FITC) 1 of 320 or swine anti-goat IgG FITC (1 of 50, Caltag Laboratory, Burlingame, CA, USA) and goat anti-mouse IgG TRITC at room temperature for 1 h. 4′,6-diamidino-2-phenylindole (DAPI) staining was used to observe nuclei. The slides were washed three times and mounted with Vectashield (Vector, Foster City, CA, USA).

Elastin and collagen staining

Staining to reveal the elastic fibres and collagen was performed by Weigert's elastin stain (Weigert's Resocin-Fuchism) and Van Gieson's collagen stain, as previously described (Chen *et al*., 2005).

Statistical analysis

Statistical analysis was performed using unpaired *t*-tests. The level of significance was set at $P < 0.05$. A non-parametric analysis (Wilcoxon/Kruskal–Wallis Test) was also used because of the small sample size. Both parametric and non-parametric analyses yielded the same significant results.

Results

Our immunohistochemistry stains show that LTBP-1, LTBP-2, TGF-β1 and fibrillin-1, fibrillin-2 are all expressed in the ECM of vaginal wall tissue from both the case group (data not shown) and the asymptomatic control group (Figure 1). Their distribution patterns are also very similar (Figure 1). The co-localization of LTBP-1, LTBP-2, TGF-β1 and fibrillin-1, fibrillin-2 suggests that LTBPs have a structural role during the remodelling of the ECM in vaginal wall tissue, in addition to that of storing and regulating TGF-β.

To confirm that fibroblasts do express LTBPs, TGF-β and fibrillins, vaginal wall fibroblasts were cultured, and double-colour immunofluorescence was performed, as previously described (Chen *et al*., 2005). Cultured vaginal wall fibroblasts from both case (data not shown) and control women (Figure 2) expressed LTBP-1, LTBP-2, TGF-β1 and fibrillin-1, fibrillin-2 proteins.

We used real-time quantification PCR analyses on total RNA to determine and compare the level of expression of LTBP-1, LTBP-2, TGF-β1, fibrillin-1 and fibrillin-2 in controls as well as cases in both proliferative and secretory phases of the menstrual cycle. LTBP-1 messenger RNA (mRNA) expression level was higher in the control compared to the case group in the proliferative phase (a 4-fold increase, $P = 0.04$, Figure 3A), but its level was lower in control compared to the case group in the secretory phase (a 19-fold decrease, $P = 0.04$, Figure 3B). The mRNA expression levels of TGF-β1 (11–fold increase) and LTBP-2 (2-fold increase) also tended to be higher in the control

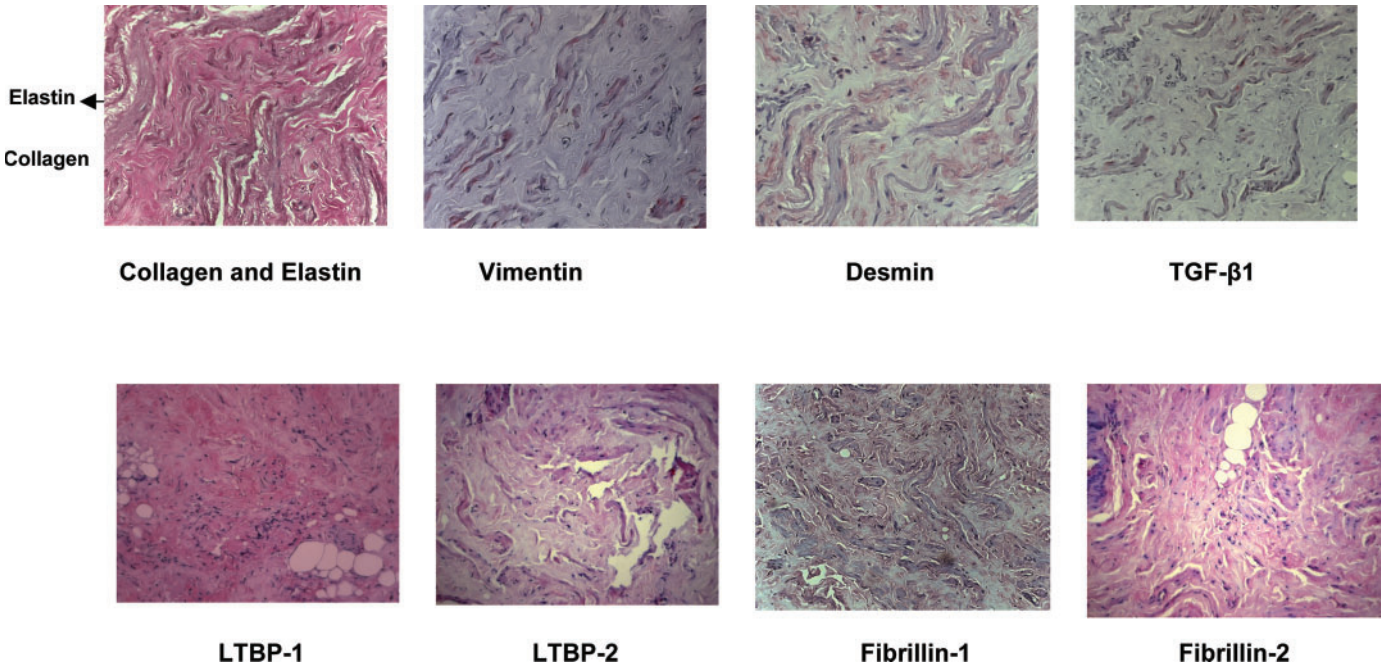

Figure 1. Latent transforming factor-binding proteins (LTBP)-1, LTBP-2 and transforming growth factor (TGF)-β1 immunolocalization and co-localization with fibrillin-1, fibrillin-2 in the vaginal wall tissue from a control patient. LTBPs, TGF-β1 and fibrillins were detected mainly in extracellular matrix of vaginal wall tissue as well as of elastin and collagen (20X). The pattern of the distribution of LTBPs, TGF-β1 and fibrillins is similar. No differences of expression and localization of these proteins were found between control and case groups (data not shown). The vimentin and desmin were used to stain fibroblasts and smooth muscle cells, respectively.

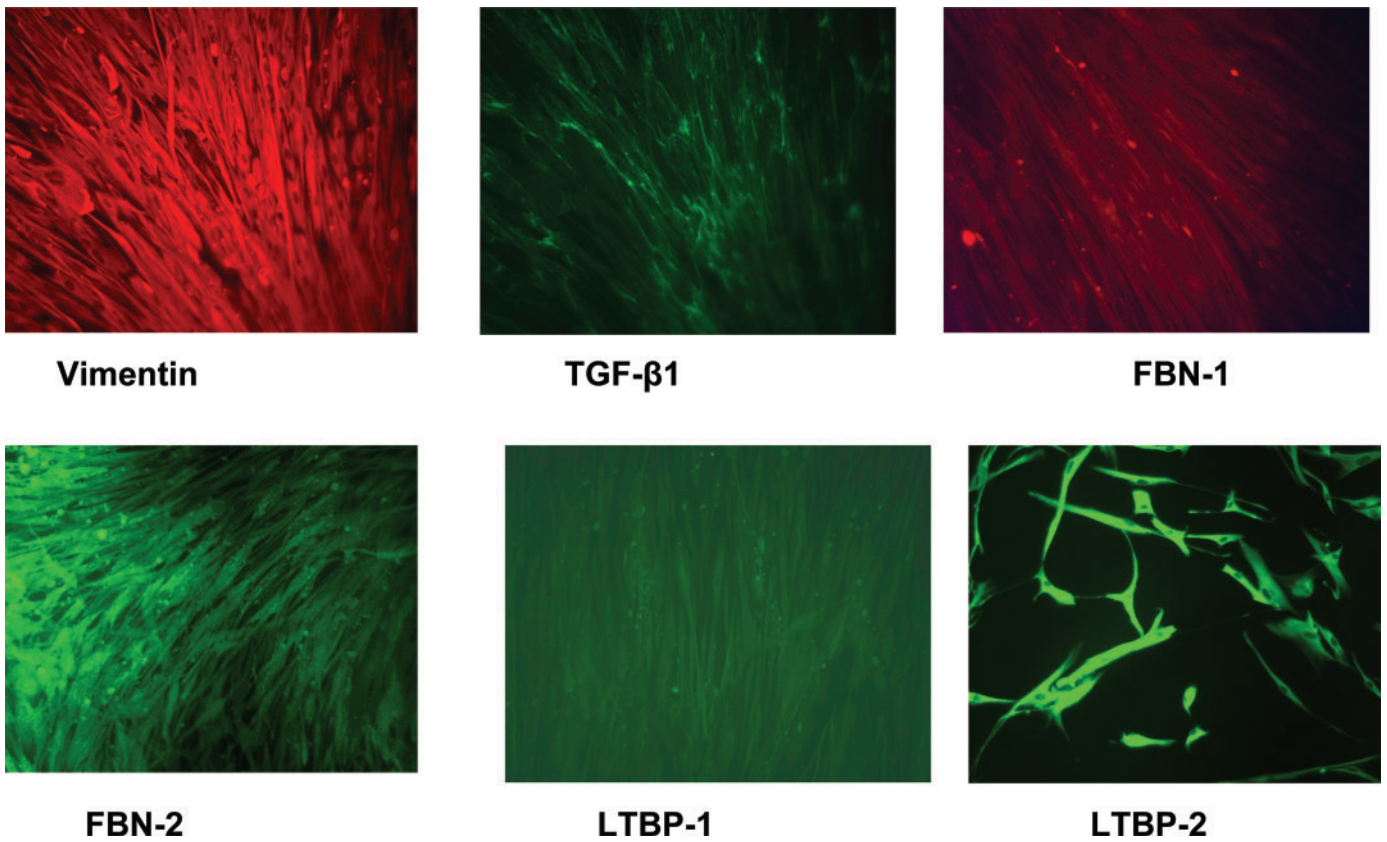

Figure 2. Fibroblasts from the vaginal wall of a normal control patient were cultured. These cells were fixed and subjected to immunofluorescence analysis using LTBP-1, LTBP-2, TGF-β1 and fibrillin-1, fibrillin-2 antibodies. The primary antibody was deleted in negative controls. Vimentin was used to confirm that more than 95% of cultured cells were fibroblasts. Immunoreactivity for LTBP-1, LTBP-2, TGF-β1, fibrillin-1 and fibrillin-2 was all detected in the fibroblasts.

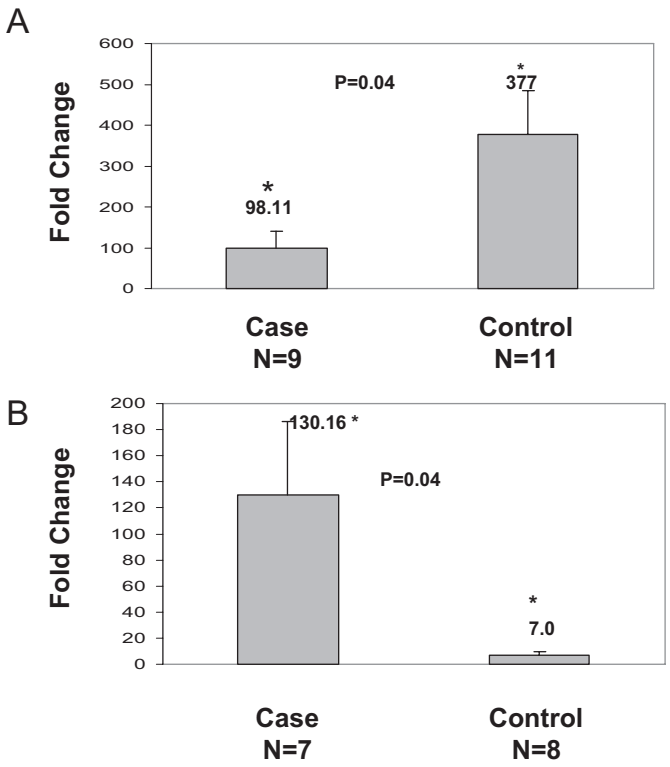

Figure 3. Comparative relative real-time quantification PCR analyses were performed on total RNA using the QuantiTect SYBR Green PCR Kit to determine and compare the level of expression of LTBP-1 in vaginal wall tissues from both asymptomatic controls and cases with stress urinary incontinence. Messenger RNA (mRNA) expression was quantified relative to the expression of hypoxanthine phosphoribosyl transferase 1 (HPRT1), whose expression remained constant in all the specimens. LTBP-1 mRNA expression level was higher in the control group than in the case group in the proliferative phase (a 4-fold increase, $P = 0.04$, **A**) but was lower in the control group than in the case group in the secretory phase (a 19-fold decrease, $P = 0.04$, **B**). The results shown are the mean ± S.E.M.

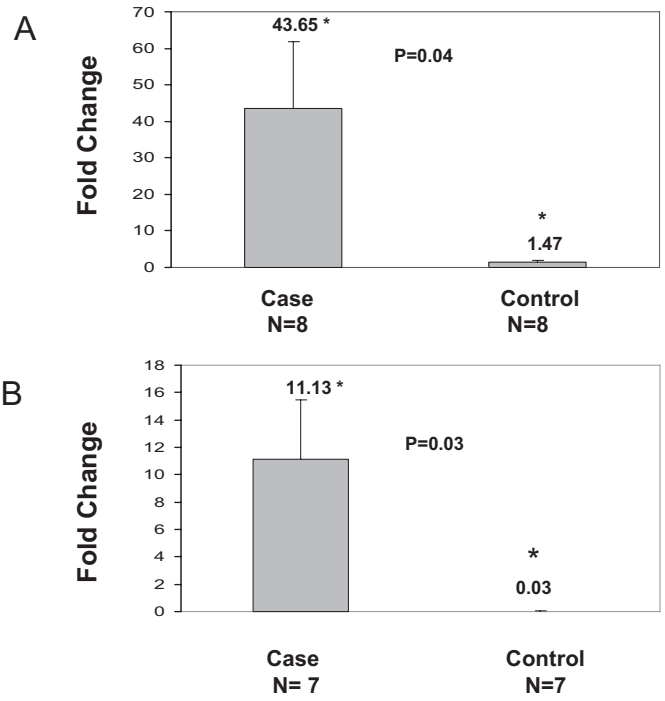

Figure 4. Comparative relative real-time quantification PCR analyses were performed on total RNA to determine and compare the level of expression of fibrillin-1 in vaginal wall tissue from both asymptomatic controls and cases. The level of fibrillin-1 mRNA expression was higher in the case group than in the control group in both the proliferative phase (a 30-fold increase, $P = 0.04$, **A**) and the secretory phase (a 371-fold increase normalized with HPRT1, $P = 0.03$, **B**). The results shown are the mean ± S.E.M.

group in the proliferative phase and lower in the control group in the secretory phase, with an 8-fold decrease for TGF-β1 and a 5-fold decrease for LTBP-2. However, these relative fold changes were not statistically significant. The level of fibrillin-1 mRNA expression was higher in the case group versus the control group in both the proliferative (a 30-fold increase, $P = 0.04$, Figure 4A) and secretory phases (a 371-fold increase, $P = 0.03$, Figure 4B). Fibrillin-2 mRNA expression levels were similar between the two groups of women.

Western blot analysis showed LTBP-1 protein expression that was higher in the control compared to the case group in the proliferative phase ($P = 0.0001$, Figure 5), but no difference was observed in the secretory phase (data not shown). LTBP-2 expression showed the same pattern as LTBP-1, with a 2-fold increase in the proliferative phase ($P = 0.03$) and no difference in the secretory phase (data not shown). Although fibrillin-1 mRNA levels were higher in case groups in both proliferative and secretory phases, no differences in protein expression were found (data not shown). The total TGF-β1 protein expression by Western blot analysis and ELISA showed no differences between control and case groups in either phase (data not shown). We were unable to detect fibrillin-2 in the vaginal wall tissue by Western blot analysis.

Discussion

LTBPs belong to the family of fibrillin/LTBP glycoproteins. The majority of the protein structure in this family is composed of repetitive epidermal growth factor-like domains and 8-cysteine repeats, both of which are believed to mediate protein–protein interactions. LTBP-1 is involved both in the sequestration of latent TGF-β in the ECM and in the regulation of its activation in the extracellular environment. TGF-β is critical to ECM metabolism since it stimulates expression of ECM components such as fibronectin, collagen and elastin. LTBPs and TGF-β1 have been reported to co-localize with fibrillins, the major component of elastic fibres in skin (Karonen *et al.*, 1997), skeleton (Dallas *et al.*, 2000), kidney (Sterzal *et al.*, 2000) and vasculature (Sinha *et al.*, 2002). However, this relationship has not been described in pelvic connective tissues. Neither is detected in anetoderma, which is characterized by the absence of elastic fibres in the dermis (Karonen *et al.*, 1997). Thus, LTBP not only serves as an important repository for latent TGF-β, but also as a force-bearing element in connective tissue as well as a scaffolding for elastin deposition. The expressions of LTBP-1 and TGF-β have been found to be elevated in several fibrotic conditions, such as in chronic rejection of cardiac allografts in

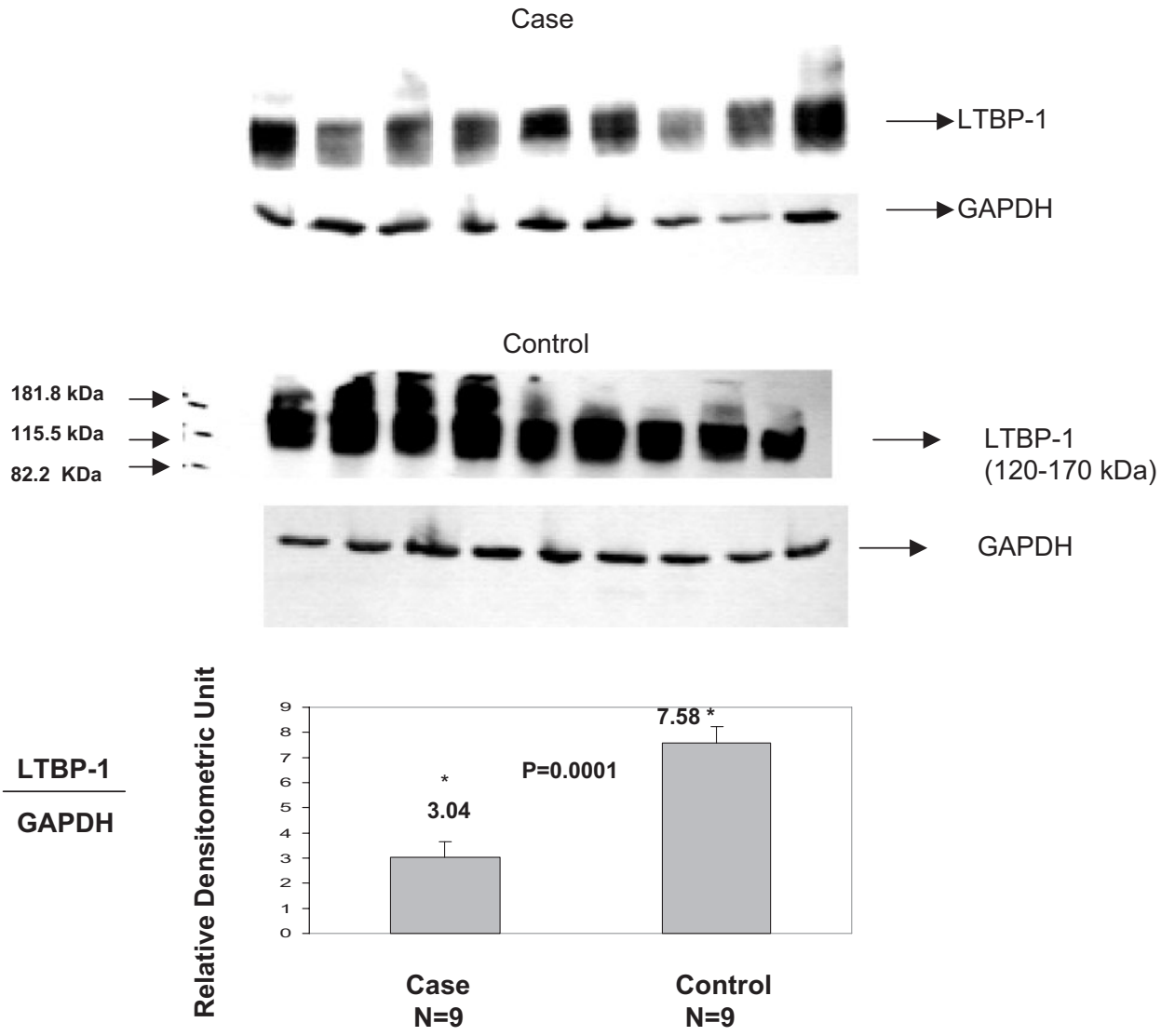

Figure 5. Western blot analysis. Twin LTBP-1 bands (marked) were detected in the tissue extraction from the proliferative phase vaginal wall at 120–170 kDa. The expression of LTBP-1 protein was higher in the asymptomatic control group than in the case group ($P = 0.0001$). The values represent the value for the LTBP-1 bands (170–120 kDa) versus the value for glyceraldehyde-3-phosphatedehydrogenase (GAPDH, 82.2 kDa). The results shown are the mean ± S.E.M.

rats and tuberculous pleurisy (Maeda *et al*., 1993; Waltenberger *et al*., 1993).

The present study has shown that fibroblasts from the vaginal wall do express LTBP-1, LTBP-2, fibrillin-1, fibrillin-2 and TGF-β1, and that asymptomatic controls expressed significantly more LTBP-1, LTBP-2 and TGF-β mRNA and protein compared to cases in the proliferative phase. This suggests that, in addition to increased connective structural components in the ECM, more TGF-β may be bound to LTBP in controls compared to cases. However, the expression ratios are reversed in the secretory phase, with the tissues of women affected by SUI showing increased LTBP-1 and TGF-β expressions compared to controls. These data indicate that hormonal environment is important in this mechanism. Tissues of women affected by SUI may respond to injury in similar fashion to tissues with pathologic fibrotic conditions, with increases in LTBP-1 and/or TGF-βs. A relative increase in active TGF-β resulting from decreased LTBP in SUI tissues may explain the increase in matrix metalloproteinase protein expression and the elastase activity observed in our previous work (Chen *et al*., 2004, 2005) where we documented increased proteolytic activity in tissues of women affected by SUI during the secretory phase of the menstrual cycle.

ECM is assembled, in principle, by two different mechanisms: self-assembly—for example, collagen fibres—and cell-mediated assembly. Cell-mediated assembly can be further divided into two types depending on whether the cells are assembling soluble monomers diffusing around cells, as in the case of fibronectin, or the assembly is coupled directly to the surface of cells that make the monomers. The latter may be the mechanism for elastin matrix assembly. The protein elastin is formed as a result of the reticulation of tropoelastin monomers over a framework of fibrillin-rich microfibrils (Schwartz and Fleischmajer, 1986) and LTBPs (Mecham and Heusar, 1991).

Although microfibrils may be assembled independently of the presence of elastin, tropoelastin monomers require the presence of microfibrils for their assembly (Robb *et al*., 1999). Animal experiments show that LTBP interferes with the normal development of elastic fibres in a tissue-specific manner (Sterner-Kock *et al*., 2002). Recent animal data demonstrated that new elastin deposition in postpartum mice also requires an intact lysyl oxidase-like 1 (LOXL1) enzyme (Liu *et al*., 2004). Mice with a deletion in the LOXL1 gene developed pelvic organ prolapse shortly after delivery, while no prolapse was observed in the non-pregnant mice. It appears that the mechanism for elastin remodelling subsequent to acute injury may be different from that of elastin deposited during embryogenesis and early postnatal life. Alterations in either mechanism could lead to elastin abnormalities.

Epidemiologic data indicate that race and genetic factors, in addition to birth trauma, are involved in the development of SUI. We have documented differential gene expressions in pelvic tissues from women affected with SUI compared to asymptomatic controls, with increased TGF-β3 expression in cases (Chen *et al*., 2002). In women who are predisposed to developing pelvic floor dysfunction, it is possible that their specific genetic differences are those affecting elastin microfibril scaffolding and TGF-β regulation.

Our data showed increased mRNA expression of fibrillin in case compared to control tissues. There was, however, no difference detected in protein expression. Similarly, while a lower level in LTBP-1 mRNA expression was observed in control tissues in the secretory phase, no differences in protein expression were detected. This may be because of the small sample size in our study. It is also possible that Western blot analysis is unable to detect the small differences measured by real-time PCR. Additionally, the small size of our biopsies limited our ability to conduct all the assays on every tissue specimen.

We will be conducting further confirmation with larger sample sizes and proteomics experiments.

In these experiments, we verified that TGF-β and LTBPs/fibrillins are secreted by pelvic fibroblasts. They also co-localize with elastin microfibrils in pelvic tissues, providing further evidence of their involvement in pelvic elastin metabolism. Tissues from women with SUI express differential amounts of these proteins compared to asymptomatic controls. This process appears to be mediated by reproductive hormones, which opens the possibility for early intervention through hormone modalities for a condition that currently has no preventive treatments.

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