A COMPREHENSIVE PROTEOMIC ANALYSIS ON CARDINAL LIGAMENTS TISSUE IN PATIENTS WITH PELVIC ORGAN PROLAPSE

Hypothesis / aims of study
Pelvic organ prolapse (POP) is a common yet distressing disease negatively affecting the live of women at all ages. The ligamentous and vaginal tissues serve as the major support attaching uterus and vagina to pelvic wall. Current research suggests that abnormalities of pelvic floor connective tissues predispose women to prolapse with unknown cause. Simultaneously, evidence from our gene expression studies and biochemical studies reported by others indicate that not only genes involved in extracellular matrix activity and collagen metabolism are differentially affected, but also the balance between proteolytic enzymes and cell proliferation may play an important role in the diseases pathogenesis. We conducted a large-scale protein profiling study to comprehensively characterize the biochemical changes in connective tissues during the development and progression of POP.

Study design, materials and methods
To delineate the biochemical composition of cardinal ligament in POP, we performed 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE) analysis, in duplicate, on 6 cardinal ligamentous tissues isolated from second degree prolapse and compared with 6 age matched healthy control subjects. In the 1st-dimension separation, proteins were separated by isoelectric point by isoelectric focusing in a nonlinear isoelectric focusing strip (Bio-Rad) with a voltage gradient for 15 h. In the 2nd-dimension separation, proteins were separated by molecular weight on a precast PAGE gel (Bio-Rad) assembled in a running cassette of a PROTEAN II XL. Silver staining (Amersham) was used to visualize the ligamentous proteins separated in 2D-PAGE. The gel images were acquired by a GS-700 Imaging Densitometer with Quantity One software (Bio-Rad). Protein spots were quantified with Discovery Series PDQuest 2D Analysis Software. Quantities of matched spots were normalized with the sum of spot quantities between the 1st and 3rd quartile and analyzed by Significance Analysis of Microarrays (Stanford University). This program used a moderated t statistic, whereby a constant was added to the denominator of the t-statistic between samples from normal and POP. We manually excised protein spots with a blade and subjected them to in-gel overnight trypsin digestion with 20 mg/L trypsin at 37 °C. We spotted 3 µL concentrated peptides on a metallic plate covered by α-cyan-4-hydroxy cinnamic acid (Fluka) in 5 mmol/L ammonium dihydrogen phosphate solution (Fluka). The peptide mass and intensity were then measured with a 4700 Proteomics Analyzer (Applied Biosystems) consisting of matrix-assisted laser desorption/ionization time of flight mass spectrometer and tandem mass spectrometer. The experimental peptide mass list was compared with the theoretical mass list for each entry in the database. To confirm identification, the most intense peptides from the peptide mass lists were subjected to further fragmentation. Peptide mass and fragment mass spectra unique to the peptide were generated, and respective database searches performed using Profound (http://prowl.rochelel.edu) and Mascot (http://www.matrixscience.com).

Results
We compared 6 paired ligament samples from POP patients and age matched healthy subjects. Age group ranged form 69 to 78 yrs old. The patients were all have second degree prolapse. We detected over 2000 spots and identified at least 32 significantly up or down regulated proteins present in the ligament samples from POP patients (Figure 1).

Interpretation of results
The identified proteins in addition to scaffold cytoskeletal proteins, we identified proteins implicated with cellular motility and membrane trafficking, stress and folding enzymes, extracellular proteins, and cell cycle regulation proteins. We are currently matching the proteomic data with our results from gene expression profiling study in cardinal ligaments from POP patients. Our preliminary data suggested that not only genes involved in collagen metabolism but also genes function as proteolytic enzymes and proteins in the immune response or cell proliferation may play an important role in the diseases pathogenesis. Nevertheless how they might play a role in connective tissue remodeling has not yet been defined. We are currently examining a set of 30 ligamentous tissue from POP patients by western blotting for evaluation of our findings.

Concluding message
Our sensitive proteomic analysis opens new avenues to study the molecular mechanisms involved in the pathogenesis and progression of prolapse.
Figure 1. 2D-PAGE of healthy control and POP patient. Ligamentous proteins from a pair of age matched control (Top left) and POP (Top right) patient were separated and visualized. Silver stained 2D-PAGEs after protein separation are shown, and two differential protein spots identified in POP case are highlighted. Spot 6112: isoelectric point = 7.04, $M_r = 17.1$kDa; Spot 9413: isoelectric point = 9.15, $M_r = 35.3$kDa.

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HUMAN SUBJECTS: This study was approved by the The Joint CUHK-NTEC Clinical Research Ethics Committee and followed the Declaration of Helsinki. Informed consent was obtained from the patients.