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URINARY PROTEOMIC PATTERN IN FEMALE STRESS URINARY INCONTINENCE: A PILOT STUDY

Hypothesis / aims of study
Women suffering from stress urinary incontinence (SUI) present an altered urinary proteomic profile compared to controls. We were aiming to identify a urinary proteomic pattern of stress urinary incontinence which may help to elucidate the diseases' pathophysiology.

Study design, materials and methods
Patients included in this prospective case-control study were all diagnosed with a history of stress urinary incontinence for at least 3 months and with a positive provocative stress test (at 300ml bladder filling) diagnosed in our urogynecology outpatient clinic, and with a history of at least one vaginal delivery (case group). Exclusion criteria were previous treatment for SUI (surgical, pharmacological or physical), overactive bladder or any incontinence other than SUI, neurological diseases, pelvic organ prolapse ≥ II, any history of lower urinary tract conditions (e.g. recurrent urinary tract infection, bladder cancer, urinary retention), renal or hepatic insufficiency, alcohol or drug abuse and pregnant or lactating patients. Same criteria applied for the control group, except for presence of SUI. After informed consent, the following examinations were carried out in the case group: patient history, gynecologic/urogynecologic routine examination, provocative stress test, measurement of residual urine volume and urine analysis (dip stick). All patients filled in the International Consultation on Incontinence Modular Questionnaire (ICIQ-UI Short Form) and were asked to deliver one urine sample. Case and control group were matched for age. Proteomic analysis of urine samples was carried out using chromatographic separation and mass spectrometry (nano High Performance Liquid Chromatography for peptide separation and ion- trap and time-of-flight mass spectrometry for peptide identification and label- free quantitation). All measurements were performed in triplicate. Database search for protein identification was performed using Human SwissProt Database and final data search using Mascot and X!Tandem. Sample size was calculated to 20 patients per group (n=40) (false discovery rate 0.05, power of 80%, assumed proportion of true null hypotheses of 0.95 and assumed standardized effect size of 1; based on two sample paired t-test) and patients were matched for age. Statistical analysis: Similarities to tag count based mRNA technologies lead us to employ an overdispersed Poisson model combined with empirical Bayes methods as commonly used to estimate mRNA-tag abundance. Count data were loaded into R (version 3.1.3) and protein abundance was estimated by calculating peptide counts normalized to counts per million (cpm). Log2 fold change was estimated based on variance stabilized average log2 cpm values using the package edgeR.

Results
Demographic data is presented in (Table 1). We identified 829 different proteins of which 167 are uncharacterized proteins. Six proteins showed a significant difference in abundance between SUI and controls (qvalue < 0.25) (Table 2). Three of these proteins are known, whereas three are uncharacterized proteins. The three known proteins show a higher abundance in SUI samples compared to controls: Plasma serine protease inhibitor (logFC 1.11), Leucine-rich alpha-2-glycoprotein (logFC 3.91) and Lysosomal alpha-glucosidase (logFC 1.24). One uncharacterized protein (PPIA) also shows higher abundance in SUI samples (logFC 1,96), whereas the other two uncharacterized proteins (UMOD, KIAA0586) present a lower abundance in SUI samples compared to controls (logFC -4.87, logFC -1.99, respectively).

Interpretation of results
This is a pilot study in which six putative SUI specific urine proteins are reported for the first time. SERPINA5 is usually present in urine in very low concentrations and serves, among other functions, as proinflammatory factor. LRG1, a secreted protein normally present in plasma, is involved in unspecific inflammatory and cancer processes. GAA is a protein essential for the degradation of glycogen to glucose in lysosomes, which is present in all tissue cell types. All three proteins are higher expressed in SUI urine samples. One uncharacterized identified protein is also higher expressed and is associated with the gene PPIA, which transcribes for the protein Peptidyl-prolyl cis- trans isomerase A. This protein has been described to accelerate the folding of proteins, and is involved in inflammatory processes and induction of interleukin-6 release from macrophages. Two of the identified uncharacterized proteins, which are associated with the genes UMOD and KIAA0586, are lower expressed in SUI samples. UMOD encodes for the protein Uromodulin, which is among other functions involved in the prevention of urinary tract infection and usually highly abundant in urine. KIAA0586 encodes for the protein TALPID3, which is required for ciliogenesis and sonic hedgehog/SHH signaling.

Concluding message
An overexpression of Plasma Serine Protease inhibitor, Leucine- rich alpha-2-glycoprotein, lysosomal alpha-glucosidase and one uncharacterized protein associated with the gene PPIA, as well as an underexpression of two "uncharacterized proteins associated with the genes UMOD and KIAA0586 may be associated with female Stress urinary incontinence. The relevance of these results regarding the pathogenesis of SUI, focusing on protein interactions, needs to be broadly investigated and results of this pilot study need to be replicated in a different collective before drawing further conclusions.

Table 1 Demographic Data of patients with Stress Urinary Incontinence (SUI) versus controls

	SUI	**control**	**p-value**
	N= 20	N=20	
(mean ± SD)			
Age (years)	49 (±9)	49 (±10)	ns
BMI (kg/cm^2)	28 (±6)	25 (±5)	ns
ICIQ* sum score	13 (±4)	0 (0)	-
Vaginal deliveries (n)	2,1 (±0.9)	1,9 (±0,7)	ns
Chronic diseases (n)**	10/20 (50%)	11/20 (55%)	ns
Menopause			ns
premenopausal	15/20 (75%)	12/20 (60%)	
postmenopausal	5/20 (25%)	8/20 (40%)	

*ICIQ-UI Short Form (International Consultation on Incontinence Modular Questionnaire); **number of patients with chronic diseases (incl. hypertension, coronary heart disease, colitis, depression, gastritis, diabetes type II, glaucoma, chronic atrial fibrillation, asthma, Hashimoto thyroiditis, hyperthyroidism, tricuspidal valve insufficiency, factor V Leiden)

Table 2 Proteins with a significantly different abundance in urine of patients with Stress Urinary Incontinence (SUI) compared to controls

Protein	Gene Symbol	logFC	qvalue
Plasma serine protease inhibitor	SERPINA5	1,111	0,029
Leucine-rich alpha-2-glycoprotein	LRG1	3,909	0,019
Lysosomal alpha-glucosidase	GAA	1,237	0,062
Uncharacterized protein	UMOD	-4,867	0,002
Uncharacterized protein	PPIA	1,962	0,227
Uncharacterized protein	KIAA0586	-1,992	0,227

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