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WHOLE GENOME GENE EXPRESSION IN BLADDER TISSUE FROM WOMEN WITH DETRUSOR OVERACTIVITY

Hypothesis / aims of study

Detrusor overactivity has multiple aetiologies. Modern anticholinergic therapies target the M3 receptor in the urothelium, bladder nerve fibres, and detrusor muscle [1]. The whole genome microarray approach offers the potential to discover differential gene expression independent of any prior hypothesis of aetiology, and to suggest new therapeutic strategies. Therefore the aim of the study was to assess differential expression of all known and predicted human gene transcripts in bladder tissue, between well phenotyped female patients with detrusor overactivity and stress incontinent controls.

Study design, materials and methods

Premenopausal adult female patients scheduled for cystoscopy were enrolled following written informed consent. Participants underwent multichannel urodynamics, filled out a 3-day bladder diary and completed the ICIQ-FLUTS questionnaire for symptom evaluation. Women were included as cases if they had systolic or provoked detrusor overactivity, with symptomatic urinary urgency and urge incontinence, >=10 voids per day, no symptomatic stress incontinence, and a negative cough stress test. Control patients were included if they symptomatic stress incontinence, with positive cough stress test, no systolic or provoked detrusor overactivity, with no symptomatic urgency or urge incontinence, <=6 voids per day, and cystometric capacity >=450ml. Participants with >= stage 2 prolapse, previous continence or prolapse surgery, acute or recurrent UTI, bladder pain, or voiding difficulties, were excluded from both groups. Cystoscopy was perfomed using a rigid 11mm cystoscope under general anaesthesia. Single biopsies of 5-10mg, including urothelium, lamina propria, and detrusor muscle, were obtained from the posterior bladder wall using cold-cup biopsy forceps, and placed immediately in ice cold RNAlater, and stored at -80°C. Total RNA was isolated using RNA STAT-60. Samples that demonstrated acceptable concentration (>12.5ng/μl), purity (260:280>1.6), and integrity (Agilent 2100 Bioanalyser), were accepted for amplification and labelling using the Ovation™ RNA Amplification System V2 and Encore™ Biotin Module (NuGEN Inc.). Samples were then fragmented and hybridised to the Human Genome HgU133 Plus 2.0 array (Affymetrix Inc.). The array data was normalised using the Rosetta Resolver Error Model, and used to generate pathway information via the MetaCore™ Knowledgebase (GeneGo Inc.).

Results

147 patients were screened and 37 recruited between October 2009 and February 2010. After optimisation of the protocol, 18 biopsies were extracted, yielding the planned sample size of 5 cases and 5 controls [2], that passed all stages of quality assurance. There were no significant differences in age, parity, or BMI, between cases and controls. By stringent criteria (significant at $p<0.005$) 29 genes (Table 1) were differentially expressed with at least 2 fold change, and 55 genes were differentially expressed at least 1.5 fold. By these criteria, CHRM3, coding for the M3 muscarinic receptor, was the single most upregulated gene, with 4.23 fold change. By less stringent criteria (significant at $p<0.01$), 1115 genes were differentially expressed, with up to 10 fold difference between cases and controls (Figure 1). Pathway analysis identified the most significant pathways as being involved in cytoskeleton remodelling; cell adhesion; smooth muscle contraction; and cholinergic, G-protein coupled, and Ca^{2+} dependent signalling (Table 2).

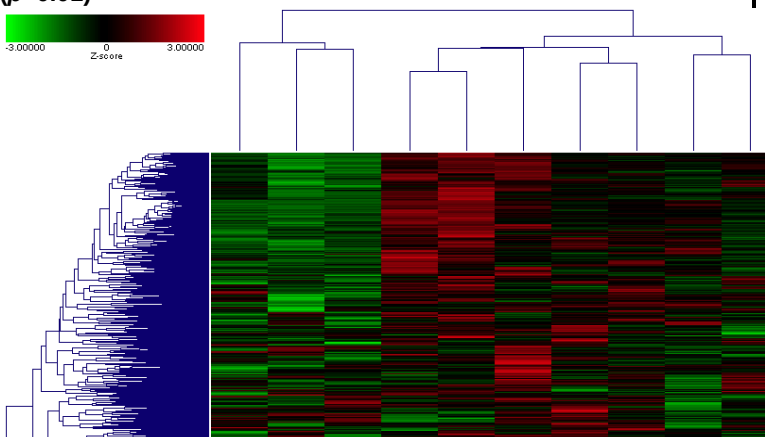
Interpretation of results

Upregulated genes coded for the major existing drug target in detrusor overactivity, and many other receptors that may be potential future drug targets. These included 8 different potassium channels, and the ryanodine receptor 2 (RYR2), a key regulator of beta-adrenergic signalling, which has not been previously identified in bladder. The type-1 cyclo-oxygenase (PTGS1) was also upregulated suggesting a role for prostaglandins. Fibronectin 1 (FN1), along with other upregulated genes that code for elements of the urinary proteome [3], might represent novel urinary biomarkers for detrusor overactivity.

Table 1: Most significantly ($p<0.005$) differentially expressed genes

Gene Symbol	FC*	*p*
FAM69C	-2.44	0.0016
MYOM2	-2.35	0.0036
SLC5A9	-2.11	0.0019
C3orf16	-2.04	0.0019
RUNX1	-2.02	0.0047
GAN	-2.01	0.0036
PWRN1	-2.01	0.0044

Figure 1: Heat map of expression levels across cases and controls for 1115 significantly differentially expressed genes ($p<0.01$)



PDE5A	2.00	0.0009
NCAM1	2.01	0.0044
LOC100126784	2.09	0.0046
MYLK4	2.16	0.0006
GFRA3	2.17	0.0042
SPTBN1	2.20	0.0011
NCAM1	2.21	0.0046
S100B	2.38	0.0001
PTGS1	2.46	0.0036
RGS11	2.47	0.0012
MYOM1	2.48	0.0047
PLN	2.63	0.0030
NRP2	2.63	0.0032
FXYD7	2.71	0.0018
RYR2	3.52	0.0026
MAEL	3.58	0.0017
NCAM1	3.82	0.0039
DCAF12L1	4.01	0.0014
CHRM3	4.23	0.0007

Control Detrusor Overactivity Control

Table 2: Top ten enriched pathways for differentially expressed genes

MetaCore Pathway Name	*p*	Ratio**
Cytoskeleton remodelling: TGF, WNT and cytoskeletal remodeling	5.12×10^{-9}	17 of 111
Cytoskeleton remodelling: Cytoskeleton remodeling	1.03×10^{-8}	16 of 102
Cell adhesion: Histamine H1 receptor signaling in the interruption of cell barrier integrity	1.85 x10-^{8}	11 of 45
Muscle contraction: G-protein coupled receptors in the regulation of smooth muscle tone	3.42×10^{-8}	14 of 83
Cell adhesion: integrin-mediated cell adhesion and migration	3.83×10^{-8}	11 of 48
Neurophysiological process: Cholinergic regulation of nerve impulse	2.85×10^{-6}	9 of 46
Muscle contraction: Cholinergic regulation of smooth muscle contraction	1.56×10^{-5}	9 of 56
Cardiac Hypertrophy: Ca(2+)-dependent NF-AT signaling in Cardiac Hypertrophy	2.09×10^{-5}	9 of 58
G-protein signalling: Regulation of cAMP levels by ACM	2.17×10^{-5}	8 of 45
Cell adhesion: Endothelial cell contacts by junctional mechanisms	5.13×10^{-5}	6 of 26

*control vs. case fold change **ratio of significantly differentially expressed genes to total genes in canonical pathway

Concluding message

We identified 1115 genes that are differentially expressed in women with detrusor overactivity and controls, most of which have never been studied in bladder. These genes are implicated in a wide range of pathways in the urothelium, bladder nerve fibres and detrusor muscle. They may represent promising candidates as drug targets or urinary biomarkers. Future work will establish the role of these genes in the pathogenesis of detrusor overactivity.

References

1. J Urol 2006;176:367-73
2. Statist Med 2002;21:3543–3570
3. Proteomics 2005;5:4994-5001

Specify source of funding or grant	**The study was supported by charitable grants from the UK Continence Society and the Institute of Obstetrics and Gynaecology Trust.**
Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**Hammersmith Research Ethics Committee**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**