BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) GENE EXPRESSION IN HUMAN BLADDER

Hypothesis / aims of study
Urinary Brain Derived Neurotrophic Factor (BDNF), is considered a leading biomarker in LUTS research, as it may be elevated in patients with overactive bladder or painful bladder syndrome, and some studies have reported treatment responsiveness. BDNF may activate bladder sensory signalling pathways contributing to inflammatory pain, and potentially also resulting in detrusor overactivity. BDNF could be present in the urine due to renal filtering, or expressed from bladder nerves, or might be expressed from the urothelium. Animal models of cystitis have demonstrated BDNF expression in urothelial cells, and human studies have reported increased bladder expression of other neurotrophic factors, in association with bladder pain[1]. The aim of this study was to determine BDNF gene expression in bladder biopsies from women with a range of lower urinary tract symptoms.

Study design, materials and methods
Pre-menopausal women attending for cystoscopy, for indications including refractory OAB, insertion of mid-urethral slings, suspected IC/PBS, intravesical botulinum toxin injections, and recurrent UTI were recruited into the study. Biopsies were obtained from the bladder dome using cold cup biopsy forceps, including urothelium, lamina propria and detrusor muscle. Biopsies were transferred immediately to sterile ice cold RNAlater, and subsequently stored at -80°C in RNase free vials. Biopsies were homogenised in TRIzol at 0°C with a QIAGEN TissueLyser LT, and RNA was extracted using an Invitrogen iPrep robot. RNA quality was assessed using both Nanodrop spectrophotometry, and an Agilent 2100 Bioanalyser. cDNA was prepared using the Life Technologies High Capacity RNA-to-cDNA™ kit. PCR was performed using an Applied Biosystems 7900HT Fast Real-Time System. Delta Ct values were derived from a mean of three house keeping genes (18s ribosomal RNA, actin beta, and glyceraldehyde-3-phosphate dehydrogenase). Finally the delta-delta Ct values were calculated comparing each experimental condition. We set case definitions for urgency incontinence, bladder pain, and stress incontinence, based on the ICIQ-FLUTS questionnaire.

Results
A total of 137 women were recruited into the study, and we prioritised the best 40 RNA samples on the basis of Bioanalyser RIN scores for analysis. The mean BMI was 27 (range 15.43) and median parity was 1 (range 0.5). BDNF was strongly expressed in all 40 samples (mean delta Ct=6.0). BDNF expression was negatively correlated with both BMI (r=-0.30, p=0.05) and parity (rho=-0.30, p=0.05). We found that BDNF gene expression was entirely uncorrelated with symptom severity for UUI, SUI and bladder pain (rho all <0.05). Dichotomised symptom scores demonstrated marginal non-specific elevation in each group (UUI delta-delta Ct 0.15, p=0.76; SUI delta-delta Ct 0.24, p=0.60; bladder pain delta-delta Ct 0.05, p=0.93). These non-significant differences were robust to different case definitions based on symptom severity.

Interpretation of results
The BDNF gene is consistently highly expressed in bladder biopsies, but expression does not vary across major LUTS subgroups. Some, but not all, studies that have reported increased urinary BDNF expression in association with OAB wet or bladder pain, and these results suggest that such increases are not from BDNF produced by the bladder itself. It may be either that BDNF transported to the bladder from the spinal cord results in abnormal bladder activity, or that renally filtered BDNF has an additional effect on the urothelium. Future work should correlate BDNF gene expression with urinary BDNF expression.

Concluding message
Bladder BDNF gene expression does not vary across major LUTS subgroups. If BDNF has a role in the pathophysiology of OAB or bladder pain, these results make it more likely that variation in BDNF is extrinsic to the bladder itself. BDNF is unlikely to be the singular cause for the development of symptoms, and an unlikely target for therapeutic interventions based on these findings.

References

Disclosures
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