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HUMAN ALPHA-SYNUCLEIN TRANSGENIC MICE: A NOVEL ANIMAL MODEL OF LOWER URINARY TRACT DYSFUNCTION IN PARKINSON'S DISEASE

Hypothesis / aims of study

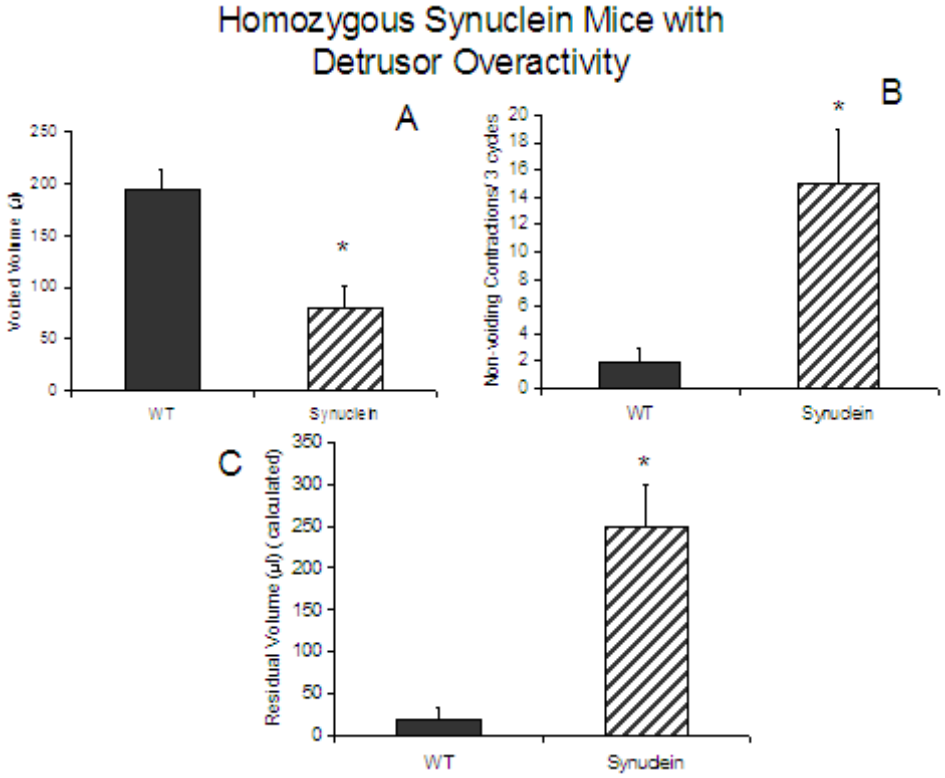
Parkinson's disease (PD) is a progressive neurological disorder known to result in profound disturbances in both motor and autonomic function. Lower urinary tract symptoms (LUTS) are common in patients with PD and result in significant morbidity.

Though the clinical and urodynamic spectrum of lower urinary tract dysfunction (LUTD) in PD have been well described, the mechanisms underlying these dysfunctions are poorly elucidated. To date, an animal model has not been described for the study of functional lower urinary tract changes occurring as a result of PD. Such a model would certainly play an important role in future studies directed at understanding the neuropathophysiology of LUTD in this disorder. Recently, transgenic (Tg) mouse lines which constitutively over-express, human alpha-synuclein (a peptide found in high concentrations in Lewy body neuronal inclusions, the histopathologic hallmark of PD) have been developed. These Tg mouse lines exhibit neuro-pathologic changes and clinical motor symptoms typical of human PD. Whether these animals develop LUTD similar to human PD patients has not been established. We aimed to characterize voiding function in human alpha-synuclein Tg mice and determine if these animals exhibit substantial LUTD similar to humans affected with PD. In addition, we investigated the expression of nerve growth factor (NGF) in the bladder of these animals to examine whether this neurotrophin may play a role in the development of LUTD in PD.

Study design, materials and methods

Breeding pairs of Tg mice hemizygous (HZ) for human alpha-synuclein were obtained from a commercial supplier (The Jackson Laboratory; Strain: B6;C3H-Tg(SNCA)83Vle/J). A colony was developed using a hemizygous (HZ) breeding scheme with subsequent genotyping to identify HO Tg, HZ Tg and wildtype (wt) littermates. Motor testing (rotorod performance at varying frequencies of rotation, gait analysis by footprinting and videotape, and forelimb extension) aimed at identifying the onset of clinical PD was performed on all genotypes, initiated at five months of age and repeated on a monthly basis. Bladder function was characterized monthly in HO Tg and wt mice beginning at five months of age and continuing through fifteen months of age (n = 4 - 8 at each age for HO and wt) utilizing conscious cystometry with continuous intravesical instillation of saline. Cryostat sections of the urinary bladders of eight month old HO Tg (n=3) and wt mice (n = 3) were analyzed for expression of NGF utilizing immunohistochemistry and enzyme linked immunoassays (ELISAs).

Figure 1: Urodynamic findings in homozygous Tg human alpha-synuclein mice with detrusor overactivity as compared with wildtype.



Results

Motor analysis did not demonstrate any differences between HO Tg, HZ Tg and wt mice until twelve months of age when HO Tg mice exhibited motor dysfunction typical of the human alpha-synuclein Tg mouse phenotype (clinical PD). Changes in bladder function were revealed in HO Tg mice compared to wt mice at the earliest age examined (five months) prior to the onset of motor dysfunction. A variety of urodynamic observations suggestive of LUTD were revealed with conscious cystometry and included: detrusor overactivity with increased frequency of voiding (terminal detrusor overactivity) and non-voiding bladder contractions, urinary incontinence suggestive of an incompetent urethral closure mechanism (evidence of leakage of saline from the urethra without evidence of increases in detrusor pressure) and detrusor underactivity associated with decreased frequency of voiding and increased bladder capacity. To date, we have not observed a clear association of any particular type of bladder dysfunction with HO Tg mouse age. The realm of described LUTD's was observed at each age examined. Terminal detrusor overactivity associated

with voiding was the most commonly observed urodynamic finding (67%). Homozygous Tg mice exhibiting terminal detrusor overactivity also demonstrated significantly decreased cystometric bladder capacity (volume per void) and elevated residual volumes as compared to wt mice ($p \leq 0.01$ and $p \leq 0.01$ respectively). The appearance of non-voiding bladder contractions during the filling phase was also significantly ($p \leq 0.01$) increased in HO Tg mice as compared to wt mice. (Figure 1)
Increased expression of the neurotrophin, nerve growth factor (NGF), was histochemically observed in cryostat sections of the urinary bladders of eight month old HO Tg mice ($n = 3$) as compared with age-matched wt mice. NGF protein content as shown with ELISAs complemented the histological studies and demonstrated a significant ($p \leq 0.05$) increase in NGF expression in HO Tg mice as compared to wt.

Interpretation of results

Transgenic mice HO for human alpha-synuclein who exhibit the PD motor phenotype also develop a range of urodynamically observable LUTD as compared with age-matched wt mice. Of interest is that LUTD appears to develop substantially earlier than observable motor dysfunction in these animals (as early as five months of age as compared with twelve months of age respectively). Detrusor overactivity appears to be the most commonly observed urodynamic finding in these animals and is associated with a reduction of bladder capacity and elevated post void residuals. These findings appear to mimic the urinary storage and voiding abnormalities observed in humans with LUTD related to PD. Nerve growth factor appears to be overexpressed in the bladders of HO Tg mice and this suggests that LUTD in these animals may be associated with local alterations in bladder innervation presumably induced by autonomic nervous system disturbances related to PD.

Concluding message

Our study has demonstrated that Tg mice homozygous for human alpha synuclein have functional abnormalities of the lower urinary tract similar to humans with PD. We believe that this novel animal model will provide a platform for future studies directed at unravelling the pathophysiologic mechanisms underlying LUTD in this disease and assist in the development of novel diagnostic and therapeutic treatment strategies.

Specify source of funding or grant	**None**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**University of Vermont animal care and use committee**