Animal Models of Diabetic Complications Consortium
(U01 DK076162)

Annual Report
(2010)

Diabetic Uropathy Pathobiology Site
Case Western Reserve University

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Progress Report Summary

Background and Summary-
Funding received through this grant is intended to support research activities related to participation of the PI in the Animal Models of Diabetic Complications Consortium (AMDCC) as the Diabetic Uropathy Pathobiology Site. Under this grant, we have proposed to create the following mice model of diabetic uropathy. The AMDCC has approved the concept of creation of the $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$. For all the experimental studies, we will use the following groups of mice:

1. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ treated with OHT to activate $CreER^{T2}$ to abolish MnSOD expression.
2. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ treated with OHT and with STZ to induce diabetes.
3. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$, sham treated.
4. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ treated with STZ.

A. Specific Aims

Our specific aims for the life of grant are as followings.

Specific aim #1: To examine the temporal alterations in the in-vivo bladder function by evaluation of 24 hours micturition habits and conscious cystometry in the above groups of mice at two time points of 8 and 12 weeks after induction of diabetes.

Specific aim #2: To examine the temporal course of morphological changes in neurogenic and myogenic components of the bladder remodeling in the above groups of mice by:

Examination the changes of bladder tissue components and their contribution to remodeling of the wall and chamber of the bladder
Examination of the changes in bladder innervations markers.

Specific aim #3: To examine the temporal alterations in the contractile function of the detrusor in the above groups of mice by:

Examination of the contractile responses of the detrusor.
Examination of the contractile and regulatory proteins of the detrusor.
Examining the alterations of the L-type Ca^{2+} channel.
Examining the alterations in the capacitive calcium entry (CCE).
Examining the IP3- and RyR-induced calcium release.
Examining the Ca^{2+} sensitivity in permeabilized detrusor strips.

Specific aim #4: To examine the temporal alterations induced by STZ in afferent and efferent autonomic pathways innervating the bladder in the in the above groups of mice by:

Assessment of afferent autonomic function by measurement of Current Perception Threshold (CPT)
Examining the relative contribution of cholinergic and purinergic components to the contractile response to transmural electrical stimulation.
Examining the alterations in ATP-P2X3, VR-1 afferent pathway in the bladder.
Examining the alterations in muscarinic receptors (M2, M3) and/or purinergic receptors (P2X1, P2X2).
Examining the connexin 43-containing gap junctions in the bladder.

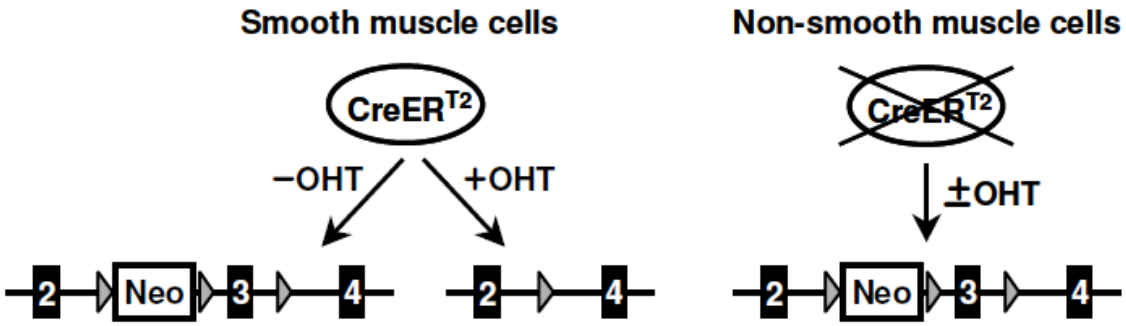
In July 2009, the PI moved from Upstate Medical University (UMU) to Case Western Reserve University (CWRU). Our entire research team was relocated to CWRU. No significant delays were caused in our work related to this grant. The PI continued to actively participate at AMDCC Steering Committee semi-annual Meetings and monthly conference calls.

B. Studies and Results

Creation and breeding of healthy MnSOD$^{lox/lox}$ SM-CreER^{T2}(ki)$^{Cre/+}$ mice- Evidence strongly suggests a key role for exaggerated Oxidative Stress (OS) in decompensated phase of diabetic bladder dysfunction (DBD). As the SA#1 of our 'Diabetic Uropathy Pathobiology site" we have aimed to generate a smooth muscle-specific manganese superoxide dismutase (MnSOD) knockout mouse to examine the role of reactive oxidative stress (ROS) in DBD.

Results:
We have completed the breeding of MnSODlox/lox mice with SM-CreERT2(ki)Cre/+ mice and subsequently treated with OHT to activate CreERT2 to delete exon 3 of the MnSOD gene (SOD2) (Figure 1). Further, we have verified the deletion of MnSOD by its protein and activity in our mice model (see below)

Figure 1: Cre-lox recombination system with or without OHT activation

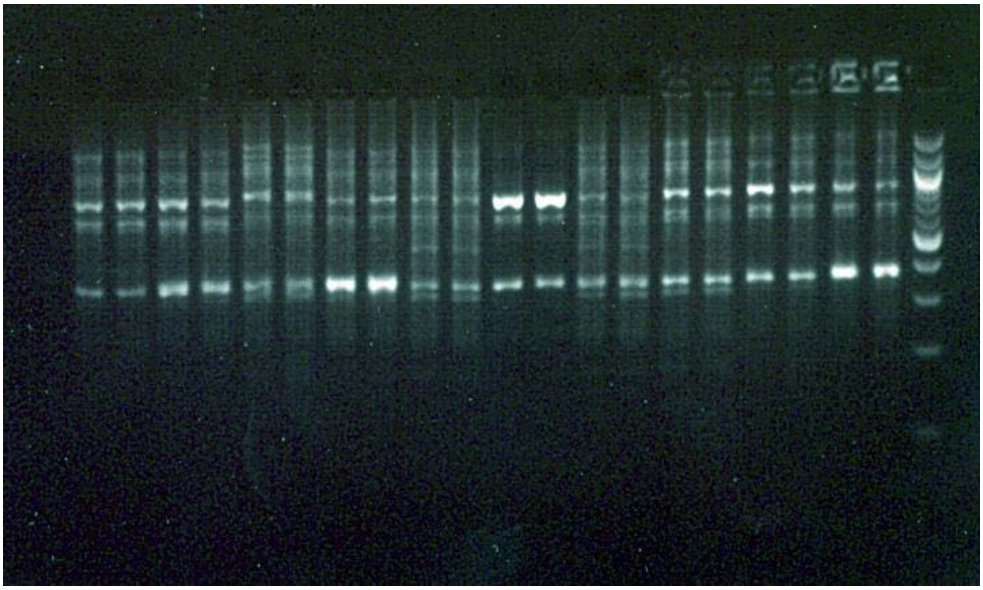


The strategy used for creation of our conditional, smooth muscle-specific MnSOD KO mice (Kuhbander, 2000). The lines at the bottom show the "floxed" MnSOD gene locus at exon 3. Exons 2-4, loxP sequences and the neomycin resistance gene are indicated by the numbered black boxes, grey triangles and boxed Neo, respectively. The circled CreERT2 indicates the CreERT2 protein. The OHT-dependent CreERT2 is only expressed in smooth muscle cells under control of the 'SM' promoter and only active in the presence of OHT.

To validate our mouse model, mature offspring (8 weeks after birth) were injected with OHT at 40 mg/kg for 5 consecutive days. Three days after the final injection, 31 male mice were sacrificed, and tissues of detrusor of the bladder, urothelium, aorta, heart, liver, skeletal muscle and skin of the tail were examined for MnSOD exon 3 by polymerase chain reaction (PCR). The phenotypical characterization of the created MnSOD lox/lox, SM-CreERT2(ki)Cre/+ mice show normal growth and function with no gross abnormalities. Three days after OHT injection, the PCR of the harvested tissues show deletion of MnSOD exon 3 in the bladder smooth muscle and aorta of the MnSODlox/lox, SM-CreERT2(ki)Cre/+ mice. The MnSOD exon 3 was present in the heart, liver, skeleton muscle,

urothelium, and tail of the mice, suggesting a conditional and smooth muscle specific deletion of the MnSOD exon 3 in the created mice
(Figure 2).

Figure 2: PCR result of MnSODlox/+,SM-CreERT2Cre/+ treated mice with OHT



Lanes 1-2: tail before OHT
Lanes 3-4: tail after OHT
Lanes 5-6: skeletal muscle
Lanes 7-8: bladder smooth muscle
Lanes 9-10: urothelium
Lanes 11-12: urethra
Lanes 13-14: ureter
Lanes 15-16: liver
Lanes 17-18: heart
Lanes 19-20: aorta
Lane 21: ladder

P1 band: without MnSOD exon 3 excision
P3 band: MnSOD exon 3 was excised

Gene Sequencing of DNA from band @400 bp (P3 band) in above agarose gel after PCR verified the MnSOD gene had been knocked-out. The work was presented at the NIDDK-sponsored workshop in March 2009 in Baltimore, MD titled “Urological Complications of Obesity and Diabetes”, where our research fellow, Dr. Nan Xiao, won the award for Best Basic Science Research poster.

Next, we began to create the following strains and subject them to the indicated treatments:

Genotype	OHT Treatment	Time Point after Treatment
wt/wt wt/wt	Yes	6 weeks
		16 weeks
	No	6 weeks
		16 weeks

lox/lox wt/wt	Yes	6 weeks
		16 weeks
	No	6 weeks
		16 weeks
wt/wt wt/cre	Yes	6 weeks
		16 weeks
	No	6 weeks
		16 weeks
wt/lox wt/cre	Yes	6 weeks
		16 weeks
	No	6 weeks
		16 weeks
lox/lox wt/cre	Yes	6 weeks
		16 weeks
	No	6 weeks
		16 weeks

In collaboration with Jackson Laboratory, we established two colonies of this mouse model: one kept at JAX and the other in our laboratory. Upon further genotyping of the breeding colony at Jackson Laboratory, our genotyping analysis by PCR revealed the JAX mice showed a fragment at 550bp to represent the lox allele, which is in contrast to the published result of 358bp (Ikegami, 2002) which has been seen in our lab's mice both before and after shipment of a sample of our colony to JAX. Both sets of mice do exhibit the wt allele at 500bp.

Jackson Laboratory stated that this difference is not uncommon and that the region of interest should still be intact. However there are no published reports to confirm this. Thus, we analyzed the data for expression (Figure 3) and activity (Figure 4) of the MnSOD from both colonies, as shown below.

Figure 3: Western Blot Results of MnSOD Expression

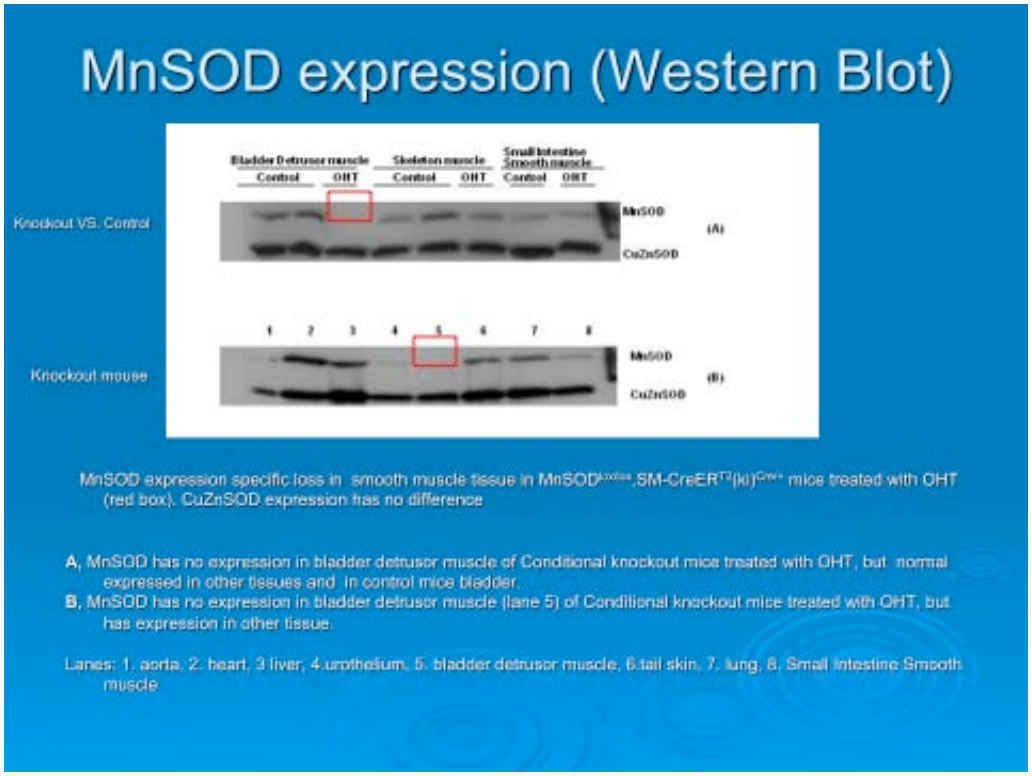
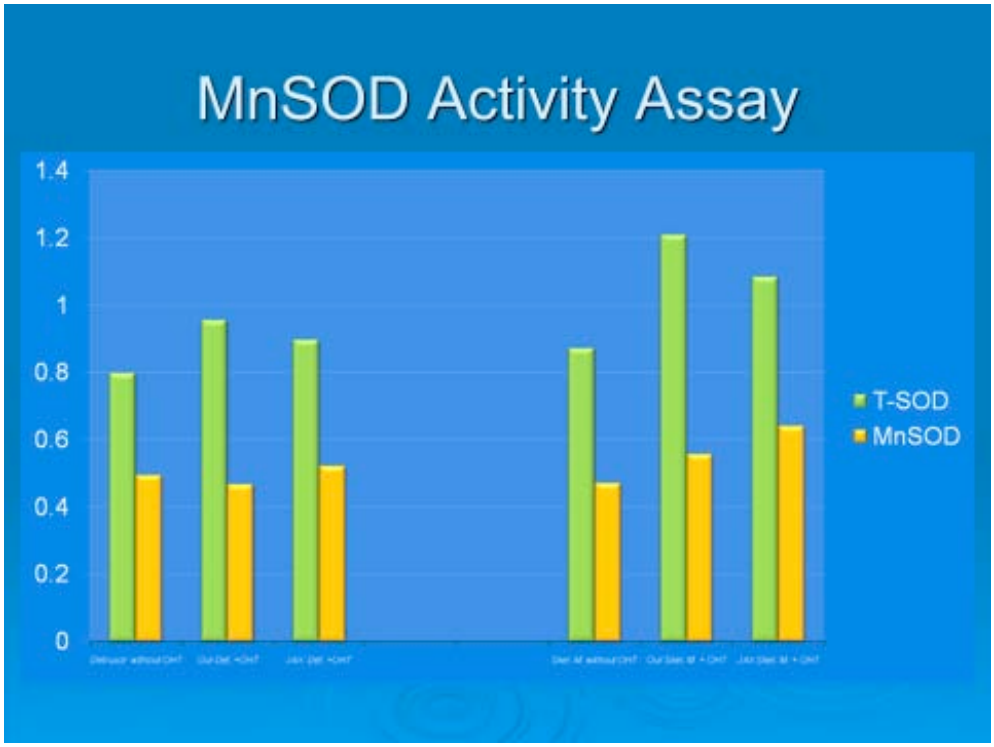


Figure 4: Results of MnSOD Activity Assay



These results indicate that the two colonies are functionally similar as it relates to deletion of SOD activity. However, with the mix up in genotype of the colony raised at Jackson Laboratory, we have had a set back

as we have had to breed more mice from our colony than anticipated and suspended our importation from Jackson until the differences could be sorted out. As a result we did obtain the adequate number of mice sufficient for completion of our experiments.

We have begun to analyze the functional capacity of these mice via 24 hour micturition testing and cystometry. To date, we do not have enough data points to make any statistically significant statements about the differences observed among the groups. Our breeding colony is producing additional animals at a rate to generate significant data in the next few months.

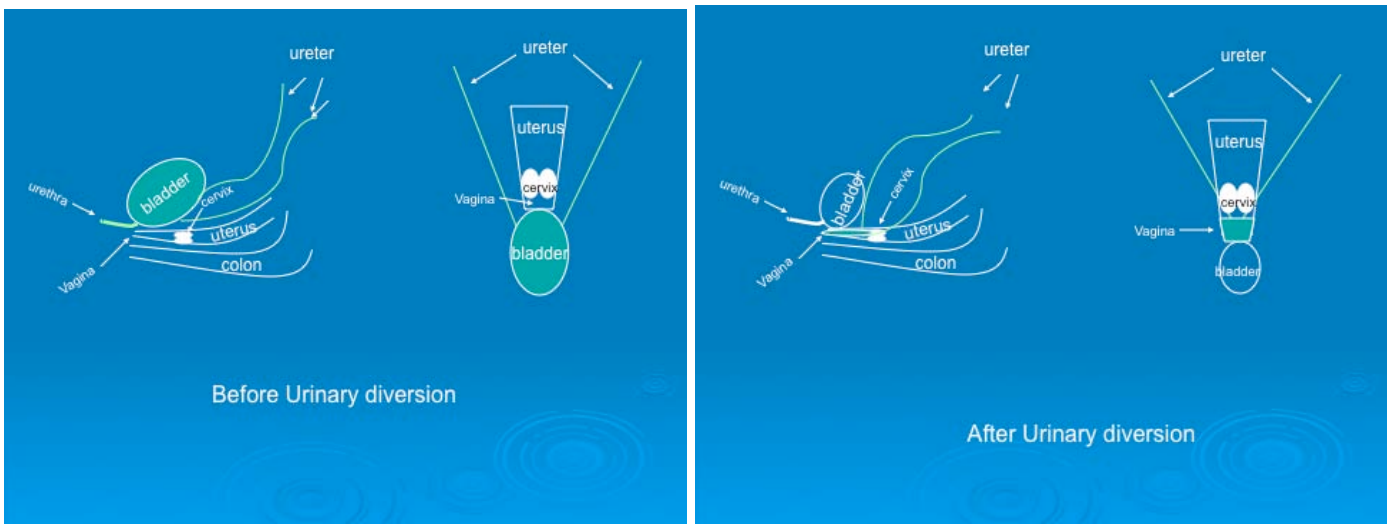
Conclusions: We have successfully deleted MnSOD exon 3 in the detrusor smooth muscle bladder of MnSODlox/lox, SM-CreERT2(ki)Cre/+ mice in a time selective manner by activation of the Cre recombinase system. Upon induction of diabetes in these mice, we will be able to examine the mechanistic role of ROS in remodeling of the bladder in a time specific manner according to the temporal alteration of the DBD previously described by us and other investigators.

Continuation of major progress on studies of pathophysiology of diabetic bladder dysfunction (DBD). Our lab continues to be at the forefront of examination of mechanisms of DBD. We have completed the following studies during 2009-2010:

Creation of a urinary diversion model (<u>accepted for publication- Journal of Urology March 2010</u>)-

To examine the impact of polyuria on LUT, we have developed and maintained a model of urinary diversion (UD) in rats and characterized UD effects on the bladder in non-diabetic (completed experiments) and diabetic (experiments in progress) animals. UD model is created by diverting the ureters to the cervix, which immediately drains the urine into the vagina (Figure 5). The epithelium of the vagina consists of keratinized squamous cells, similar to skin, with less permeability to urine compared to other choices of diversion such as colon or uterus. We have examined the functional (CMG), morphological and molecular profiles of the bladder and the urethra 1 wk and 8 wks after UD in female Sprague-Dawley rats compared to normal and sham diversion controls. Bladder weight in UD group (43.5 ± 2.4) reduced to about half of that in controls (90.4 ± 1.8) 8 wks after UD. Morphological quantification showed detrusor regression, as the percentages of smooth muscle and urothelium in the bladder wall were decreased, while the percentage of collagen was increased after UD (see the preliminary data below). The most obvious changes in CMG of UD rats relative to the sham controls were shortened inter-micturition intervals, reduced voiding volumes and compliance. The expressions of muscarinic receptor-3 (M3), mainly responsible for the micturition contraction, are decreased in bladder after 1-wk and 8 wks UD compared with those of control and sham UD rats. We believe this new animal model will be a very important tool for elucidating the pathogeneses of DBD.

Figure 5: Anatomical View of UD Model



Functional and morphological changes in UD and UD-DM rats (*submitted for publication*). To test the feasibility of this application, we did a pilot experiment to induce DM 10 days after UD in rats. At the time of writing this proposal, we examined CMG and morphology of the bladder 20 wks after DM in some of UD rats. Morphologically (Figure 6), we can see the obvious changes in urethelium, collagen and lumen size in UD and UD+DM rats. UD leads to progressively detrusor regression and relatively increased connective tissues. Interestingly, we found the urothelium deep folds disappeared in 20-wk UD+DM rats, and connective tissues are increased more in 20-wk UD+DM rats compared with those in 20-wk UD only animals. Functionally (Figure 7), both 20-wk UD and 20-wk UD+DM result in significantly reduced bladder capacity, and the bladder capacity in 20-wk UD + DM is significantly smaller than that in 20-wk UD only animals. Obviously, the different manifestations between UD and UD+DM animals resulted from hyperglycemia, but not polyuria.

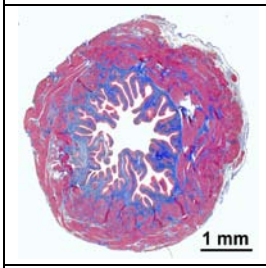
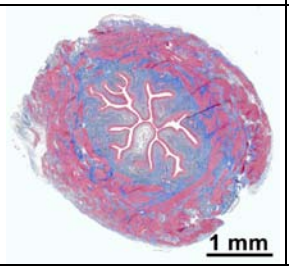
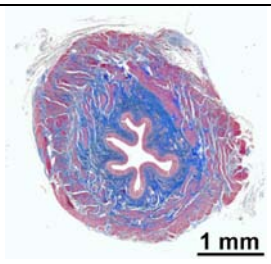
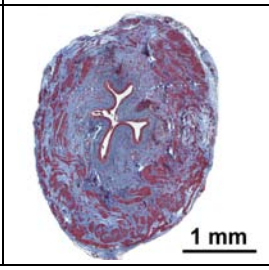
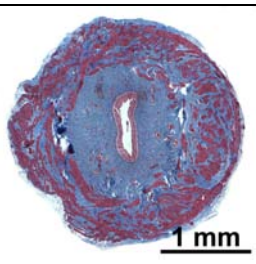
control	1-week UD	8-week UD	20-week UD	20-week UD+DM
				

Figure 6. Representative images of Masson's trichrome staining of equatorial sections of urinary bladders from control, 1-wk UD, 8-wk UD 20-wk UD and 20-wk UD + DM rats, showing the changes of smooth muscle (magenta), collagen (blue), urothelium (inner light magenta) and lumen area.

Temporal diabetes- and diuresis-induced alteration of nerve and vessel of the urinary bladder (<u>accepted for publication British Journal of Urology March 2010</u>)

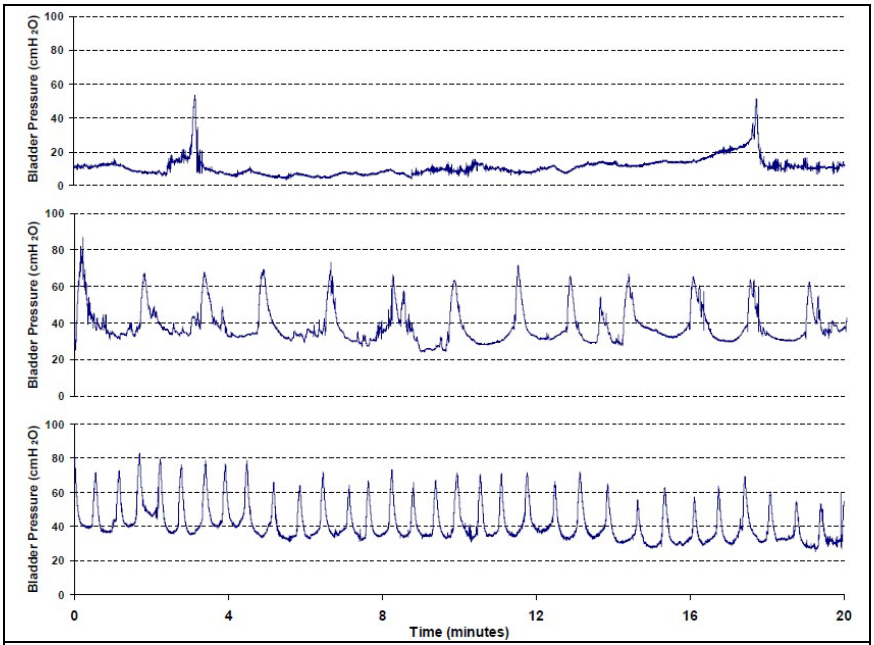


Figure 7. Representive tracings of constious Cystometrogram (CMG) from an age-matched control (upper panel), UD-20 wk (middle panel) and UD + DM-20 wk (lower panel) rat.

In this study, we aimed to characterize the temporal changes of the nerves and vasculature of the bladder. Thirty-six Sprague-Dwaley rats were divided into 3 groups: streptozotocin-induced diabetics, 5% sucrose-induced diuretics, and age-matched controls. Characteristics of the nerves and microvasculature in the equatorial cross-sectional areas of the bladder were examined by immunofluorescence staining of the specific markers, neurofilament 200 (NF200) and CD31 in 1 week, 9 weeks, or 20 weeks after induction of disease. The distribution of the nerve and blood vessels were observed under fluorescence microscopy. Digital image analysis was used to quantify the nerve and vascular density.The results indicate that DM caused significant reduction of body weight. Bladder weight increased significantly in diabetic and diuretic rats, but not in controls over the course of diabetes. Total cross-sectional wall areas and detrusor muscle area at the equatorial midline increased significantly in bladders of diabetic and diuretic rats compared with controls. Neurofilament-immunoreactive nerves were mainly distributed in the detrusor muscle area (Figure 8a). CD31- immunoreactive blood vessels were distributed in the mucosa/submucosa area (Figure 8b). There were no significant differences in the absolute value of the NF 200-immunoreactive nerve terminals in either muscle area or mucosa/submucosa area among control, diabetic and diuretic groups within investigated period. When expressed as percentage of the detrusor muscle area, nerve density decreased at 9 and 20 wks after induction in muscle area, but only 20 weeks after induction in mucosa/submucosa area in diabetic and diuretic animals. Blood vessel density decreased in diabetic and diuretic group only in muscle area in 20 weeks when expressed as percentage of the detrusor muscle area. We concluded that diabetes induced time-dependent changes of density of the nerve terminals and microvasculaure in the bladder tissues. Both polyuria and hyperglycemia may play roles in the alterations.

Figure 8a: Representative image of immunofluorescence staining of neurofilament 200-immunoreactive nerves (green) and CD31 immunoreactive blood vessels (pink) in cross-sectional areas of the bladder. Left side is mucosa/submucosa layer (muc/submuc). Right side is detrusor smooth muscle area.

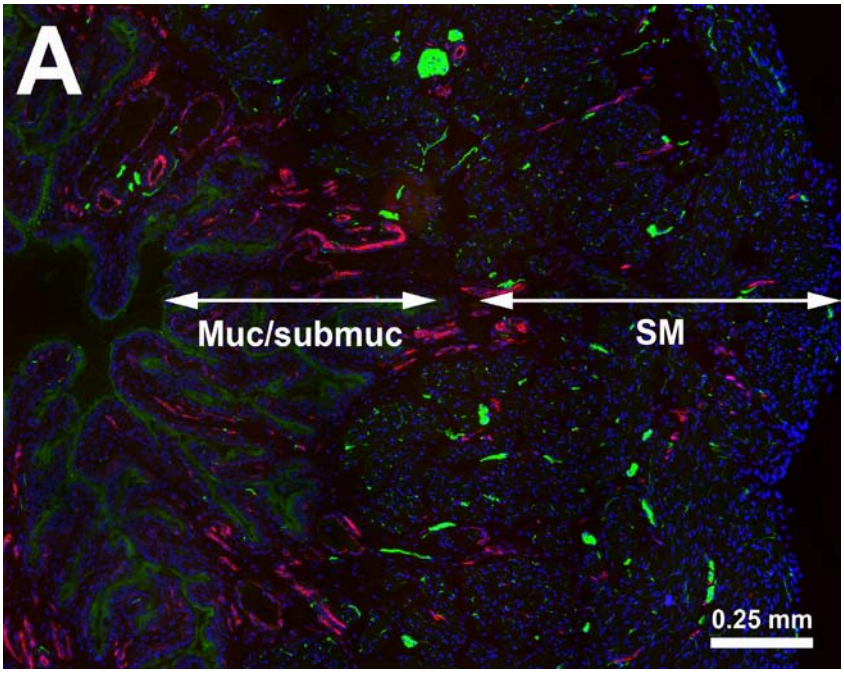
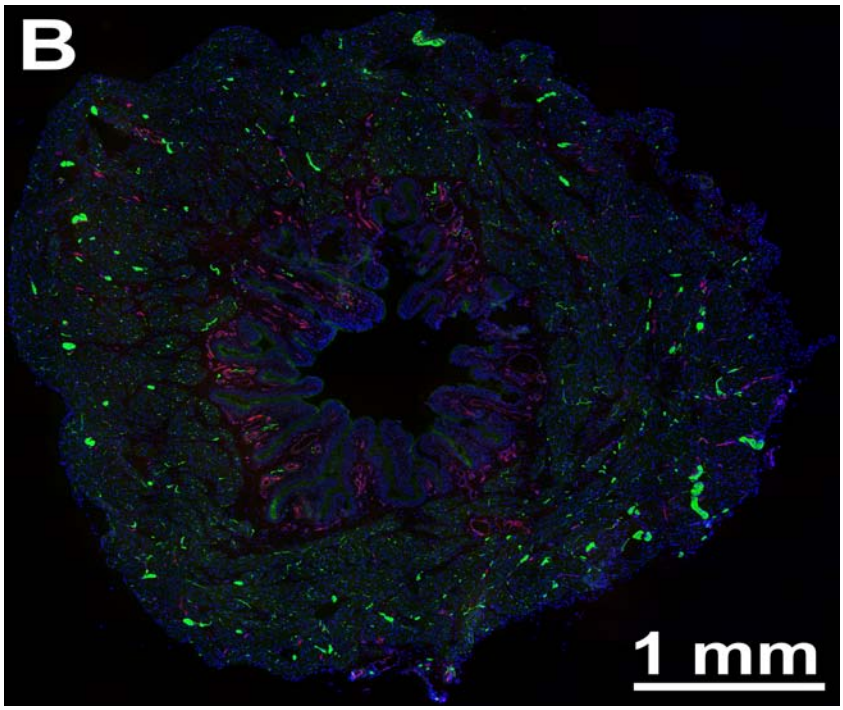


Figure 8b: Representative large field-of-view image of CD31- and neurofilament-immunoreactive nerves in equatorial sections of the bladder in an age-matched control rat.



Development of methods of simultaneous studies of cystometrogram and electromyogram of the bladder and external urethral sphincter- In several pilot studies, we have profiled the urodynamic functions of EAE mice during various stages of neurological deficits. The findings revealed significant distributions of either areflexia and/or hyperreflexia bladder across the various clinical stages of EAE (Figure 9). Below figures are representative traces of continuous cystometrogram (**CMG**) in urethane-anaesthetized age-matched naïve control, CFA control and EAE injected mice. For each group n = 3.

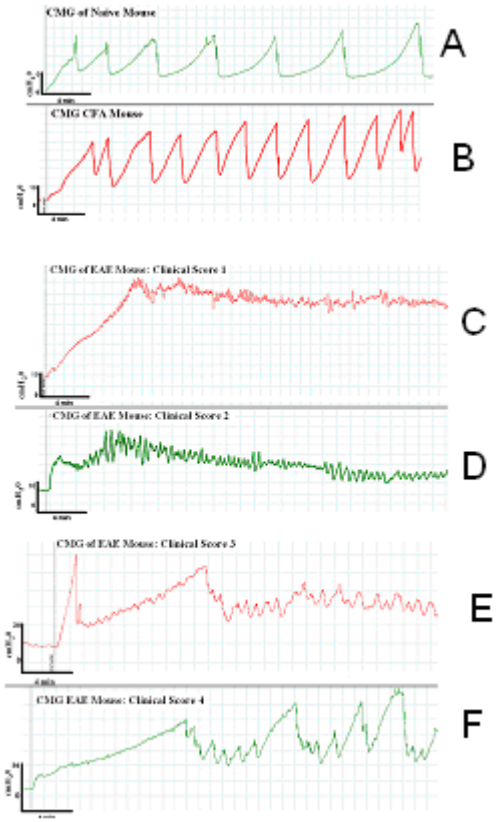


Figure 9: Representative traces of continuous cystometrogram (CMG) in urethane-anesthetized EAE mice in comparison to controls (age-matched naïve, and age-matched injected only with Freund's adjuvant). CMG profile of **naïve mice (A)** characterized by short filling latency, relatively low baseline pressure, strong voiding contraction preceded by obvious voiding threshold, and consistent inter-voiding intervals with reasonable intervals. **CFA mice (B)** exhibited increase in voiding frequency.

CMG profile of EAE mice with **Clinical Score 1 (C) and 2 (D)** exhibited characteristics of areflexic bladder with overflow incontinence, characterized by elevated baseline pressure, long filling latency with steep increase in detrusor pressure which are an indication of low compliance bladder with weak and poorly sustained contractions.

EAE mice with **Clinical Score 3 (E) and 4 (F)** exhibited characteristics of hyperreflexic bladder with involuntary voiding contractions, characterized by long filling latency, elevated baseline pressure, and non-synchronized voiding pattern.

Construction of Tuoyo electrode for electromyogram **(EMG) recording (unpublished data)-** An ideal model for detection of DSD however, should allow simultaneous assessment of **CMG** and **EMG** of External Urethral Sphincters (**EUS**). **We have successfully developed surgical techniques and created bipolar micro-electrodes named 'Tuoyo'**.. The Tuoyo electrode was constructed in-house from curved suture needle (10-0 Ethicon, CS 160-6 Needle, reduced to approximately 0.38 mm in length, Ethicon, Inc. Somerville, NJ) that was carefully soldered onto a fine Teflon® coated flexible stainless steel wire (0.078 – 0.102 mm, diameter, Cooner Wire Inc, Chatsworth, CA, USA). Copper-braid was soldered to the end of the wire, distal to the electrode for connection to either the stimulator or amplifier. The total length of the electrode is 8 inch. Tuoyo.electrode allow simultaneous recording of mouse CMG and EUS-EMG under continuous transvesical infusion (Figure 10). **To our knowledge, feasibility of this technique in mouse has never been reported.**

A B

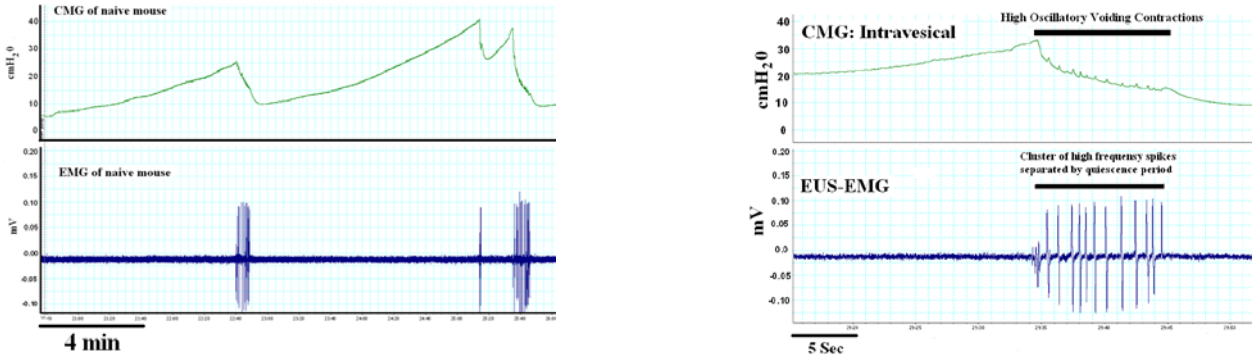


Figure 10: Trace illustrating simultaneous recording of cystometrogram (top tracing) and EUS/EMG (bottom) in urethane anesthetized female SJWX mouse. EUS exhibited low activity during bladder filling and in between micturition (A). EUS activity was markedly increased in amplitude during bladder contractions. During micturition (B) a bout of long bursting period of phasic EUS activity characterized by clusters of high-frequency spikes separated by a quiescence period were in display.

Based on the above work we have been awarded a R03 (HD061825) to study the characteristics of bladder in mouse model of neurogenic bladder. This project will further assist our work on diabetic uropathy, as the diabetic bladder dysfunction is recognized as a type of 'neurogenic bladder' in which similar methodology will be used.

Assessment of bladder function in T2D mice. (Data not shown) We have completed studies of LUT dysfunction in monogenic mice models of T2D and obesity in relation to our work within the AMDCC and related to studies of animal models of urinary incontinence[8]. The studies have included 24 hr micturition habits, CMG, measurements of leak point pressure (LPP). The models have included C57Bl6/db/db and C57Bl6/ob/ob mice with their respective age and sex-matched controls. The initial aim of these experiments was to assess the presence or absence of urinary incontinence in these animals as measured by LPP. Further, the animals underwent survival surgery (vaginal distension and implantation of suprapubic tube) for 20 days[9].

C. Plans-

Our plans for the next year are along two parallel pathways: a) continue our investigation of pathophysiology of diabetic bladder dysfunction; b) phenotype and genotype characterization of our created $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}$ mice, begin the experiments related to the following specific aims. For all the experimental studies, we will use the following groups of mice:

1. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ treated with OHT to activate $CreER^{T2}$ to abolish MnSOD expression.
2. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ treated with OHT and with STZ to induce diabetes.
3. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$, sham treated.
4. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ treated with STZ.

Specific aim #1: To examine the temporal alterations in the in-vivo bladder function by evaluation of 24 hours micturition habits and conscious cystometry in the above groups of mice at two time points of 8 and 12 weeks after induction of diabetes.

We plan to further test $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ mice for blood pressure (via tail cuff) and perform smooth muscle contractility testing. We also will examine the oxidative and antioxidant status in bladder smooth muscle, urothelium, and other control organs (heart, brain, aorta, liver, skeletal muscle,

small intestine, tail). In addition we will measure total antioxidant ability, SOD activity, and Cu-ZnSOD and MnSOD immunoblotting.

Once these tests are complete, our next goal is to induce diabetes in these same mice and examine the additive effect upon diabetic bladder dysfunction.

Specific aim #2: To examine the temporal course of morphological changes in neurogenic and myogenic components of the bladder remodeling in the above groups of mice by:

1. Examination the changes of bladder tissue components and their contribution to remodeling of the wall and chamber of the bladder
2. Examination of the changes in bladder innervations markers.

D. Publications-

The following publications were completed by the PI and his collaborators during 2009-2010 funding period:

1. Fry CH, **Daneshgari F**, Thor K, Drake M, Eccles R, Kanai AJ, Birder LA. Animal models and their use in understanding lower urinary tract dysfunction. Neurourol Urodyn. 2010 Apr;29(4):603-8.**Daneshgari F**, Leiter EH, Liu G, Reeder J. Animal models of diabetic uropathy. J Urol 182(6): S8-13, 2009. PMID 19846143.
2. Gasbarro G, Lin DL, Vurbic D, Quisno A, Kinley B, **Daneshgari F**, Damaser MS. Voiding function in obese and type 2 diabetic female rats. Am J Physiol Renal Physiol 298(1): F72-7, 2010. PMID 19889955.
3. **Daneshgari F**, Liu G, Birder L, Hanna-Mitchell AT, Chacko S. Diabetic bladder dysfunction: current translational knowledge. J Urol 182(6): S18-26, 2009. PMID 19846137.
4. Chen CC, Hijaz A, Drazba JA, Damaser MS, **Daneshgari F**. Collagen remodeling and suburethral inflammation might account for preserved anti-incontinence effects of cut polypropylene sling in rat model. Urology 73(2): 415-20, 2009. PMID 18829083.
5. Nager CW, Brubaker L, **Daneshgari F**, Litman HJ, Dandreo KJ, Sirls L, Lemack GE, Richter HE, Leng W, Norton P, Kraus SR, Chair TC, Chang D, Amundsen CL, Stoddard AM, Tennstedt SL. Design of the Value of Urodynamic Evaluation (ValUE) trial: A non-inferiority randomized trial of preoperative urodynamic investigations. Urinary Incontinence Treatment Network. Contemp Clin Trials 30(6): 531-9, 2009. PMID 19635587.
6. Barber MD, Spino C, Janz NK, Brubaker L, Nygaard I, Nager CW, Wheeler TL. The minimum important differences for the urinary scales of the Pelvic Floor Distress Inventory and Pelvic Floor Impact Questionnaire. Am J Obstet Gynecol 200(5): 580e1-7, 2009. PMID 19375574.
7. Lin YH, Liu G, Li M, Xiao N, **Daneshgari F**. Recovery of continence function following simulated birth trauma involves repair of muscle and nerves in the urethra in the female mouse. Eur Urol 57(3): 506-12, 2010. PMID 19346061.
8. **Daneshgari F**, Brown JS, Kusek JW, Nyberg LM. Urological complications of obesity and diabetes. J Urol 182(6): S1, 2009. PMID 19846132.
9. Kefer JC, Liu G, **Daneshgari F**. Pubo-urethral ligament injury causes long-term stress urinary incontinence in female rats: an animal model of the integral theory. J Urol 181(1): 397-400, 2009. PMID 19010492.

10. Liu G, Li M, Vasanji A, **Daneshgari F**. Temporal diabetes and diuresis-induced alteration of nerves and vasculatures of the urinary bladder in the rat. BJU Intl, accepted 2010.
11. Liu G, Lin YH, Li M, Xiao N, **Daneshgari F**. Temporal morphological and functional impact of complete urinary diversion on bladder: a model of bladder disuse in rats. J Urol, accepted 2010.

E. Response to EAC Comments:

- "With regard to these studies in the lower urinary tract, the combination of SOD KO with DM may worsen injury but its relevance needs to be interpreted with circumspection (for reasons noted above in the NEPHROPAHTY general comments regarding combining injurious mechanisms vs. combinations relevant to human disease). Has Dr. Daneshgari ever examined the effects of diuresis induced by sugar or saccharin dissolved drinking water or ADH deficient rats/mice and compared these to diabetes?"

RESPONSE- Yes, indeed we have. In majority of our experiments related to role of DM, we have (and will continue to do so) used diuresis induced by 5% sucrose as a control for diuresis caused remodeling of the bladder. This model has been used in the following studies:

1. Liu G, Lin YH, Yamada Y, Daneshgari F: External Urethral Sphincter Activity in Female Diabetic Rats. Neurourology and Urodynamics 2008 March 19 (Epub)
2. Daneshgari, F., Huang, X., Liu, G., Bena, J., Saffore, L., Powell, C. T.: Temporal differences in bladder dysfunction caused by diabetes, diuresis, and treated diabetes in mice. Am J Physiol Regul Integr Comp Physiol 2006 Jun;290(6):R1728-35. Epub 2006 Jan 26
3. Liu G, Daneshgari F: Alternations in Neurogenically Mediated Contractile Responses of Urinary Bladder in Rat with Diabetes. American Journal of Physiology Renal Physiol. June 2005 288(6):F1220-6. Epub 2005 Feb 1
4. Liu, G. and Daneshgari, F.: Temporal diabetes- and diuresis-induced remodeling of the urinary bladder in the rat. Am J Physiol Regul Integr Comp Physiol, 2006 March 2

- "Dr. Daneshgari is strongly encouraged to collaborate with the new AMDCC P&F awardees"

RESPONSE- we have collaborations with all three recipients of P&F awards. Currently, we have active collaborations with Drs Birder and Fraser (sharing specimen, concepts); and have started our collaboration with Dr. Olumi (sharing protocols on studies of type II DM mice models)

- "Great progress again. The PI's continued development of the mouse as a model system for studying bladder function is invaluable. But it is also good to see that he is getting to the stage were he can apply his pioneering work to look at his diabetes/oxidative stress models. This should yield interesting results."

RESPONSE- Thank you for your encouragement.

F. Project Generated Resources-

Dr. Guiming Liu has received a Faculty Developmental Award from the American Diabetic Association for 2010-2013.

In addition, based on preliminary data developed from the current grant, we have developed a collaboration with Lori Birder, Ph.D. from Departments of Medicine and Pharmacology of the University of Pittsburgh- Our collaboration stated from studies of role of urothelium and reactive oxidative stress products in mechanisms of diabetic bladder dysfunction and led to Dr. Birder's receiving a Pilot & Feasibility Award from AMDCC in 2009.

G. Research Development-

As above

H. Other Activities/Achievements-

We have developed an extensive collaboration within and outside AMDCC as the followings:

1. Lori Birder, Ph.D. and Anthony Kanai, Ph.D. from Departments of Medicine and Pharmacology of the University of Pittsburgh- Our collaboration stated from studies of role of urothelium and reactive oxidative stress products in mechanisms of diabetic bladder dysfunction and led to our joint project funded by JDRF for 2006-2008.
2. Timothy Kern, Ph.D.- Case- Department of Medicine and Ophthalmology- We have extensive collaboration with Dr. Kern extending from sharing animals for joint experiments to monthly joint lab meetings that are alternatively held at Case or CCF campus.
3. Fernando Casas, Ph.D.- LRI- BME- to study the integration of vocalization of animal models into the assessment of afferent function of the bladder.
4. Aria Olumi, M.D.- From Harvard School of Medicine and MGH- we have collaborated on characterization of a mouse model of type 2 DM
5. Matthew Fraser, Ph.D.- We have shipped samples of urethra from diabetic animals to Dr. Fraser at Duke University. Dr. Fraser also received an AMDCC Pilot and Feasibility Award in 2009.
6. Mark Chance, Ph.D.-Dr. Chance is Professor of Biochemistry and Physiology and Director of Proteomic Core Center of the CWRU. We have developed a collaboration with Dr. Chance and are in the process of identifying the protein targets in DBD.

I. Research Development and other activities planned for the next year.

See above

Respectfully submitted,

Firouz Daneshgari, M.D.