AMDCC Annual Report (2011)

PI: DANESHGARI, FIROUZ
Project Title: Diabetic Uropathy Pathobiology Site
Grant Number: U01 DK076162

Abstract: Diabetic Uropathy is a term for a range of debilitating urologic complications such as bladder dysfunction, urinary incontinence, urinary tract infection and sexual dysfunction, that are among the most common and costly, yet understudied complications of diabetes mellitus (DM), an incurable disease that affects at least 20 million people in the U.S. and is rising in prevalence with the rapidly rising prevalence of obesity. Therapeutic options for diabetic uropathy are inadequate and have not improved over the last 50 years. In response to RFA-DK-05-011, we propose to work with the Animal Models of Diabetic Complications Consortium's organizational structure to function as the 'Diabetic Uropathy Pathobiology Site" to participate in development of two novel mice models of diabetic uropathy and to investigate the mechanisms of the pathophysiology of diabetic bladder dysfunction in these animals. Based on the observed temporal effects of diabetes on the bladder function in small animals, we hypothesize that depletion of manganese superoxide dismutase (MnSOD) specifically in smooth muscle of adult mice will exacerbate accumulation of free radicals in smooth muscle during STZ-induced diabetes and accelerate the onset of the decompensated phase of diabetic bladder dysfunction. We hypothesize further that limiting depletion of MnSOD to arterial smooth muscle will have a lesser effect on STZ-induced diabetic bladder dysfunction by limiting exacerbation of STZ-induced free radical accumulation to the vasculature. Depletion of MnSOD selectively in total and arterial smooth muscle in the MnSODlox/lox, SMCreERT2 mice and MnSODlox/lox, ASM-CreERT2 mice, respectively, will be accomplished by administration of 4-hydroxytamoxifen to activate Cre recombinase expressed in the smooth muscle. The animals will be further treated with 4-hydroxytamoxifen treatment, and half of them will be injected with STZ to induce diabetes. The bladder function in the animals will be studied via four specific aims to examine: 1) the temporal alterations in the in-vivo bladder function by micturition habits and conscious cystometry; 2) the temporal course of morphological changes in diabetes-induced bladder hypertrophy; 3) temporal alterations in the contractile function of the detrusor muscle; 4) the temporal alterations in afferent and efferent autonomic pathways innervating the bladder. The Principal Investigator and the research team have a productive track record in being a part of the existing AMDCC since 2003 and have functioned well under the auspices of the NIH, and the Steering and External Advisory Committees of the AMDCC.

1. Program Accomplishments:

Hypothesis- our application to AMDCC consists of the following hypotheses related to diabetic bladder dysfunction:

i. In the early stage of DM (<9 weeks in rodents), osmotically-induced polyuria initiates a cascade of events leading to marked hypertrophy and remodeling of the bladder, involving urothelium, detrusor and connective tissue. The remodeled bladder causes compensatory storage problems such as urinary incontinence.

ii. In the later stage of DM (>12 weeks in rodents), accumulative effects of prolonged hyperglycemia, including Advanced glycation end products (AGEs), oxidative stress, and inflammation cause irreversible damages to the phenotype and functions of the bladder tissues, leading to decompensatory voiding problems of the bladder.

iii. Depletion of manganese superoxide dismutase (MnSOD) specifically in smooth muscle of adult mice will exacerbate accumulation of free radicals in smooth muscle during STZ-induced diabetes and accelerate the onset of the decompensated phase of diabetic bladder dysfunction. This hypothesis was the leading elements for our proposal for creation of MnSODlox/lox SM-CreERT2(ki)Cre/+ mice

Progress toward stated aims-

Our specific aims for our AMDCC applications were to create and use the following groups of mice:

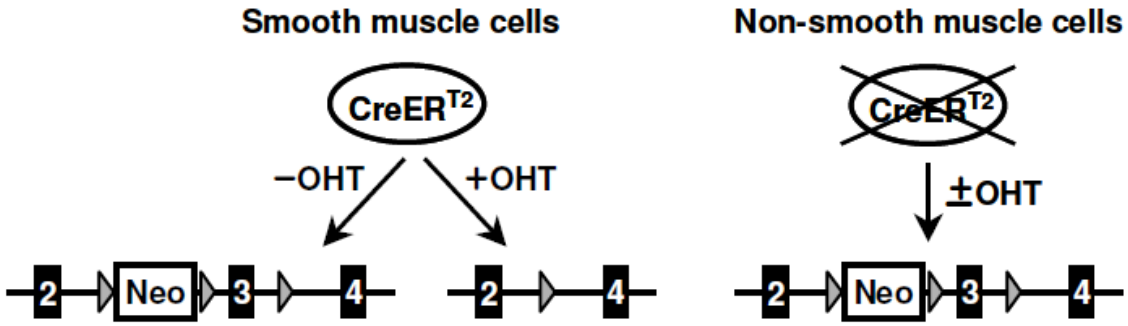
1. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ treated with OHT to activate $CreER^{T2}$ to abolish MnSOD expression
2. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ treated with OHT and with STZ to induce diabetes
3. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$, sham treated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

We are pleased to report the following progress toward those specific aims:

A. Creation and breeding of MnSODlox/lox SM-CreERT2(ki)Cre/+ mice

As reported in our 2010 annual report, we completed the breeding of MnSODlox/lox mice with SM-CreERT2(ki)Cre/+ mice in 2009 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 is published (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.

Further and 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. Based on the results reported in 2010, our investigation indicate that the two colonies are functionally similar as it relates to deletion of SOD activity.

Over the past year, the following two sets of experiments have been performed in parallel in our lab. The experiments are performed based on the availability of the mice.

1. Lower Urinary Tract Phenotype of the conditional smooth muscle-specific deletion of MnSOD mice

The following mice strains have been bred in our lab (Table 1). We are examining function (cystometry), histology, manganese superoxide dismutase (MnSOD) protein levels and activity in the lower urinary tract function in liver, skeletal muscle, heart, aorta, intestine at 6 and 16 weeks after 4-hydroxytamoxifen (OHT) injection in these mice. OHT is injected when the mice are 8 weeks old. Thus far, we have completed the following measures for four groups of mice (n=40 for each group) including wt/wt wt/wt; lox/lox wt/wt; wt/wt wt/cre; wt/lox wt/cre; lox/lox wt/cre:

- Frequency volume charts (FVC) and CMG
- SOD assays 6 weeks after OHT treatment
- FVC and CMG 9 weeks after OHT treatment and 8 weeks after STZ treatment, and harvested for histology.
- 40 mice have been harvested (for SOD assays) 9 weeks after OHT treatment and 8 weeks after STZ treatment

At the time of this writing, we are completing the extraction and analysis of the data. Interpretation of this large data set will allow us to: 1) confirm MnSOD knockout is smooth muscle-specific; 2) examine the response of oxidative and anti-oxidative capacity to MnSOD deletion in smooth muscle; 3) observe the effects of conditional knockout MnSOD in smooth muscle on the lower urinary tract function, 4) observe the effects of conditional knockout MnSOD in smooth muscle on other organ containing smooth muscle.

Two time points were chosen based on the previous studies (Kuhbandner S et al. *Genesis*, 2000, 28: 15-22), in which the efficiency of tamoxifen injection significantly decreased 16 weeks after injection (1). We'd like to observe the short-time and long-time effects of smooth muscle-specific deletion of the MnSOD on the lower urinary tract.

Table 1. Experimental groups

Genotype	OHT or vehicle (at age of 8 wks)	Wks after OHT or vehicle treatment	
		6	16
wt/wt	vehicle	x	
	vehicle		x
	OHT	x	
	OHT		x
lox/lox	vehicle	x	
	vehicle		x
	OHT	x	
	OHT		x
Cre	vehicle	x	
	vehicle		x
	OHT	x	
	OHT		x
wt/lox Cre	vehicle	x	
	vehicle		x
	OHT	x	
	OHT		x
lox/lox Cre	vehicle	x	
	vehicle		x
	OHT	x	
	OHT		x

2. Examining the role of oxidative stress in diabetic bladder dysfunction by using conditional smooth muscle-specific deletion of the MnSOD mouse model.

To explore the role of oxidative stress in the pathogenesis of diabetes-induced bladder dysfunction, we have conducted two series of studies:
a- induced diabetes in the conditional smooth muscle-specific deletion of the MnSOD mouse model (Table 2). We inject OHT when the mice are 8 weeks old. One week

later, STZ is injected to induce diabetes. We examine the temporal alterations of the bladder function, neurogenic and myogenic components of the bladder at 5 and 15 week after diabetes induction. At the time of this writing, we have induced DM in these animals and are awaiting their reaching to the 5 and 15 weeks post DM induction.

Two time points after induction of DM were chosen based on our previous work, in which we developed the following hypothesis of diabetes bladder dysfunction (DBD) to describe how diabetes affects the bladder in a temporal fashion: Osmotically-induced polyuria and chronic hyperglycemia are the two main pathogenesis mechanisms of DBD. In the early stage of DM, osmotically-induced polyuria causes rapid hypertrophy and remodeling of the bladder, leading to compensatory bladder function. In the late stage of DM, long-term hyperglycemia and polyuria-induced accumulation of oxidative stress products cause damage to nerves and muscles, leading to decompensatory voiding problems of the bladder.

Table 2. Experimental groups

Genotype	OHT or vehicle (at 8 wks old)	STZ or vehicle (1 wk after OHT)	Wks after STZ or vehicle treatment	
			5	15
lox/lox	vehicle	vehicle	x	
	vehicle	STZ	x	
	vehicle	vehicle		x
	vehicle	STZ		x
	OHT	vehicle	x	
	OHT	STZ	x	
	OHT	vehicle		x
	OHT	STZ		x
lox/lox Cre	vehicle	vehicle	x	
	vehicle	STZ	x	
	vehicle	vehicle		x
	vehicle	STZ		x
	OHT	vehicle	x	
	OHT	STZ	x	
	OHT	vehicle		x
	OHT	STZ		x

b- completion of proteomic studies. In collaboration with laboratories of Dr. Mark Chance, we have completed proteomic analysis of the urothelium and bladder detrusor muscle in series of animals with DM, diuresis and controls. Please see below for further description.

Plans for the year and completion of the project

- Our plans for the next year are along two parallel pathways: a) finish the experiments described above and completion of analysis of the available data. b) Continue our investigation of pathophysiology of bladder dysfunction in type 2 diabetes mice models (se below).

Given the higher prevalence of type II DM and frequency of diabetic uropathy in subjects with type II DM, and in expansion of our aims as the pathobiology site for "diabetic urolpathy", we have completed the following experiments:

1. Investigation of the temporal changes of bladder function in type 2 diabetic mice and identify molecular targets related to these temporal changes using proteomic analysis.

To compare and identify which mice model better mimics DBD in humans, we have used a monogenic and a polygenic T2D mice models and their background species (Table 3). Two different time points were chosen to examine the bladder function in the early and late phases of DM in different mouse models. All of the FVB ob/ob mice exhibited a T2D phenotype by 10 weeks of age, and more than 90% of the NONcNZO10/LtJ mice exhibited a T2D phenotype by 8 weeks of age when fed a diet with 11% fat. We are examining the functional, histologic, and molecular changes in the designed time points. Importantly, we want to identify the molecular targets involved in bladder hypertrophy occurring in the early phase and oxidative stress occurring in the later phase using a quantitative proteomic method, proteolytic ^{18}O labeling (Miyagi, 2007).

Table 3. Experimental groups

Genetics	Group	Strain	Phenotype
Monogenic	1	FVB/NJ	non-diabetic parental strain
	2	FVB ob/ob	diabetes
Polygenic	3	NON/ShiLtJ	non-diabetic parental strain
	4	NONcNZ010/LtJ	diabetes

2- Proteomic Analysis of Bladder Tissues- We recently completed a pilot proteomic analysis of detrusor muscle and urethelium in one 20-wk monogenic FVB (ob/ob) diabetic mouse and an age-matched control mouse (FVB/NJ). A total of 128 proteins in detrusor muscle and 193 proteins in urothelium were quantified. Pathway analysis using the Ingenuity Pathway Analysis suite (IPA) suggested proteins that were found greater amount in diabetic detrusor were related to muscle contraction and oxidative stress. In addition, we also found proteins associated with cellular assembly and organization were altered. In urothelium, proteins involved in nucleic acid metabolism, tissue morphology, free radical scavenging, cellular growth and proliferation and cell cycle were altered in the diabetic mouse. The top 10 proteins altered in detrusor muscle and urothelium in 20-week diabetic relative to control mouse are listed inTables 4 and 5.

Table 4. Top 10 proteins altered in detrusor muscle in 20-week diabetic relative to control mouse

Top 10 proteins found in greater amount in detrusor muscle in 20-wk	Top 10 proteins found in lesser amount in detrusor muscle in 20-wk

diabetic relative to control mouse		diabetic relative to control mouse	
Protein name	**Fold change**	**Protein name**	**Fold change**
Myosin light chain 4	>10	Telomerase Cajal body protein 1	<0.1
Protein phosphatase 1 regulatory subunit 14A	>10	Alpha-1-antitrypsin 1-1	0.11313
Heat shock protein beta-7	5.77034	Serum albumin	0.12886
Adapter molecule crk	5.43774	Serotransferrin	0.15587
Thymosin beta-10	5.37924	Protein AMBP	0.24362
Heat shock protein beta-1	5.11247	Elongation factor 1-alpha 1	0.33097
SH3 domain-binding glutamic acid-rich-like protein	4.9975	40S ribosomal protein S19	0.35935
Protein S100-A6	4.57038	Kininogen-1	0.3867
Cystatin-B	4.50857	Histone H4	0.39118
Zyxin	4.3802	Fibrinogen beta chain	0.40487

Table 5. Top 10 proteins altered in urothelium in 20-week diabetic relative to control mouse

Top 10 proteins found in greater amount in urothelium in 20-wk diabetic relative to control mouse		Top 10 proteins found in lesser amount in urothelium in 20-wk diabetic relative to control mouse	
Protein name	**Fold change**	**Protein name**	**Fold change**
E3 ubiquitin-protein ligase RNF216	>10	Prostaglandin E synthase 2	<0.1
Glutathione S-transferase P 1	5.6509	Serine/threonine-protein kinase MARK2	<0.1
ATP synthase subunit beta, mitochondrial	4.6119	LIM and SH3 domain protein 1	<0.1
Annexin A1	4.4371	Collagen alpha-1(I) chain	<0.1
Fibrinogen gamma chain	3.7937	Heterogeneous nuclear ribonucleoprotein K	0.1159
Histone H1.0	3.3789	Transthyretin	0.1302
Isocitrate dehydrogenase [NADP] cytoplasmic	3.3201	Protein S100-A8	0.1438
Fibrinogen beta chain	2.7347	Liver carboxylesterase N	0.223
Eukaryotic initiation factor 4A-I	2.6733	Tropomyosin alpha-4 chain	0.2796
Alkaline phosphatase, tissue-nonspecific isozyme	2.6694	Apolipoprotein A-II	0.3068

In addition, we have also completed proteomic analysis of tissues from animals of type I DM (data not presented here). This investigation is along our hypothesis to search for molecular targets responsible for time-course alterations of the bladder remodeling and function observed in both types of DM of cell cycle, inflammatory, and oxidative pathways including systems biology modeling of deregulated protein sub-networks.

The data from all these experiments will be uploaded to the ADMCC site upon completion and confirmation of data.

3. Confirmation of fiber and site specific nociceptive stimulation of the bladder by our Bladder Sensory Threshold (BST) device.

As reported previously, we created a device (BST) by which the neuroselective afferent sensation of the bladder could be assessed (Abouassaly 2008). We recently completed a set o studies in which the efficacy of sine-wave electrical stimulation of the bladder was assessed by measuring expression of Fos-immunoreactive (IR) cells in rat spinal cord regions. Thirty-seven female Sprague-Dawley rats were divided into eight groups: sham stimulation; 5, 250, and 2,000 Hz stimulation in 1.5 mA or 2.0 mA; and a group instilled with capsaicin in bladder. Using a recently developed bladder sensory perception threshold device, sine-wave electrical stimulation was applied for 90 min to rat bladder. Spinal cord was harvested after sacrifice. Fos-IR cells in the spinal regions of the medial dorsal horn, lateral dorsal horn, dorsal commissure (DCM), and sacral parasympathetic nucleus (SPN) were measured. The distributions of Fos-IR neurons were compared.The maximum expression of Fos-IR cells, induced by 250-Hz and 5-Hz stimulation of the bladder, was found at L6 of the spinal cord and was significantly higher than in the control group ($p < 0.01$). Stimulation with 2,000 Hz did not induce any Fos-IR cells. Fos-IR neurons were predominantly seen in the SPN region in response to 250-Hz stimulation and in the DCM region in response to 5-Hz stimulation. The distribution of Fos-IR cells in rat spinal cord segments L1 to S1 was determined after application of two different types of stimuli to the bladder mucosa: Neurometer®-SPT stimulation at 5 Hz and intravesical infusion of the C-fiber selective activator and neurotoxin capsaicin. Figure below is the quantification data, which showed the two types of stimuli yielded similar distribution patterns of Fos-IR expression, with a major peak in L6 and lesser expression in S1 and L1. From these studies, we have concluded that frequency-specific sine-wave electrical stimulation of the rat bladder induced the expression of Fos-IR cells in a neuroselective manner. Therefore, the bladder sensory threshold device could be used for explore the pathophysiology of the diseases with disturbances of afferent pathway of the bladder, including diabetic bladder dysfunction.

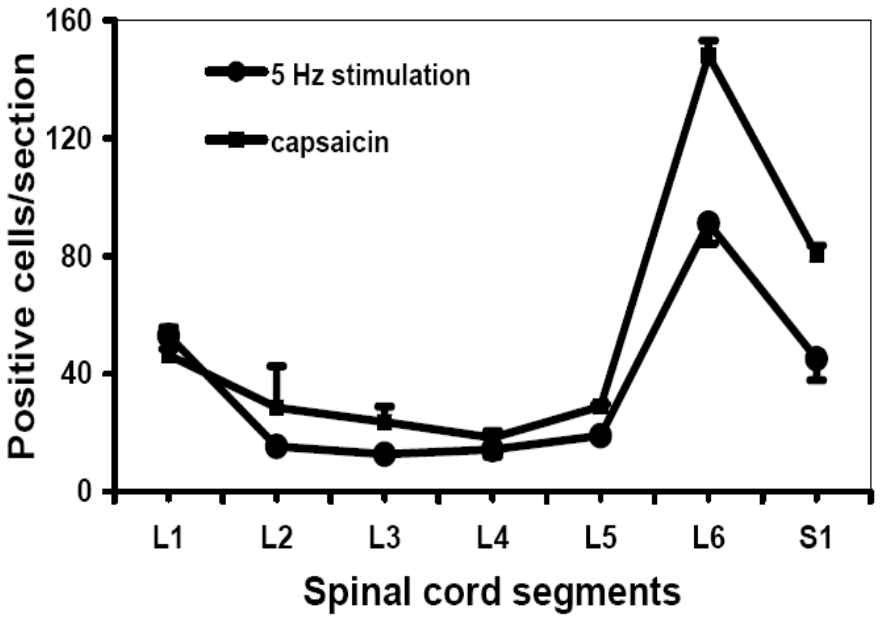


Figure. Fos-IR cells in L1 to S1 spinal cord sections after bladder stimulation. Following Neurometer®-SPT stimulation of the bladder mucosa at 5 Hz (2.0 mA, 90 min, n=6), or intravesical capsaicin treatment (1 mM, 30 min, n=4), Fos was detected by immunohistochemistry and the numbers of Fos-immunoreactivecells per section were counted.

Further validate and improve our BST device for use in mice. Transformation of the BST method to mice thus far has not been an easy task, mainly due to the size differences and challenges in implantation of the device in mice. Recently, in collaboration with Department of Biomedical Engineering, we have made a breakthrough and created a new electrode design for use in mice. We will report our design and results of this electrode in the near future.

4. Collaborations:

With Jax

We have continued to collaborate with JAX. Initially we established two colonies of our mouse model: one kept at JAX and the other in our laboratory, and as mentioned earlier, worked closely with JAX to settle the issue of the 550bp fragment.
In addition, we have responded periodically to JAX queries in regard to Uropathy phenotypes in other models.

With the MMPCs-

At our new Institution at Case Western Reserve University (CWRU), we have started active collaboration with CWRU- Mouse Metabolic Phenotypic Center (MMPC; PI-Henri Bruenengraber). In this collaboration, our expertise and laboratory resources in phenotyping of lower urinary tract and diabetic uropathy are used by customers of the CWRU-MMPC. In addition, we have participated in generation of new data and submission of a renewal of the CWRU-MMPC application in October 2010.
In addition, we have collaborated with the following AMDCC P&F investigators:

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. As results of this collaboration, we have generated remarkable data and are in the process of submission of a joint proposal for studies of bladder remodeling in DM.

2. **Aria Olumi, M.D**.- From Harvard School of Medicine and MGH- we have collaborated on characterization of a mouse model of type 2 DM

3. **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.

With other non-AMDCC PIs

We have active collaborations with the following PIs on topics related to 'diabetic uropathy':

1. **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. 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. This collaboration led to receiving the P20 Grant on "Studies of Urological Complications of Obesity and Diabetes" in September 2010 (PI: Daneshgari)
2. **Sanjay Gupta, Ph.D.-** is an NIH funded investigator on prostate diseases. We have established an active collaboration with Dr. Gupta to study the effects of diabetes on prostatic diseases. This collaboration led to receiving the P20 Grant on "Studies of Urological Complications of Obesity and Diabetes" in September 2010 (PI: Daneshgari)
3. **Mark Chance, Ph.D**.- during the last year, we have established an extensive collaboration with Dr. Chance, the director of Proteomic Core of Case Western Reserve University. These collaboration has resulted in receiving the P20 grant on "Studies of Urological Complications of Obesity and Diabetes".
4. **Margot Damaser, Ph.D**.- we have continued our collaboration with Dr. Damaser on studies of role of diabetes on urinary incontinence. This collaboration has led to 2 publications in 2010 (see list of publications below)

5. Address previous EAC comments:

- It is disappointing that this work remains focused primarily on rats and has not moved more quickly toward the mouse models proposed in the original application. That said, you have significant rat data. Efforts should be made to upload this data to the website.

RESPONSE- During the last year, we have been able to move quicker on our mouse model studies. We agree with the reviewer and wish that we could have moved faster. A number of reasons contributed to that slowness:

1- Mix up of in genotype of the colony raised at Jackson Laboratory. As reported in 2010, we established two colonies of our mouse model (MnSOD 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. Therefore, we had to analyze our data for expression and activity of the MnSOD from both colonies prior to proceeding with any investigations on this model

2- For our urinary diversion model- which was a part of our hypothesis related to early changes seen in diabetic bladder dysfunction (DBD), we had to use rats simply because of size difference and the fact that doing a complicated urinary diversion surgery on a mouse would have been more difficult if not possible

3- Thus far, all of our studies on bladder remodeling resulted from DBD indicate similarities between changes in rats and mice

- Overall good progress despite a delay in the breeding of the MnSOD model. However, this seems to be on track now and Dr. Daneshgari has begun testing the smooth muscle specific MnSOD KOs. We expect significant progress in the area over the next year.

RESPONSE- Thank you and Yes, we are making significant progress in the area. As described earlier, we have have completed a large set of experiments on phenotyping and other measurements of MnSOD KOs. In addition, we have completed a sizable set of experiments on monogenic and polygenic models of Type II DM. In addition to examination of the hypothesis related to our AMDCC work, these data have (for example the P20 award) and will led to submission of future proposal.

- Dr. Daneshgari continues to add to and improve his research tools with the development of the urinary diversion model and a new electrode design to simultaneously measure CMG and EMG.

RESPONSE- Thank you and Yes, we continue our search in completion of research tools for studies of 'diabetic uropathy'

- Dr. Daneshgari has extended his collaborative efforts to new P&F members. This has already led to one joint project with Dr. Birder.

RESPONSE- Thank you and Yes, we are currently collaborating with all three (Drs. Birder, Fraser and Olumi) funded AMDCC P& F investigators. We intend to submit several new proposals for new funding based on the results of our collaboration with these investigators.

- The nature of the "misup in genotyping" is unclear from the written report. In particular its practical effect is unclear but seems detrimental. The diversion model is interesting. The plans for its follow-up and use are not clear. The other plans seem reasonable. Productivity is difficult to judge with mixture of relevant and irrelevant publications.

RESPONSE- I hope our explanation to the first comment has clarified the issue of the mixup in gengotyping.
In regard to our future plans for the diversion model, we have: a) completed and published the feasibility and methods of diversion and its impact on the bladder (see list of publications); and b) have completed this model in diabetic animals, and are awaiting review of the manuscript. So, we have completed testing of our hypothesis in regard to the role of polyuria on bladder remodeling during the early stage of DBD. Completion of our studies on our MnSOD model will complete our studies on the second part of the hypothesis related to role of oxidative stress on DBD.
Some parts of these studies have led to our new collaboration and receiving of a P20 grant for studies of 'Urological Complications of Obesity and Diabetes" by the PI in 2010
We are submitting two new research proposal based on findings of these studies. In addition, we have published 5 new and original manuscript in 2010 related to DBD (see below).

- Productive lab. Plans for upcoming year should focus on the original peer-reviewed aims.

RESPONSE- Thank you and Yes, as indicated earlier, we expect to complete the studies related to MnSOD mouse by the end of this year.

Below is the updated list of **publications** from 2010-2011 periods:

1. Liu G, Li M, Vasanji A, Daneshgari F.- Temporal diabetes and diuresis-induced alteration of nerves and vasculature of the urinary bladder in the rat. BJU Int. 2010 Nov 18. Doi: 10.111/j1464-410X.2010.09840.x [Epub ahead of print]

In this study, we showed that Diabetes induced time-dependent changes in the density of the nerves and thatdiabetes-related polyuria and vasculature in the bladder tissueplay important roles in these changes.

2. Vricella GJ, Tao M, Altuntas CZ, Liu G, Kavran M, Daneshgari F, Hijaz AK. Expression of monocyte chemotactic protein 3 following simulated birth trauma in a murine model of obesity. Urology. 2010 Dec;76(6):1517.e12-7. Epub 2010 Oct 23.

In this study, we observed that MCP-3 is significantly over-expressed in the urethral tissues of both wild-type and obese mice immediately after any urethral manipulation.

3. Pan HQ, Lin DL, Strauch C, Butler RS, Monnier VM, Daneshgari F, Damaser MS. Pudendal nerve injury reduces urethral outlet resistance in diabetic rats. Am J Physiol Renal Physiol. 2010 Dec;299(6):F1443-50. Epub 2010 Sep 29.

In this study, we observed that diabetic rats with pudendal nerve crush (PNC) demonstrated increased muscle fiber disruption and atrophy of the external urethral

sphincter. AGEs were significantly elevated in diabetic rats. PNC relieves a functional outlet obstruction in diabetic rats. AGEs are elevated in diabetic rats and could play a role in urinary dysfunction and recovery from PNC.

4. Liu G, Lin YH, Li M, Xiao N, Daneshgari F. Temporal morphological and functional impact of complete urinary diversion on the bladder: a model of bladder disuse in rats. J Urol. 2010 Nov;184(5):2179-85. Epub 2010 Sep 17.

In this study, we reported that creation of a urinary diversion model by ureterovaginostomy in the rat is feasible. Urinary diversion causes distinct functional and morphometric bladder alterations.

5. 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.

In this article, we reviewed the appropriate animal models for the study of a number of disorders involving changes to lower urinary tract function

6. 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. 2010 Jan;298(1):F72-7.

In this study, we observed that histology of the external urethral sphincter in obese rats showed increased fibrosis, leading to disruption of the skeletal muscle structure compared with control. Additionally, the bladder wall of the obese+nondiabetic and obese+diabetic rats demonstrated edema and vasculopathy. Voiding dysfunction was evident in both obese groups but with no significant differences due to DMII, suggesting that voiding dysfunction in DMII may be attributable at least in part to chronic obesity.

Below are the previously published articles related to our work at AMDCC:

1. Temporal morphological and functional impact of complete urinary diversion on bladder: a model of bladder disuse in rats
Guiming Liu, Yi-Hao Lin, Mei Li, Nan Xiao, Firouz Daneshgari
The Journal of urology, 2010 (184)
2. Animal models and their use in understanding lower urinary tract dysfunction.
Fry CH, Daneshgari F, Thor K, Drake M, Eccles R, Kanai AJ, Birder LA
Neurourol Urodyn. 2010 Apr;29(4):603-8
3. Voiding function in obese and type 2 diabetic female rats.
Gasbarro G, Lin DL, Vurbic D, Quisno A, Kinley B, Daneshgari F, Damaser MS
American journal of physiology. Renal physiology, 2010 (298), F72 - F77
4. Animal models of diabetic uropathy.
Daneshgari F, Leiter EH, Liu G, Reeder J
The Journal of urology, 2009 (182), S8 - 13

5. Diabetic bladder dysfunction: current translational knowledge.
Daneshgari F, Liu G, Birder L, Hanna-Mitchell AT, Chacko S
The Journal of urology, 2009 (182), S18 - S26
6. Urological complications of obesity and diabetes.
Daneshgari F, Brown JS, Kusek JW, Nyberg LM
The Journal of urology, 2009 (182), S1
7. Bladder dysfunction in mice with experimental autoimmune encephalomyelitis.
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Respectfully submitted,

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