# Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Children's Hospital of Pittsburgh of the UPMC Health System

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 - 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** David H. Perlmutter, MD

4. **Grant Contact Person's Telephone Number:** 412-692-8071

5. **Grant SAP Number:** 4100047629

6. **Project Number and Title of Research Project:** 1 - A Genetic Model of Congenital Obstructive Nephropathy

7. **Start and End Date of Research Project:** 1/1/2009 – 9/15/2011

8. **Name of Principal Investigator for the Research Project:** Carlton M. Bates, MD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 692,235.63

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name | Position Title | % of Effort on Project | Cost |
|---|---|---|---|
| Bates, C | Principal Investigator | 11%Yr 1;21%Yr 2; 20% Yr3 | 111,886.35 |
| Giovanni, V | Post-Doc | 53% Yr3 | 18,454.20 |
| Cusack, B | Lab Mgr | 9% Yr 1; 50% Yr2 | 36,035.00 |
| Baust, J | Lab Mgr | 33% Yr 1 | 25,281.31 |

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name | Position Title | % of Effort on Project |
|---|---|---|
| Shaefer, C | Research Tech | 10 |

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

| Type of Scientific Equipment | Value Derived | Cost |
|---|---|---|
| Leica Stereomicroscope | This allowed us to dissect embryos and kidneys for use in both project 1 and project 2. | 30,503.75 |
| Leica DM 2500 Microscope | This allowed us to perform light and fluorescence microscopy for general stains and immunofluorescence in both projects 1 and 2. | 26,358.20 |
| MBF Bioscience Microscope | This scope was used during paraffin sectioning to determine if we were sectioning in the region of interest (embryonic urinary tract and adult kidneys, ureters and bladders)for both projects 1 and 2. | 107,066.00 |

**10. Co-funding of Research Project during Health Research Grant Award Period.** Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes____x_____ No_____

If yes, please indicate the source and amount of other funds:

NIH (NIDDK) R01 DK085242-01, "Model of Congenital Obstructive Nephropathy-Biomarker & Therapeutic Development." co-PI (Bates, Kirk McHugh): Yearly direct costs: $250,000. Indirect costs: $128,750. 03/01/10-02/28/15.

**11. Leveraging of Additional Funds**

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No___X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

| A. Title of research project on grant application | B. Funding agency (check those that apply) | C. Month and Year Submitted | D. Amount of funds requested: | E. Amount of funds to be awarded: |
|---|---|---|---|---|
| None | ☐NIH<br>☐ Other federal (specify:_____ _____)<br>☐ Nonfederal source (specify: _____) | | $ | $ |

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____x_____ No_____

If yes, please describe your plans:

At the end of the period covered by the current NIH grant, we plan to submit a competing renewal to continue the research project.

**12. Future of Research Project.** What are the future plans for this research project?

We have made significant progress on our understanding about how the bladder defects in the *mgb-/-* mouse lead to chronic kidney injury. We plan to continue these studies to elucidate possible therapeutic interventions to complement the surgical interventions in children with this physiology (which is only effective in about 50% of patients). In time, we envision that the data we acquire from the mouse model will lead to translational or clinical studies in children with obstructive nephropathy.

**13. New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X____ No_____

If yes, how many students? Please specify in the tables below:

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Male | | | | 1 |
| Female | | | 1 | |
| Unknown | | | | |
| **Total** | | | **1** | **1** |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Hispanic | | | | |
| Non-Hispanic | | | 1 | 1 |
| Unknown | | | | |
| **Total** | | | **1** | **1** |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| White | | | 1 | 1 |
| Black | | | | |
| Asian | | | | |
| Other | | | | |
| Unknown | | | | |
| **Total** | | | **1** | **1** |

**14. Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

Yes____ _____ No____x_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

**15. Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____x_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

Some of the techniques used in this project (e.g., the 3D reconstructive imaging of the developing and adult bladder) were techniques we began using in other projects focused on defects in the lower urinary tract.

**16. Collaboration, business and community involvement.**

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes____x_____ No_____

If yes, please describe the collaborations:

We worked on this project in collaboration with Dr. Kirk McHugh, Associate Professor of Pediatrics at Nationwide Children's Hospital/The Ohio State University College of Medicine. Dr. McHugh is an expert on smooth muscle biology, genetics, and development including the bladder. His expertise has complemented my expertise in kidney development and my clinical understanding of pediatric chronic kidney disease, including obstructive nephropathy. The design and implementation of the experiments were largely collaborative. This partnership has also led to other collaborative efforts on different projects within our respective labs.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____x_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____x_____

If yes, please describe involvement with community groups that resulted from the research project:

**17. Progress in Achieving Research Goals, Objectives and Aims.**

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

**There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.**

**Summary of Research Completed** (Divided by Specific Aims)

Specific Aim 1. Complete characterization of the development of *in utero* obstructive nephropathy in the *mgb* mouse including the identification of pathophysiological changes associated with kidney development.

3 Dimensional Reconstructions Reveal Significant Bladder Defects in *mgb-/-* Mice:
Using 3D reconstructive imaging (Microbrightfield, Vermont), we found that while total bladder volumes between mutant and controls were equivalent at embryonic day (E) 13.5, *mgb-/-* volumes were well below control volumes at later developmental stages. Next we compared developmental changes in volumes of the urothelium and detrusor smooth muscle in control and *mgb-/-* mice. From E13.5-E17.5, controls had a relatively constant volume of urothelium at ~5%, while detrusor muscle volumes increased from 32% to 67%. In contrast, mutant bladders had an increase in urothelial volume from 6% at E13.5 to 12% at E17.5, while detrusor volumes started at 6% and only increased to 18% (reflecting the poor smooth muscle differentiation in the *mgb-/-* mice). Finally, we compared the pattern of smooth muscle differentiation in control and *mgb-/-* mice. In controls, smooth muscle differentiation started at E13.5 at the upper bladder dome with a projection extending down the right posterior surface; by E14.5, controls had smooth muscle encompassing the upper half of the bladder; by E15.5 the entire bladder was encased in smooth muscle that increased in thickness with advancing age. In *mgb-/-* mice, bladder muscle development initiated at E13.5 in a manner similar to controls with a projection of differentiation along the right posterior surface; however, by E15.5, the *mgb-/-* muscle remained highly fenestrated and did not fully extend to caudal regions as it had in controls. Due to massive distension of the *mgb-/-* bladders starting at E16.5 (when urine was present), it was technically not feasible to interrogate detrusor muscle development in *mgb-/-* bladders.

The *mgb-/-* Mutation Has No Direct Genetic Effect on Kidney Development
At embryonic day (E) 15.5, when the abnormal bladder phenotype first becomes evident (loss of smooth muscle actin staining and poor myoblast formation), all other organ systems including the kidneys appear normal by hematoxylin and eosin staining (Figure 1A, B). Furthermore, we bred *mgb-/-* mice with heterozygous Hoxb7GFP transgenic mice (*Hoxb7Gfp*$^{Tg/+}$) that express green fluorescent protein (GFP) throughout all of the ureteric bud derived structures including the renal collecting ducts, renal pelvis, and ureters. To date, seven mice that were *mgb-/-Hoxb7GFP*$^{Tg/+}$ compound mutants had normal appearing GFP-positive ureteric bud trees at E15.5 (Figure 1C, D). Furthermore, the gene responsible for the *mgb-/-* phenotype has no known role in kidney development or function (not shown). These collective observations indicate that the mutation associated with the development of megabladder in *mgb-/-* mice has no direct genetic effect on kidney development. Therefore, any renal abnormalities seen at later stages of development or post natally are associated with the pathogenic progression of obstructive nephropathy in *mgb-/-* mice.

*mgb-/-* Kidneys Have Increased Cell Proliferation, α-Smooth Muscle Actin Staining (Myofibroblasts), and Fibrosis
We observed increases in proliferative cell nuclear antigen (PCNA) staining in *mgb-/-* kidneys, indicating a high number of proliferating cells in adult *mgb-/-* kidneys (Figure 2). Many proliferating nuclei are in the interstitial space consistent with expansion of myofibroblasts.

To determine whether there were activated myofibroblasts, we stained sections for a-smooth muscle isoactin (ASMA). Although we saw no differences at E15.5 or E18.5 (not shown), we detected abnormal staining in *mgb-/-* mice starting in newborns and progressing with age

(Figure 3). This correlated with our previous observation that hydronephrosis first occurs in newborn *mgb-/-* mice. In newborn control mice, ASMA is confined mostly to the renal medulla and papilla (in vascular muscle), but mutants have significant staining well into the cortical interstitium (Figure 3A). In adults, excessive ASMA staining in *mgb-/-* mice becomes more intense, throughout the kidney compared to controls (Figure 3C,D). Thus, there is myofibroblast transformation in *mgb-/-* mouse kidneys, starting in newborns and worsening with age.

We also noted that the ASMA staining pattern in *mgb-/-* mutants varied with the degree of hydronephrosis. In *mgb-/-* mice with more mild hydronephrosis, the most significant staining is in the sub-epithelial region adjacent to the renal pelvis (Figure 4A). In *mgb-/-* mice with more severe hydronephrosis, ASMA signal is very pronounced in sub-epithelial and sub-capsular areas, and diffuse signal is present in the parenchyma in between (Figure 4B). Closer views of severely affected kidneys reveal not only interstitial staining (suggestive of myofibroblast transformation) but also ASMA positive tubular cells (suggesting epithileal-mesenchymal transformation- EMT)(Figure 4C). Many adult *mgb-/-* mice also had disrupted E-cadherin immunostaining, consistent with early EMT changes (not shown). Thus it appears that injury in the *mgb-/-* mice occurs first in pelvic sub-epithelial areas, but then spreads throughout the parenchyma to subcapsular regions as well.

Fibrosis (by trichrome staining) in *mgb-/-* mice lags slightly behind the appearance of myofibroblasts, in that it is not obvious until a few weeks after birth. In adult *mgb-/-* mice, however, the pattern of fibrosis follows ASMA staining, i.e., it varies with the degree of hydronephrosis (Figure 5). In mice with mild hydronephrosis, it is most prominent in pelvic sub-epithelial areas. With worsening hydronephrosis, sub-epithelial scarring increases and fibrosis is more evident throughout the parenchyma up to sub-capsular areas. We also quantitated the changes in fibrosis in control and *mgb-/-* kidneys by Trichrome staining sampled over many regions of the kidneys. In controls, mean percentage of fibrosis in the outer cortex and inner medulla were 4.5% and 4.2% respectively. In comparison, mildly affected mutants (stratified by hydronephrosis) had 10.1% and 15.3% mean percentages of fibrosis in the outer cortex and inner medulla, respectively. Severely affected mutants had 45.2% and 85.5% mean percentages of fibrosis in the cortex and medulla, respectively. Taken with the ASMA data, it appears that the initial injury is in sub-epithelial areas, and then progressively worsens throughout the kidney.

*mgb-/-* Kidneys Have Increased CTGF Staining

Given that connective tissue growth factor (CTGF) is often implicated in scarring, we performed immunostaining in control and *mgb-/-* mice (Figure 6). Some newborn *mgb-/-* mice had evidence of significant CTGF expression compared with controls. By adulthood, CTGF expression was uniformly increased in *mgb-/-* over controls, with the most striking increases in those with severe hydronephrosis. CTGF is a well-characterized downstream target of TGF-β signaling.

*mgb-/-* Kidneys Have No Apparent Significant Interstitial Inflammation or Excessive Tubular Apoptosis:

Unlike the four-stage model of renal injury with surgical obstruction such as unilateral ureteral obstruction, *mgb-/-* kidneys have little or no interstitial inflammation by H&E or PAS staining and no excessive tubular apoptosis by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays (not shown). However, *mgb-/-* kidneys have clear signs of tubular EMT, myofibroblast proliferation, and fibrosis shortly after birth that worsens with age. Progression of fibrosis in *mgb-/-* kidneys from pelvis to subcapsular cortex and interstitium appears distinct from other surgical models of obstruction. We suspect that gradual obstruction in *mgb-/-* mice accounts for the unique renal injury pattern.

Many Histological Markers are Altered in Adult *mgb-/-* Mice:

F4/80 immunostaining (specific for macrophages) was almost undetectable in adult control kidneys (not shown). In contrast, F4/80 expression in presumptive macrophages progressively increased in moderate to severe adult kidneys, with positively staining cells observed throughout the interstitium and glomeruli (not shown). Thus, there is an inflammatory infiltrate of macrophages within adult *mgb-/-* mice.

Wt-1 immunostaining was also altered in injured *mgb-/-* mice (not shown). Adult control kidneys showed sparse Wt-1 positive cells within their glomeruli, while the glomeruli of severely injured *mgb-/-* kidneys showed a statistically significant increase in Wt-1 positive cells with a distribution similar to that observed in newborn control glomeruli. Thus, Wt-1 may be a biomarker of progressive kidney injury in *mgb-/-* mice.

Pax2 immunostaining was also altered in more severely injured *mgb-/-* mice. In adult control kidneys, Pax2 staining was reduced when compared to newborn control kidneys. Newborn control kidneys showed intense nuclear and diffuse cytoplasmic staining throughout the nephrogenic zone, in a subset of cortical and medullary ducts, and in the urothelium lining the renal pelvis (Figure 7A-D). Pax2 staining showed a mixed response in severe adult kidneys with some animals displaying high levels of Pax2 staining throughout the renal parenchyma, and the urothelium of the renal pelvis similar to that observed in newborn kidneys (Figure 7E). Adult control kidneys showed limited Pax2 staining in the cells lining Bowman's capsule (Figure 7F), while severely injured *mgb-/-* adult kidneys showed a statistically significant increase in the number of Pax2-positive cells lining Bowman's capsule to a level similar to that observed in newborn controls (Figure 7G, H).

Disease Stratification:

We next stratified renal injury in *mgb-/-* mice using high resolution renal ultrasound (RUS). As shown in Figure 8, we stratified increases in mutant renal pelvic anterior-posterior diameter (APD) into mild, moderate, and severe categories of hydronephrosis in mice at 14, 18, and 35 days of age. While we did not observe evidence of hydronephrosis in utero at embryonic day 15.5 (E15.5), we did detect hydronephrosis in mutants by RUS at E17.5. As shown in Table 1, 3D reconstructions from RUS images in E17.5 mutants show significant increases in renal pelvic volume (normalized to total kidney volume) in both right and left kidneys. Newborn mutant mice also uniformly showed mild to moderate hydronephrosis on renal ultrasonography. At the end of the renal ultrasonography, we euthanized animals and

confirmed that the apparent hydronephrosis seen on RUS correlated with real hydronephrosis by direct observation.

Urine Output and Serum Chemistry in *mgb-/-* Mice Is Altered

In general, we observed only modest differences in mean water intake and mean urine output between *mgb* genotypes (not shown). Interestingly, none of age-matched male *mgb-/-* weanlings examined produced measurable urine in metabolic cages, and only one male *mgb-/-* weanling survived the 24-hour testing period. To characterize potential urine output, we percutaneously drained male *mgb-/-* bladders removing an average of 10-15 mls of urine. Male *mgb-/-* bladders refilled with urine within 1-2 hours suggesting the presence of severe concentrating defects. To confirm that the male urethra is patent, we performed percutaneous radiocontrast cystograms on live mice. The cystograms showed grossly enlarged, trilobular bladders in *mgb-/-* mice that indeed extruded contrast reagent through the urethra with sufficient pressure.

To determine degree of kidney injury, we performed blood chemistry profiles on 30 *mgb-/-*, 17 *mgb+/-* and 12 wildtype female mice, and 30 *mgb-/-*, 23 *mgb+/-* and 16 wildtype male mice (Figure 9). We assayed BUN, creatinine, phosphorus, calcium, sodium, potassium, chloride, bicarbonate, and albumin. We compared individual test results to the median of the corresponding gender wildtypes, and used the magnitude of the log2 ratio to determine the degree of abnormality. We used an exact upper-tailed Wilcoxon rank-sum test to determine whether the abnormality measures were significantly elevated relative to the same gender wildtypes. Individual values are considered significant with an NLP value > 2.3 or a P-value of 0.005. We observed no statistically significant alterations in serum chemistries between any female mice. In contrast, we detected significant increases in BUN, creatinine, calcium, and potassium in male *mgb-/-* mice compared to controls.

Since the temporal course of kidney injury in *mgb-/-* mice is variable, individual serum chemistry values often vary between mutants. Even so, we were able to identify a subset of eight male and four female *mgb-/-* mice that displayed bicarbonate levels <5.0 mmol/L and showed dramatic increases in BUN and creatinine levels (Table 2). These animals were noted as terminally sick with eight of the twelve showing severe hydronephrosis (four mice were not recorded). Thus, the combined assessment of BUN + creatinine + bicarbonate provides a prognostic marker set that unambiguously identifies mice in the terminal stages of renal failure, which will be important as we track the responses to therapy and validate other potential biomarkers.

Additional Histological Changes Noted in Aged *mgb-/-* Mice:

We noted that many aged *mgb-/-* adult kidneys developed additional signs of chronic injury and/or dysplasia (Figure 10). Among these included simple cysts and multiple cysts within the tubules. Many mutant kidneys also contained proteinaceous casts indicative of severe kidney injury. Trichrome staining revealed that many kidneys had sclerotic glomeruli. Regions of other *mgb-/-* kidneys showed evidence of coagulation necrosis. These findings are important given that these findings are often seen in patients with congenital obstructive nephropathy as in the case of patients with posterior urethral valves.

In addition, while interstitial infiltration of macrophages in many of the younger adult mutant mice was limited, in mutants that were aged longer and more severely affected, we detected different patterns of inflammation (Figure 11). As shown, more severely affected mutants had a more significant chronic inflammatory response, often adjacent atrophic and/or dilated/cystic tubules (Figure 11A, B). Some of the mutants also developed micro-abscesses within the cortex (Figure 11C). Many of these cells appeared to be plasma cells, including Russell bodies (plasma cells with large eosinophilic immunoglobulin inclusions) (Figure 11D). These findings could be secondary to infection as is often the case in patients with chronic obstructive nephropathy

Specific Aim 2. Identify and characterize the role that TGF-β signaling plays in mediating renal injury in the *mgb-/-* mouse model of *in utero* obstructive nephropathy.

Given the evidence that CTGF (a direct target of TGF-β) is overexpressed in *mgb-/-* kidneys, we have begun examining the potential of TGF-β as a biomarker by first examining tissue expression levels by Enzyme-Linked ImmunoSorbent Assay (ELISA). Adult male *mgb-/-* mice (N=11) clearly have increased mean TGF-β1 levels compared with controls (N=8) (1.90pg/mg ± 1.14pg/mg versus 0.80pg/mg ± 0.63pg/mg; $p < 0.05$). As seen in Figure 12, there is some variability between the *mgb-/-* samples, which could represent differences in kidney injury. Moreover, some of the adult *mgb-/-* kidneys with the highest TGF-β levels had gross evidence of hydronephrosis. While at post natal day 10, there were no statistical differences between mean mutant (N=5) and control (N=5) TGF-β1 levels (1.15pg/mg ± 0.38pg/mg versus 0.92pg/mg ± 0.25pg/mg; $p = 0.11$), 3 out of 9 *mgb-/-* kidneys had TGF-β1 levels in excess of 1.5 pg/mg whereas controls were all < 1.25 pg/mg. Thus, TGF-β1 is clearly elevated in adult *mgb-/-* mice as a group and is markedly elevated in a subset of P10 *mgb-/-* mice making it an attractive biomarker candidate to gauge severity of kidney disease.

To complement the ELISA data, we performed immunostaining for TGF-β1. Moderately injured *mgb-/-* kidneys showed more intense TGF-β1 expression by immunostaining within cortical ducts, cortical and medullary interstitial cells, and glomeruli compared to controls (Figure 13A-F). Severely injured kidneys showed expanded TGF-β1 staining in glomeruli, interstitial cells, the capsular epithelium, urothelium of the renal pelvis, and cortical ducts where an apical pattern of expression was observed (Figure 13G-I). TGF-β1 staining in severe kidneys was also observed surrounding regions of necrosis and within areas containing inflammatory infiltrates (data not shown).

In addition to TGF-β1, we assayed for TGF-β3 by ELISA in the adult male mice noted above. As with TGF-β1, adult male *mgb-/-* mice have markedly elevated mean TGF-β3 levels compared to controls (2.01pg/mg ± 1.13pg/mg versus 0.81pg/mg ± 0.40pg/mg; $p < 0.005$). Overall, TGF-β3 expression levels in individual mice mimicked TGF-β1 levels. In fact, some *mgb-/-* had 4 fold or greater levels than the control mean, possibly acting as a more sensitive biomarker to kidney injury.

To determine whether TGF-β1 would be a potential biomarker from a renewal source of material, we collected urine samples over 24 hours from adult *mgb-/-* and control mice and

performed ELISAs (Figure 14). Since *mgb-/-* mice generate significant amounts of urine we assayed individual samples, whereas for controls, we pooled urine collected from 5 animals. Since we were only able to run the control assay once, we were unable to perform statistical comparisons; however, there were 4 *mgb-/-* mice out of the 10 sampled with significantly higher urinary TGF-β1 levels than the pooled control mean levels. In fact the mouse with the highest TGF-β1 levels had severe hydronephrosis (Figure 14, **). Taken together, TGF-β family members are attractive candidate biomarkers for differentiating degrees of kidney injury in *mgb-/-* mice.

<u>Specific Aim 3.</u> Develop and assess therapeutic strategies designed to ameliorate renal injury in the *mgb-/-* mouse model of *in utero* obstructive nephropathy**.**

Given that the only therapy for patients with obstructive nephropathy is surgical relief of obstruction, we performed cutaneous cystotomies (percutaneous vesicostomies) in male *mgb-/-* mice to relieve the obstruction. Of the 19 male *mgb-/-* mice having undergone the procedure, only four died of surgical complications (dehiscence, bladder prolapse, uroperitoneum) and one of a medical complication (Table 3). Thus, we have established that vesicostomy can be performed in mice with few experiencing complications.

Among the 14 mice with no peri-operative complications, 6 (43%) lived several weeks to months after surgery (see Table 3), whereas *mgb-/-* males without surgery die by 5-6 weeks of age. Examination of each animal revealed a patent stoma, well-healed abdominal wall, and no evidence of organ prolapse. Thus, the vesicostomy alone rescues a significant subset of *mgb-/-* mice, confirming that they were dying of renal failure. Some long-term survivors also bred well, suggesting significant renal recovery.

The other eight *mgb-/-* male (57%) mice died within 1-2 weeks of surgery, within the time frame that untreated male *mgb-/-* mice die (Table 3). None of these mice had evidence of surgical complications, infection, bleeding, etc. In two cases, we gave mice intraperitoneal saline to try to prevent dehydration. Based on these collective data, we strongly suspect that these mice died of renal failure despite vesicostomy.

Thus approximately 43% (6/14) of the male *mgb-/-* survive as a result of the surgery (excluding those with surgical and medical complications), whereas 57% (8/14) die as they would have without vesicostomy. This strongly mimics what happens in children with obstructive nephropathy, i.e., 30-70% develop end stage renal failure despite surgical intervention (and would die without renal replacement therapy). This model therefore offers the unique opportunity to test adjunctive pharmacologic therapies to augment surgical intervention.

**Tables and Figures:**

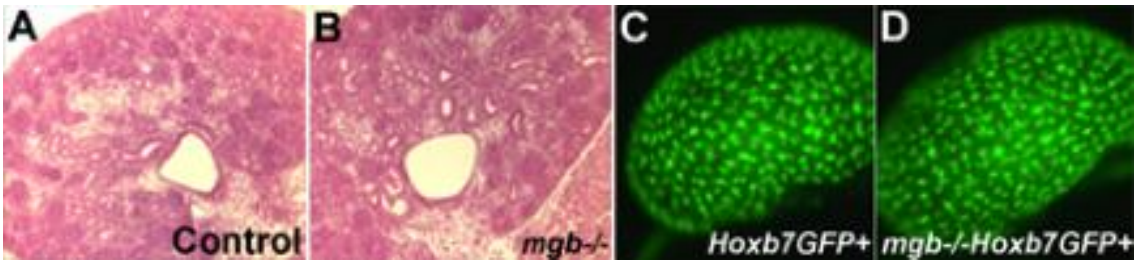



**Figure 1. Kidneys appear normal when the *mgb-/-* bladder phenotype first becomes evident at E15.5.** Representative Mason's trichrome stains of kidney in E15.5 control (A), and *mgb-/-* (B) mice show normal morphogenesis (50X). Green fluorescent protein (GFP) expression in E15 control *Hoxb7GFP*$^{Tg/+}$ (C) and *mgb-/-Hoxb7GFP*$^{Tg/+}$ (D) mice show that the all ureteric bud-derived structures appear normal.

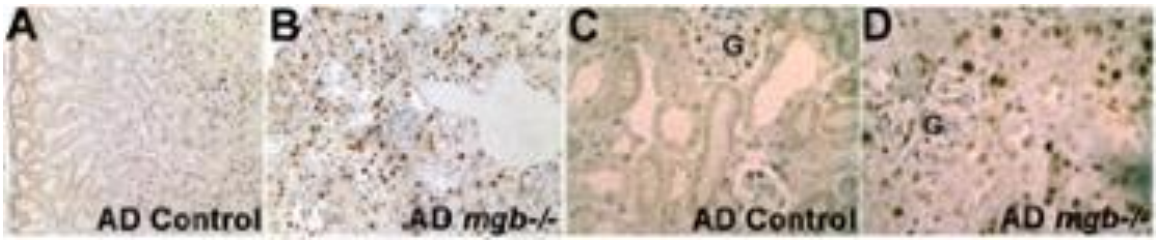



**Figure 2. *mgb-/-* kidneys have more cell proliferation**. PCNA staining of adult control (A, C) & *mgb-/-* (B, D) kidneys. Dark-brown nuclei=proliferating cells. G=glomerulus. 40x mag.

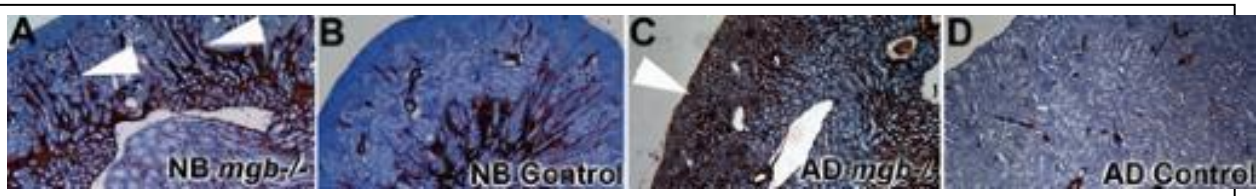
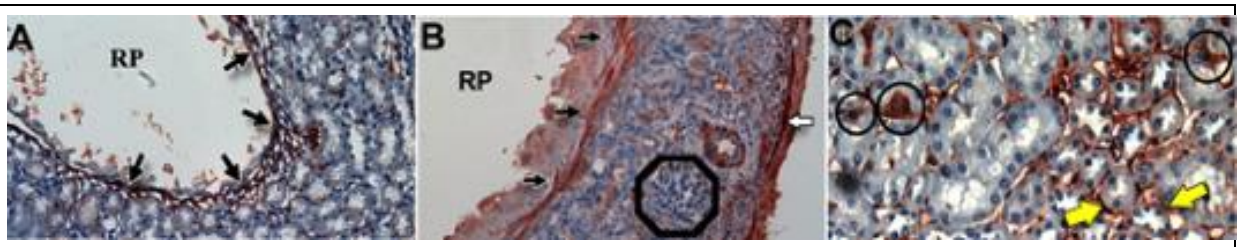
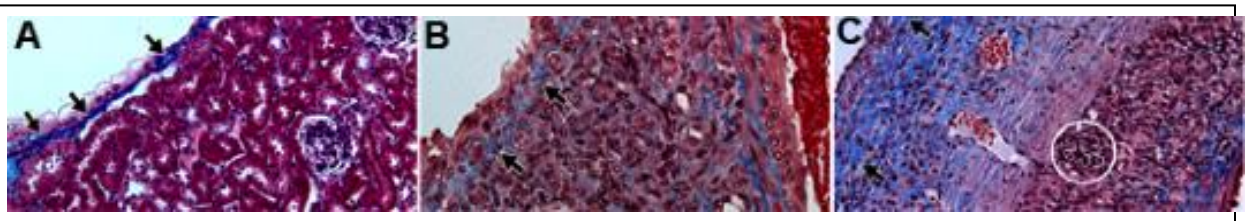


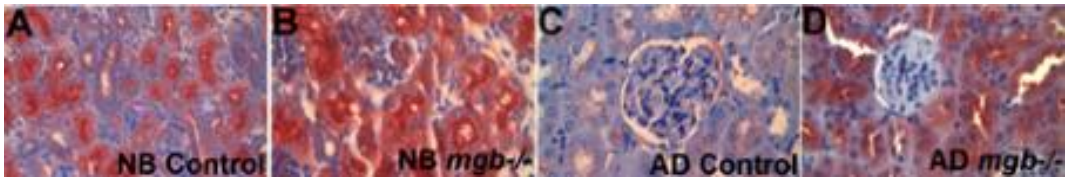

**Figure 3: *mgb-/-* kidneys have excessive ASMA staining consistent with activated myofibroblasts.** Sections of ASMA immunostaining in newborn *mgb-/-* (A) and control (B) and adult *mgb-/-* (C) and control (D). Note excessive stain in *mgb-/-* (arrowheads). All 40x.




**<u>Figure 4: *mgb-/-* mice have changes in ASMA staining with worsening hydronephrosis</u>**
ASMA staining in *mgb-/-* mouse with mild hydronephrosis (A) shows signal (arrows) near the renal pelvis (RP). Mice with more severe hydronephrosis (B,C) have intense signal near renal pelvis (black arrows) and capsule (white arrow). High power view of severely affected kidney shows significant interstitial (yellow arrows) and tubular signal (circles) suggestive of EMT. Hexagon = glomerulus. A,B = 40x, C = 100x mag.




**<u>Figure 5: *mgb-/-* mice have worsening fibrosis with increased hydronephrosis.</u>**
Trichrome staining in mice with mild (A), moderate (B), and severe (C) hydronephrosis. Sub-epithelial scarring (blue) near the pelvis (arrows) is progressively worse with increased hydronephrosis. Scarring becomes more evident in the parenchyma and subcapsular area with worsened hydronephrosis. Hexagon = glomerulus. All 40x mag.




**<u>Figure 6: *mgb-/-* mice have increased CTGF staining.</u>** Brown CTGF immunostaining in newborn control (A) & *mgb-/-* (B) and adult control (C) & *mgb-/-* (D) mice. All = 40x mag.

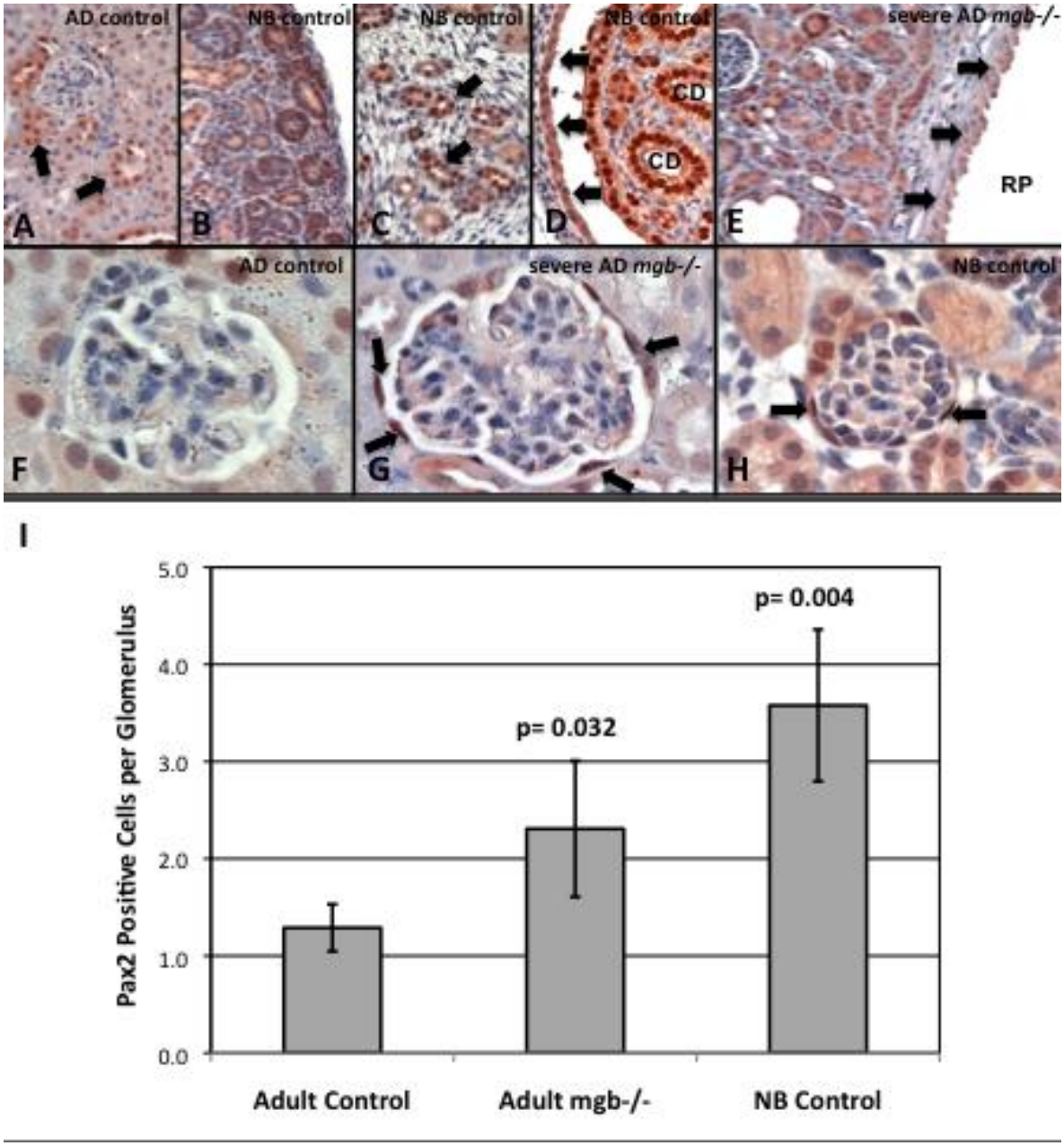



**Figure 7: Representative Pax2 expression in newborn and adult kidneys**. (A) Adult control kidney shows minimal Pax2 staining in cortical ducts (arrows), 20X. NB control kidneys show Pax2 staining in immature nephrons (B), cortical ducts (C), collecting ducts of renal papillae (D, CD), and urothelium of the renal pelvis (D, arrows), 40X. (E) Severe adult *mgb-/-* kidneys show expanded Pax2 staining in compressed parenchyma and urothelium of renal pelvis (RP, arrows) reminiscent of newborn control kidneys, 20X. (F) Severe adult *mgb-/-* kidneys show Pax2 positive cells within Bowman's capsule similar to the pattern observed in NB control (G) versus adult control (H). 100X. Individual P-values are compared to adult control.

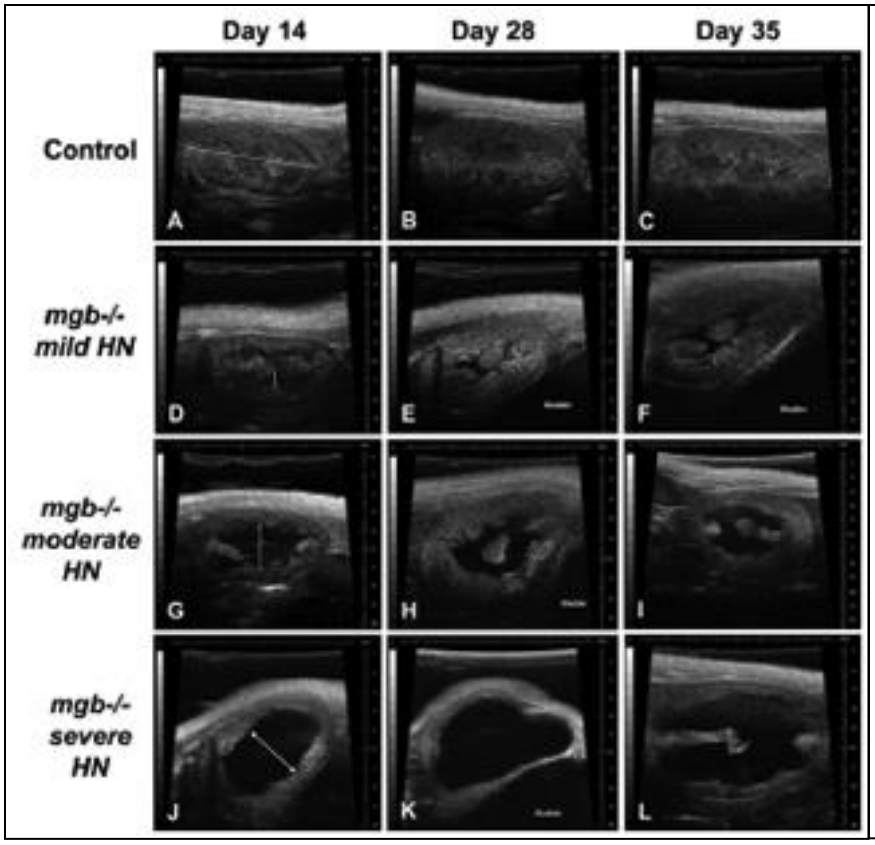



**Figure 8: Renal ultrasonography showing differences in *mgb-/-* mutant hydronephrosis (HN) at 14, 28, and 35 days of age.** A-C. Controls with normal appearing kidneys including no hydronephrosis. D-F. *mgb-/-* mice with mild hydronephrosis. G-I. mutant mice with moderate hydro-nephrosis. J-L. mutant mice with severe hydro-nephrosis. Please note that the double-headed arrows in D, G, and J indicate the pelvic APD.

Table 1: Total kidney volume vs renal pelvic volume by 3D reconstruction in E17.5 kidneys

| | *mgb-/-* right | *mgb-/-* left | control right | control left |
|---|---|---|---|---|
| kidney volume ($\mu m^3$) | $2.10 \times 10^9$ | $1.85 \times 10^9$ | $1.34 \times 10^9$ | $1.29 \times 10^9$ |
| pelvic volume ($\mu m^3$) | $8.67 \times 10^7$ | $6.27 \times 10^7$ | $1.32 \times 10^7$ | $1.31 \times 10^7$ |
| pelvic/ kidney volume (normalized to 1 in controls) | 4.1* | 3.4* | 1.0 | 1.0 |

* $p = 0.018$

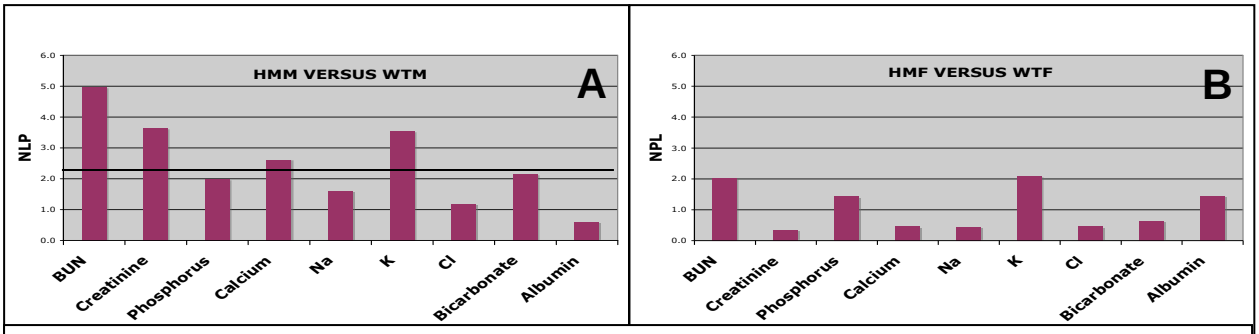
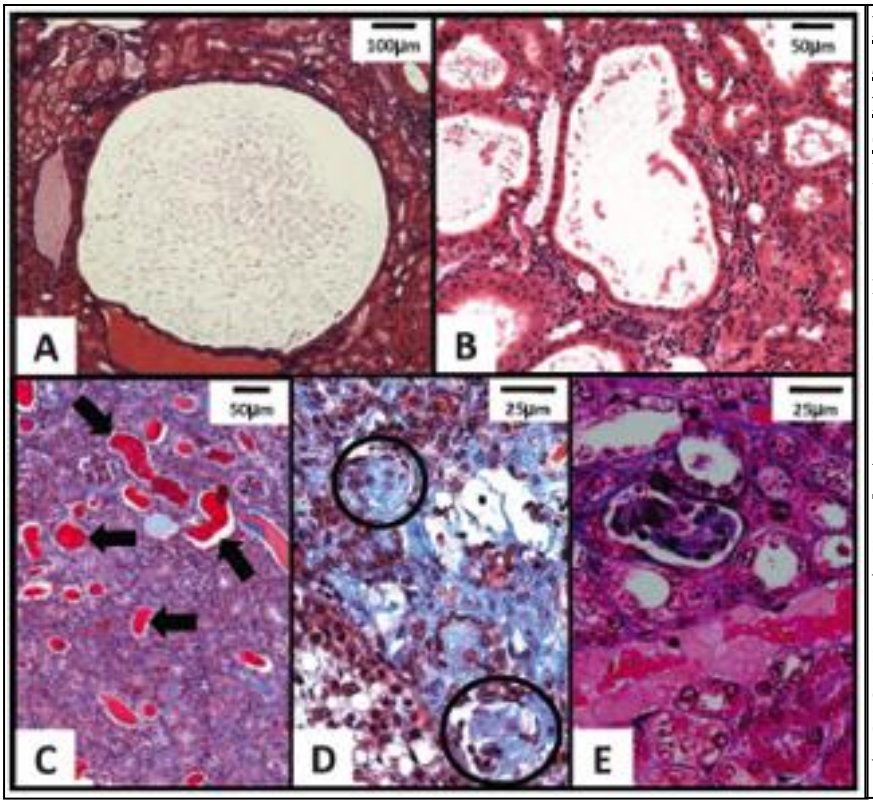



**Figure 9. *mgb-/-* mice show altered serum chemistries consistent with chronic renal failure.** Statistical analysis of serum chemistry values for *mgb-/-* male (HMM; A) and *mgb-/-* female (HMF; B) versus wildtype control male (WTM) and female (WTF) mice. Alterations are considered statistically significant with a negative Log10 P-value (NLP) value > 2.3, which is a p value <0.005 (A, bold line).

Table 2. Blood chemistry and kidney morphology in male and female *mgb-/-* mice

| Genotype[a] | BUN | Creatinine | Phosphorus | Calcium | Na | K | Cl | Bicarbonate | Albumin |
|---|---|---|---|---|---|---|---|---|---|
| 0-2 male | **153** | **2.7** | **32.7** | **10.2** | 129 | **18.3** | 87 | *<5.0* | *0.21* |
| 0-2 male | **40** | 0.4 | **12.0** | 9.2 | 132 | 5.6 | 84 | *<5.0* | 2.0 |
| 0-2 male | *20* | -- | **13.2** | **9.6** | **136** | **6.0** | **88** | *<5.0* | **3.2** |
| 3-4 male | **740** | **11.0** | **94.1** | 9.3 | *87* | **80.3** | 83 | *<5.0* | *0.8* |
| 3-4 male | **99** | 0.3 | **15.0** | 10.5 | **159** | **8.1** | **108** | *<5.0* | **3.3** |
| 3-4 male | **27** | 0.3 | **13.2** | -- | **150** | -- | 90 | *<5.0* | **3.3** |
| 3-4 male | **84** | **0.6** | **13.8** | 9.6 | 144 | **9.6** | **108** | *<5.0* | -- |
| 5+ male | **84** | **0.6** | **13.8** | **9.6** | 144 | **9.6** | **108** | *<5.0* | **4.2** |
| 0-2 female | **220** | 0.3 | 16.0 | 10.8 | **152** | **6.8** | 100 | *<5.0* | 2.8 |
| 5+ female | **366** | **3.2** | **30.2** | **12.2** | 142 | **8.8** | *92* | 7.2 | **3.6** |
| 5+ female | **164** | **1.4** | **11.8** | -- | **154** | -- | 106 | *<5.0* | 2.8 |
| 5+ female | **218** | **1.8** | **23.6** | **10.9** | 141 | **37.8** | **114** | 10.0 | -- |

[a] 0-2 week old (0-2), 3-4 week old, and 5+ week old (5+) *mgb-/-* mice. Chemistry values are statistically increased (bold), or decreased (italic) from normal (plain). --error recorded. BUN/ Creatinine/Phosphorus/Calcium=mg/dl; Na/K/Cl=mEq/L; Bicarbonate=mmol/L; Albumin=g/dl




**<u>Figure 10: Aged adult *mgb-/-* kidneys have features of dysplasia and chronic kidney disease</u>.** A, B. H&E staining shows severely affected kidneys (stratified by hydro-nephrosis) with simple cysts (A) and multiple cysts (B). C. Trichrome staining in a severely affected kidney shows many proteinaceous tubular casts (arrows). D. Trichrome stain shows a severely affected kidney with globally sclerotic glomeruli (circles). E. H&E stain shows a moderately affected kidney with coagulation necrosis and vacuolization of tubules

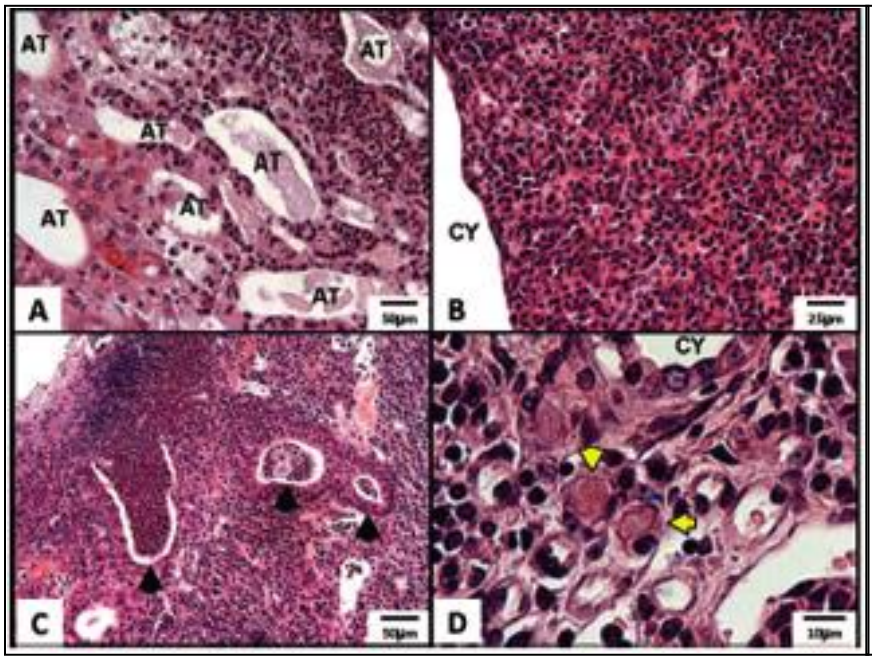



**Figure 11: Aged adult *mgb-/-* kidneys that are severely affected have significant inflammation.** A, B. Sheets of inflammatory infiltrates surround dilated atrophic tubules (A) and cysts (B). C. Many mutant kidneys have microabscesses. D. Some mutant kidneys have plasma cells including Russell bodies (yellow arrows).

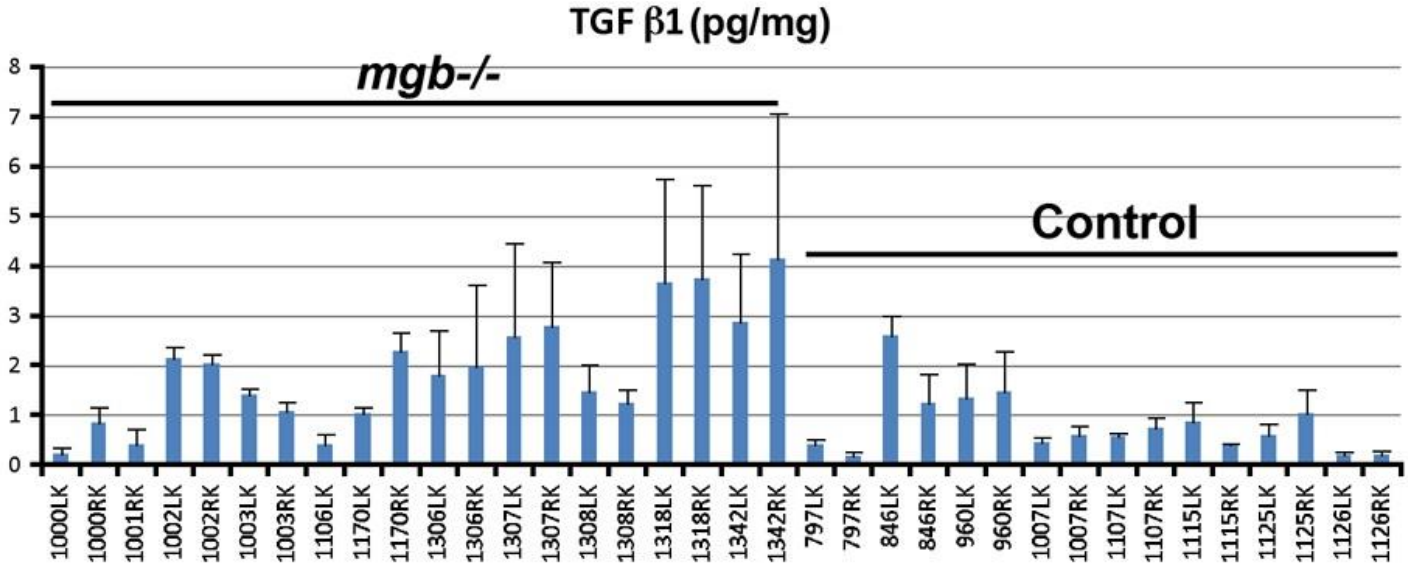



**Figure 12 Adult mgb-/- mice have increased TGF-β1 levels**
Compared with controls, adult (3 week or older) *mgb-/-* mice have increased TGF-β expression by ELISA (N=3 assays run per tissue). Some *mgb-/-* kidneys have dramatically higher levels.

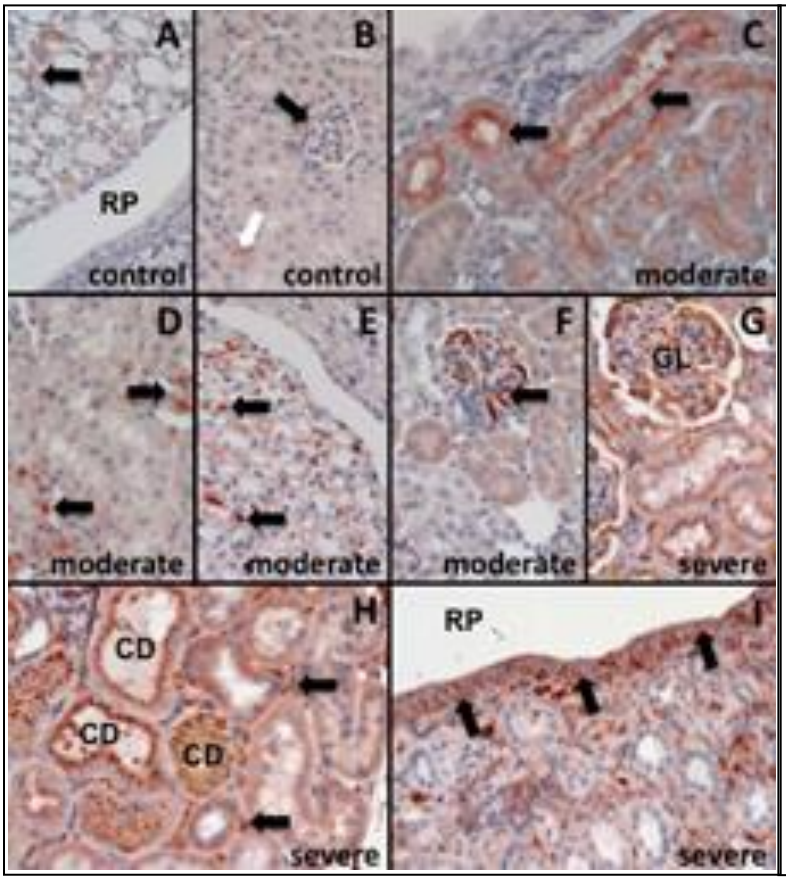
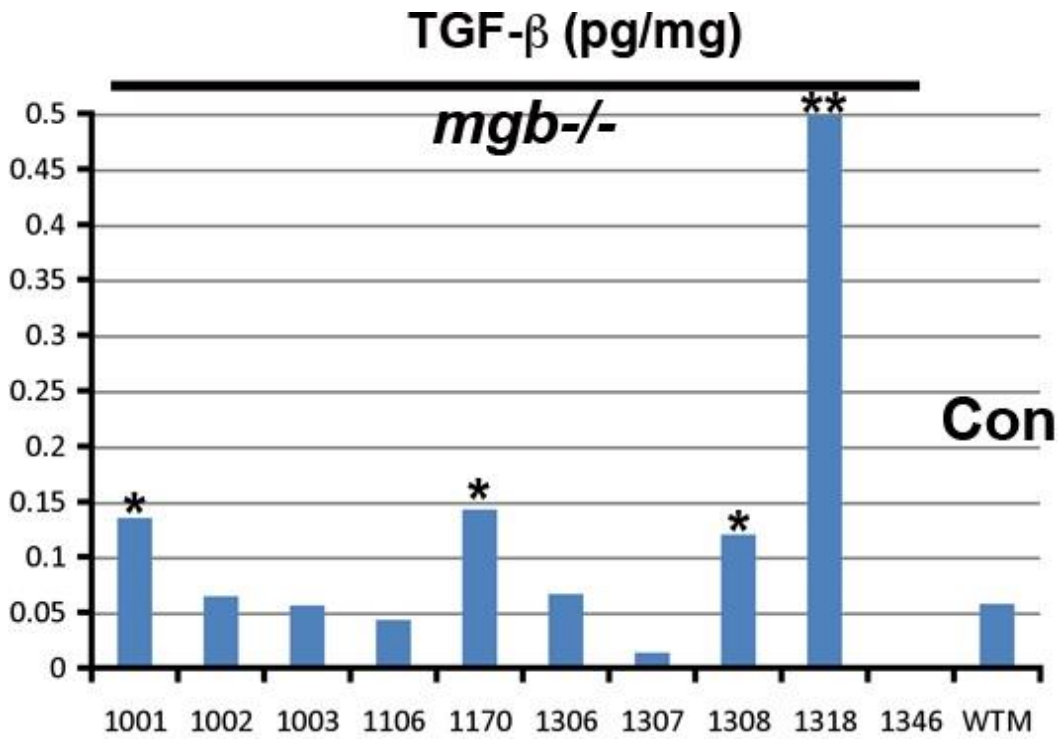



**Figure 13: Representative TGFβ1 expression in adult kidneys**. Control kidneys show TGFβ1 staining in the collecting ducts (A, arrow), interstitial cells (B, white arrow) and glomeruli (B, black arrow). Moderate mgb-/- kidneys show expanded TGFβ1 staining within cortical ducts (C, arrows), cortical and medullary interstitial cells (D,E, arrows), and glomeruli (F, arrow). Severe mgb-/- kidneys show expanded TGFβ1 staining in glomerular cells (G, GL), cortical ducts (H, CD), interstitial cells (H, arrows), and urothelium of the renal pelvis (I, arrows; RP), 40X.




**<u>Figure 14: A subset of *mgb-/-* mice have markedly elevated urinary TGF-β1 levels</u>** Four *mgb-/-* mice have TGF-β1 levels that are more than 2-fold greater than control mean levels (asterisks). The mouse with the highest urinary levels, over 10-fold the control level (******), also has severe hydronephrosis.

Table 3: Status of *mgb-/-* mice after cutaneous vesicostomy

| Animal | Age At Surgery (Days) | Status/Cause of Death |
|---|---|---|
| 1869 | 32 | lived several months and bred well |
| 1923 | 39 | lived several months and bred well |
| 2021 | 42 | lived for about 3 months |
| 2309 | 31 | lived for about 3 months (did not breed well) |
| 2264 | 28 | sacrificed at 14 weeks of age but looked well |
| 2409 | 27 | alive, stoma patent, thriving at 15 weeks of age |
| 1986 | 19 | apparent renal failure |
| 1987 | 19 | apparent renal failure |
| 1988 | 16 | apparent renal failure |
| 1989 | 16 | apparent renal failure |
| 1999 | 26 | apparent renal failure |
| 2298 | 59 | apparent renal failure |
| 2299 | 60 | apparent renal failure |
| 2419 | 30 | apparent renal failure |
| 1933 | 63 | medical demise, morphine overdose |
| 1998 | 33 | surgical complication, wound dehiscence |
| 2008 | 28 | surgical complication, bladder prolapse |
| 2348 | 27 | surgical complication, uroperitoneum |
| 2349 | 27 | surgical complication, bladder prolapse |

**18. Extent of Clinical Activities Initiated and Completed**. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___x___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___x___No

**If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed.** (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

**Note**: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics

_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

**19. Human Embryonic Stem Cell Research.** Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___x___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
___x___ No

19(C) Please describe how this project involved human embryonic stem cells:

**20. Articles Submitted to Peer-Reviewed Publications**.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include

the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

**Note:** The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

| Title of Journal Article: | Authors: | Name of Peer-reviewed Publication: | Month and Year Submitted: | Publication Status (check appropriate box below): |
|---|---|---|---|---|
| 1. None | | | | ☐Submitted<br>☐Accepted<br>☐Published |

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____x_____ No_____

If yes, please describe your plans:

We are planning a series of manuscripts on these data. First, we will describe how the evolving injury pattern in out model (from early obstruction at the onset of kidney development and urine production) differs from existing surgical models in which there is a sharp and sudden decrease in kidney function due to acute obstruction. We also plan on submitting a manuscript describing the 3D reconstructive changes in the bladders over time in the mutant mice. We also plan on submitting a manuscript on the TGF-β signaling changes in the kidneys over time. Finally, we plan on submitting a manuscript on the effectiveness of surgical interventions with and without pharmacological interventions in the mutant mice.

**21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.**

Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be

single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

There has not yet been a direct impact on disease as a result of this project (since it is a basic science project). As noted, we do expect these studies to elucidate more about the pathophysiology and mechanisms of congenital obstructive nephropathy, and that this will lead to new therapeutic interventions in children with these diseases.

**22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment**. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

**23. Inventions, Patents and Commercial Development Opportunities.**

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___x_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:

Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____x_____

If yes, please describe your plans:

**24. Key Investigator Qualifications.** Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

## *BIOGRAPHICAL SKETCH*

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person.

| NAME | POSITION TITLE |
|---|---|
| Carlton M. Bates, M.D. | Associate Professor |
| eRA COMMONS USER NAME<br>BATESCM | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| Otterbein College, Westerville, OH. | B.S. | 1982-1986 | Chemistry, Life Science |
| The Ohio State University College of Medicine, Columbus, OH. | M.D. | 1986-1991 | Medicine |
| Pediatric Residency Program, The Ohio State University, Columbus, OH. | Residency | 1991-1994 | Pediatrics |
| Pediatric Nephrology Fellowship Program, Univ of Texas Southwestern Medical Center, Dallas, TX. | Fellowship | 1994-1997 | Pediatric Nephrology |

**A. Personal Statement:**

My research is focused on the role of fibroblast growth factor receptors (Fgfrs) and other genes in kidney and urinary tract development, using mouse models. I have an extensive track record of publications and previous funding manipulating expression of Fgfrs and other genes in mouse models. These mouse lines often mimic many of the congenital forms of kidney disease that are leading causes of renal disease in children, of which I am familiar given my history as a clinical Pediatric Nephrologist for over 15 years. My research track record in renal and urinary tract maldevelopment and post-natal consequences coupled with my clinical experience, make me uniquely qualified to be the PI on this application.

**B. Positions and Honors**

**Positions**

1997-1998 Assistant Instructor, Dept. of Pediatrics, Division of Nephrology, University of Texas Southwestern Medical Center, Dallas, TX.

1998-1999 Assistant Professor, Dept. of Pediatrics, Division of Nephrology, University of Texas Southwestern Medical Center, Dallas, TX.

1999-2007 Assistant Professor, Dept. of Pediatrics, Section of Nephrology, The Ohio State University College of Medicine and Public Health, Columbus, OH.

2007-11/08 Associate Professor, Dept. of Pediatrics, Section of Nephrology, The Ohio State University College of Medicine, Columbus, OH.

12/08- Associate Professor, Department of Pediatrics, University of Pittsburgh School of Medicine, Division Chief of Nephrology, Fellowship Program Director, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA.

**Reviewer Activities (grant applications)**:

2001 *Ad hoc* reviewer for The Wellcome Trust funding agency

2005- *Ad hoc* reviewer for the Urologic and Kidney Development and Genitourinary Diseases NIH study section (9/2005, 2/2006, 6/2006, 10/2006)

2008 Reviewer for NIH/NIDDK Special Emphasis Panel ZDK1 GRB-7 (01) M.

2009 Reviewer for NIH Challenge Grant Editorial Panel 18 ZRG1 DKUS-A (58) (07/20/2009).

2009 Reviewer for NIH Special Emphasis Panel ZRG1 DKUS-F (02) (11/04/2009).

2011- Member, Basil O'Connor Research Advisory Committee (2011-2016)

**Reviewer/Editorial Activities (journals/abstracts):**

*Ad hoc* reviewer for the journals Human Molecular Genetics, Oncogene, Pediatric Nephrology, American Journal of Kidney Diseases, American Journal of Physiology-Renal Physiology, The Pediatric Infectious Disease Journal, Journal of the American Society of Nephrology, Toxicology and Applied Pharmacology, Kidney International, Methods and Findings in Experimental and Clinical Pharmacology, Pediatric Research, Clinical Journal of the American Society of Nephrology, BMC Developmental Biology, Developmental Dynamics, Journal of Urology, Life Sciences, Biochimica et Biophysica Acta, PLoS One

2004- Editorial board member for the journal Pediatric Nephrology, Journal of the American Society of Nephrology

2010- Associate Editor (Educational Reviews) of the journal Pediatric Nephrology

**Professional (last 5 years)**:

2008 Chair of the Program Committee of the American Society of Pediatric Nephrology

2008- co-Chair of the Research Committee of the American Society of Pediatric Nephrology

2009- Member, Organizing Committee, International Workshop on Developmental Nephrology, New York (8/10)

2009- Member, Organizing Committee, International Pediatric Nephrology Association meeting, New York (9/10)

2010-11 Member, Program Committee of the American Society of Nephrology

2010-13 co-Chair, Organizing Committee, International Workshop on Developmental Nephrology

2010-13 Member, Scientific Committee for the International Pediatric Nephrology Association 2013 Congress

2010- Member, Council of the International Pediatric Nephrology Association

**Invited talks/oral presentations (last 5 years)**

2008 Invited lecture: “Role of fibroblast growth factor receptors in the kidney” at the Department of Zoology, Miami University, Oxford, Ohio on September 18th, 2008.

2009 Invited Lecture: “Role of fibroblast growth factor receptors in the kidney” at the O’Brien Center lecture, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas (September 22, 2009).

2009 Invited lecture: “Congenital Diseases of the Kidney and Urinary Tract” at Pediatric Grand Rounds, Children’s Medical Center at Dallas, Dallas, Texas (September 23, 2009).

2010 Invited lecture: “Role of fibroblast growth factor receptors in the kidney” at Magee-Womens NICU Grand Rounds, Pittsburgh, PA (January 14, 2010)

2010 Invited lecture: "Role of fibroblast growth factor receptors in the kidney" at Magee-Womens Research Institute's Work-in-Progress (WIP) Conference & Research Seminar, Pittsburgh, PA (February 2, 2010).

2010 Invited lecture: “Role of fibroblast growth factor receptors in the kidney” at the Mount Sinai School of Medicine, New York, NY (April 12, 2010).

2011 Invited lecture: “Role of fibroblast growth factor receptors in the kidney” at the Renal Research Seminar, Renal-Electrolyte Division, University of Pittsburgh, Pittsburgh, PA (May 11, 2011)

2012 Invited lecture: “Roles of Mesenchymal Fgfr Signaling in Urogenital Development” at Einstein Renal Grand Rounds, New York, NY (April 5, 2012).

2012 Invited lecture: “Roles of fibroblast growth factor receptors in models of structural kidney disease” at Children’s Hospital of Pittsburgh Pediatric Grand Rounds, Pittsburgh, PA (July 12, 2012).

2013 Invited lecture: “Roles of fibroblast growth factor receptors in models of structural kidney disease” at University of California-San Francisco Renal Grand Rounds, San Francisco, CA (February 27, 2013).