Supplemental Figure S1. There was no difference in expression of *Gdnf, Bmp4, Fgfr2, Grem1, Foxc1, Foxc2, Pax2, or Ret* in mutant compared to control E10.5 dissected urogenital ridge tissue by qPCR (n=4-6 embryos per genotype from 3 litters). RNA from each embryo was analyzed as a separate sample. Black bars = control. Gray bars = mutant. Error bars represent SEM. n.s = not significant (two-tailed t-test).
Table S1. Primer sequences for genotyping and qPCR.

| Primer name  | Forward Sequence (5’-3’)                                                                 | Reverse Sequence (5’-3’)                                                                 |
|--------------|-----------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| Tbx18Cre     | CCATCCAACAGCACCTGGGCCA GCTCAACA                                                        | CCACCATCGGTGCAGGAGATGTC CTTCAGT                                                        |
| Dicer        | CCTGACAGTGACGGTCCAAAG                                                                   | CATGACTCTTCAACTCAAAACT                                                                  |
| Dicer exon 21| GAACATGCTGCACATCAAGG                                                                    | GCAACCTTTTGCAACTCAAAACT                                                                 |
| Dicer exon 24| TCCAGGGGTCTTGACTGACT                                                                     | CCAATGATGCAAAGATGGTTG                                                                  |
| GAPDH        | AGGTCCGCTGTGAACCGGATTTG                                                                 | TGTAGACCATGTAGTTGAGGTC                                                                  |
| 18S RNA      | GACAGATTGACAGATTTGAGG                                                                   | CCAGACTTCTGGTTGTTA                                                                     |
| Gdnf         | TCATAACCACGCCTCAGA                                                                     | GGCAAGGATAGAGGAAGAG                                                                     |
| Bmp4         | TGAGAGCTCTCTGCTTTTTCTGTT                                                               | TGGTGTCAGTGTCTGGTTG                                                                    |
| Fgfr2        | CTTGGCGGGAAATTTCTATCG                                                                  | TGCTGAAAGTGCTGGCTTGG                                                                    |
| Greml        | Sequences confidential; Ordered from Sino Biological (Catalog: MP200016)                |                                                                                         |
| Foxc1        | TTCCTGCTCATTGCTCTT                                                                     | GGTCCTGTAACATCCAAACT                                                                   |
| Foxc2        | CACTCTGAAAGGGACTCTA                                                                    | GGCAATCTTCTTTTTG                                                                      |
| Pax2         | GCTAAGGAAAGGACTTTGT                                                                    | TAGGCAGTTCAGGTTG                                                                       |
| Ret          | TCTTTGTCCAAACATCAGT                                                                   | ACTATGCAACAAAGCCCTCCAG                                                                  |
NAME: Melissa Jeanne Anslow (Maiden Name: Domis)

eRA COMMONS USER NAME (credential, e.g., agency login): anslowmj

POSITION TITLE: Pediatric Nephrologist, Assistant Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

| INSTITUTION AND LOCATION                        | DEGREE (if applicable) | Completion Date MM/YYYY | FIELD OF STUDY |
|------------------------------------------------|------------------------|--------------------------|----------------|
| University of Pittsburgh, Pittsburgh, PA       | B.S.                   | 05/2008                  | Chemistry      |
| Temple University, Philadelphia, PA            | M.D.                   | 05/2012                  | Medicine       |
| Cleveland Clinic Children’s Hospital, Cleveland, OH | Residency             | 06/2015                  | Pediatrics     |
| UPMC Children’s Hospital of Pittsburgh, PA    | Fellowship             | 06/2018                  | Nephrology     |

A. Personal Statement

My overall career goal is to become an independently funded pediatric physician-scientist with expertise in the role of microRNA (miRNA) biology in lower urinary tract development and vesicoureteral reflux. I am a board-certified pediatrician and completed my Pediatric Nephrology Fellowship at UPMC Children’s Hospital of Pittsburgh in June 2018. I joined as faculty on 7/1/18 with 80% protected research time with the support of my department and the NIH K12 Institutional Career Development Award.

My career in medicine began with a strong commitment to gaining clinical skills and knowledge to provide the best patient care possible. This was facilitated through my medical school education at Temple University School of Medicine and pediatric residency at Cleveland Clinic Children’s Hospital. Interactions with pediatric nephrology patients during medical school and residency compelled me to medical research. My specific interest is congenital anomalies of the lower urinary tract, including vesicoureteral reflux, which can result in reflux nephropathy, a leading cause of renal failure in children. I am interested in determining how noncoding portions of the genome (such as miRNAs) contribute to lower urinary tract development and disease, with the ultimate goal of helping to inform better diagnostic and prognostic markers of these diseases (particularly VUR and reflux nephropathy) that could be used to guide clinical practice. With these long-term goals in mind, I was fortunate that Dr. Jacqueline Ho (expert in microRNA biology of the kidney) and Dr. Carlton Bates (expert in lower urinary tract and mechanisms underlying VUR) worked with me to develop an idea that could be my independent research focus moving forward. With the support of the divisional NIH T32 grant during fellowship, I was able to show with a transgenic mouse model that microRNAs act to suppress vesicoureteral reflux and have a role in proper ureteric bud (early ureter) induction and ureterovesical junction formation.

Given my strong mentorship team with complementary expertise and the incredible institutional training environment offered at the University of Pittsburgh, my decision to stay in Pittsburgh for my faculty position was easy. I was fortunate to be selected for and appointed to the NIH Institutional K12 Career Development Award in July 2018 that funds my research and allows 80% protected research time for the first two years of my faculty position. With the support of the K12, I was further able to interrogate underlying molecular mechanisms by which VUR may occur in the mutant mice. I was also able to generate preliminary differential mRNA and miRNA sequencing data before and after ureteric bud induction that showed differential expression of three microRNAs (mir-125, let-7, and miR-126) in which copy number variants were associated with VUR in multiple, unrelated children from the RIVUR (Randomized Intervention for Children with Vesico Ureteral Reflux) study. The results from my research efforts on the T32 and the K12 form the preliminary data for this K08 proposal.
My dedication to laboratory research did not become apparent until my nephrology fellowship, so I still have knowledge and skill gaps in miRNA biology and general developmental biology, as well as the need to develop writing, presentation, and leadership skills, that need to be improved before I am ready to transition to independence. Achievement of the research plan and career goals included in this application, combined with the guidance of my highly qualified mentors and research advisory committee, will ensure that I can generate strong data, communicate my findings effectively to the broader scientific community, and supplement my current skill set to achieve my long-term career goal to become an independent R01-funded physician-scientist in pediatric nephrology with expertise in miRNA biology of the lower urinary tract and vesicoureteral reflux.

B. Positions and Honors

Positions and Employment

| Date       | Position Description                                                                 | Institution | Location       |
|------------|--------------------------------------------------------------------------------------|-------------|----------------|
| 7/2012 – 6/2015 | Pediatric Resident, Cleveland Clinic Children’s Hospital, Cleveland, Ohio |             |                |
| 7/2015 – 6/2018 | Pediatric Nephrology Fellow, UPMC Children’s Hospital of Pittsburgh, Pittsburgh, PA |             |                |
| 7/2016 – 6/2018 | Postdoctoral Scholar, University of Pittsburgh School of Medicine, Department of Pediatrics |             |                |
| 7/2018 – 6/2019 | Pediatric Nephrologist, Instructor, UPMC Children’s Hospital of Pittsburgh and University of Pittsburgh School of Medicine, Department of Pediatrics, Pittsburgh, PA |             |                |
| 7/2019 – current | Pediatric Nephrologist, Assistant Professor, UPMC Children’s Hospital of Pittsburgh and University of Pittsburgh School of Medicine, Department of Pediatrics, Pittsburgh, PA |             |                |

Other Experience and Professional Memberships

| Year       | Organization/Role Description                                                                 |
|------------|------------------------------------------------------------------------------------------------|
| 2015 – pres | Board-certified Pediatrician                                                                  |
| 2019 - pres | Nephrology and Urology Research Affinity Group (NURAG), Pittsburgh, Director                |
| 2019       | Ad hoc Review for Scientific Reports, JCI Insight, Neuourology and Urodynamics, Pediatric Nephrology, Journal of Molecular Medicine |
| 2018       | Grant Review Workshop, Mock Study Section at the American Society of Nephrology meeting, October 2018.
| 2018       | Departmental K Grant Writing course, Fall 2018                                               |
| 2018       | Ad hoc Review for Pediatric Nephrology                                                        |
| 2018       | Mentor for the Summer Undergraduate Research Program (SURP) at the University of Pittsburgh School of Medicine, Summer 2018. |
| 2017 – pres | American Society of Pediatric Nephrology (ASPN) Research Committee, Member                    |
| 2017       | Grant-Writing course (CLRES 2076: Introduction to Grant Writing for Postdoctoral Trainees)      |
| 2016       | Ad hoc Review for Journal of Pediatric Urology (Co-review with Dr. Carlton Bates)             |
| 2016 – pres | American Society of Pediatric Nephrology, Member                                               |
| 2015 – pres | International Pediatric Nephrology Association, Member                                         |
| 2015 – pres | American Society of Nephrology, Member                                                        |
| 2015 – pres | Pennsylvania Medical Society, Member                                                          |
| 2012 – pres | American Academy of Pediatrics, Member                                                        |

Presentations

| Year | Title                                                                 | Event/Location |
|------|----------------------------------------------------------------------|----------------|
| 2019 | “Loss of Dicer in the Peri-Wolffian Duct Stroma Leads to Aberrant Ureteric Budding and Increased Rates of Vesicoureteral Reflux." | Poster Presentation at American Society of Nephrology annual Kidney Week meeting, Washington D.C, Nov 8, 2019 (upcoming). |
| 2019 | “Loss of Dicer in the Peri-Wolffian Duct Stroma Leads to Aberrant Ureteric Budding and Increased Rates of Vesicoureteral Reflux.” | Oral Presentation at the CHRC Annual Retreat, Pittsburgh, PA. September 13, 2019 |
| 2019 | “The Role of microRNAs in Vesicoureteral Reflux.” | Poster Presentation at Clinical and Scientific Advances in UTIs Conference. Columbus, Ohio. June 29, 2019. |
| 2019 | “The Role of microRNAs in Vesicoureteral Reflux." | Oral Presentation at Pediatric Discovery Day, Pittsburgh PA. April 18 2019. |
| 2019 | “The Role of miRNAs in Vesicoureteral Reflux." | Oral Presentation at Nephrology and Urology Research Affinity Group (NURAG). Pittsburgh, PA. February 19, 2019. |
| 2018 | “Loss of Dicer activity in the peri-Wolffian duct stroma leads to increased rates of vesicoureteral reflux.” | Poster Presentation at the American Society of Nephrology Kidney Week Meeting. San Diego, CA. October 26, 2018. |
“Loss of Dicer activity in the peri-Wolffian duct stroma leads to increased rates of vesicoureteral reflux.” Poster Presentation at the CHRC Annual Retreat, Nashville, TN, October 11, 2018.

“Loss of Dicer activity in the peri-Wolffian duct stroma leads to increased rates of vesicoureteral reflux.” Poster Presentation at the International Workshop on Developmental Nephrology (IWDN), Tel Aviv, Israel, April 23 2018.

“Loss of Dicer activity in the peri-Wolffian duct stroma leads to increased rates of vesicoureteral reflux.” Oral Presentation at the K12 Retreat, Pittsburgh, PA, April 5 2018.

“Loss of Dicer activity in the peri-Wolffian duct stroma leads to increased rates of vesicoureteral reflux.” Oral Presentation at Nephrology and Urology Research Affinity Group (NURAG), Pittsburgh, PA, March 20 2018.

“Loss of Dicer activity in the peri-Wolffian duct stroma leads to increased rates of vesicoureteral reflux.” Oral Platform Session Presentation at the American Society of Nephrology (ASN) Meeting, New Orleans, LA, November 4 2017.

“Loss of Dicer activity in the peri-Wolffian duct stroma leads to increased rates of vesicoureteral reflux.” Oral Platform Session Presentation at the Pediatric Academic Societies Meeting, San Francisco, CA, May 8 2017.

“Loss of Dicer activity in the peri-Wolffian duct stroma leads to increased rates of vesicoureteral reflux.” Oral Presentation at Nephrology and Urology Research Affinity Group (NURAG), Pittsburgh, PA, March 21 2017.

“Red man syndrome following intraperitoneal vancomycin in a child with peritonitis.” Poster Presentation at Cleveland Clinic Children’s Hospital Research Day, Cleveland, OH, May 2015.

**Honors**

2018 NIH K12 Career Development Award Recipient (Mentors: Jacqueline Ho and Carlton Bates)

2014 Cleveland Clinic Children’s Nephrology Resident of the Year Award

2014 Cleveland Clinic Children’s Rheumatology Resident of the Year Award

2014 Cleveland Clinic Children’s Community Pediatrics/Child Health Advocacy Resident of the Year Award

2014 Chief Resident Nomination, Cleveland Clinic Children’s Hospital

2013 Cleveland Clinic Children’s Newborn Nursery Resident of the Year Award

2008 Graduation Summa Cum Laude, B.S. in Chemistry, University of Pittsburgh

2007 The Richard F. Zarilla Award for academic excellence in chemistry and involvement in the undergraduate community

**C. Contribution to Science**

1. T32 Grant (fellowship research study) and NIH K12 Institutional Career Development Award (current funding)

*The Role of miRNAs in Vesicoureteral Reflux*

This project explores the role of miRNAs in vesicoureteral reflux (VUR) and the lower urinary tract. Using transgenic mouse models to knock out Dicer, and thus mature miRNAs in the area surrounding the future ureter and vesicoureteral junction, I have shown significantly higher rate of VUR among Dicer mutants compared to controls. There is lower ureter insertion into the bladder on the side of VUR and shorter intravesicular tunnel length (the portion of ureter that traverses the bladder wall) in mutants that reflux. Mutants also have more cranially positioned ureteric buds than controls. I performed all experiments and analysis for this study under the mentorship of Dr. Ho (mentor) and Dr. Bates (co-mentor).

2. Case Report: Red Man Syndrome Following Intraperitoneal Vancomycin in a Child with Peritonitis

We presented the case of a patient who was treated for peritonitis with intraperitoneal vancomycin per guidelines and had an adverse event called red man syndrome. The case emphasized the importance of monitoring for adverse reactions since our patients are often treated in outpatient settings. We also suggested changes to dosing guidelines to prevent adverse reactions. I summarized the clinical course of our patient, reviewed the current literature, and worked with Dr. Michael Moritz on a first-authored case report.

Domis MJ and Moritz ML (2014) Red man syndrome following intraperitoneal vancomycin in a child with peritonitis. Front. Pediatr. 2:55. doi: 10.3389/fped.2014.00055
D. Additional Information:

Research Support
K12 HD052892 NIH K12 Institutional Career Development Award 07/01/2018-06/30/2020
(Mentors: Bates, Carlton and Ho, Jacqueline)
The Role of miRNAs in Vesicoureteral Reflux and the Lower Urinary Tract
This project focuses on determining the underlying molecular and genetic mechanisms that lead to abnormal ureteric bud induction site and increased rates of VUR in mutant mice with deletion of miRNAs in the peri-Wolffian duct stroma. The K12 award is institutional career development funding to support research for clinician-scientists early in their careers while they prepare to apply for K level funding. The award offers salary, research, and travel support. The key findings of the project so far are described in detail in the preliminary data section of this proposal.

2 T32 DK 91202-6 (PI: Bates, Carlton) 07/01/2016-6/30/2018
The Role of miRNAs in Vesicoureteral Reflux and the Lower Urinary Tract
This project explored the role of microRNAs in vesicoureteral reflux (VUR) and the lower urinary tract, using a conditional knockout approach for Dicer in the lower urinary tract of a mouse model. This project showed increased rates of vesicoureteral reflux in mutant mice compared to control, as well as abnormal ureteric bud induction sites. The data generated is detailed in the preliminary data section of this application.