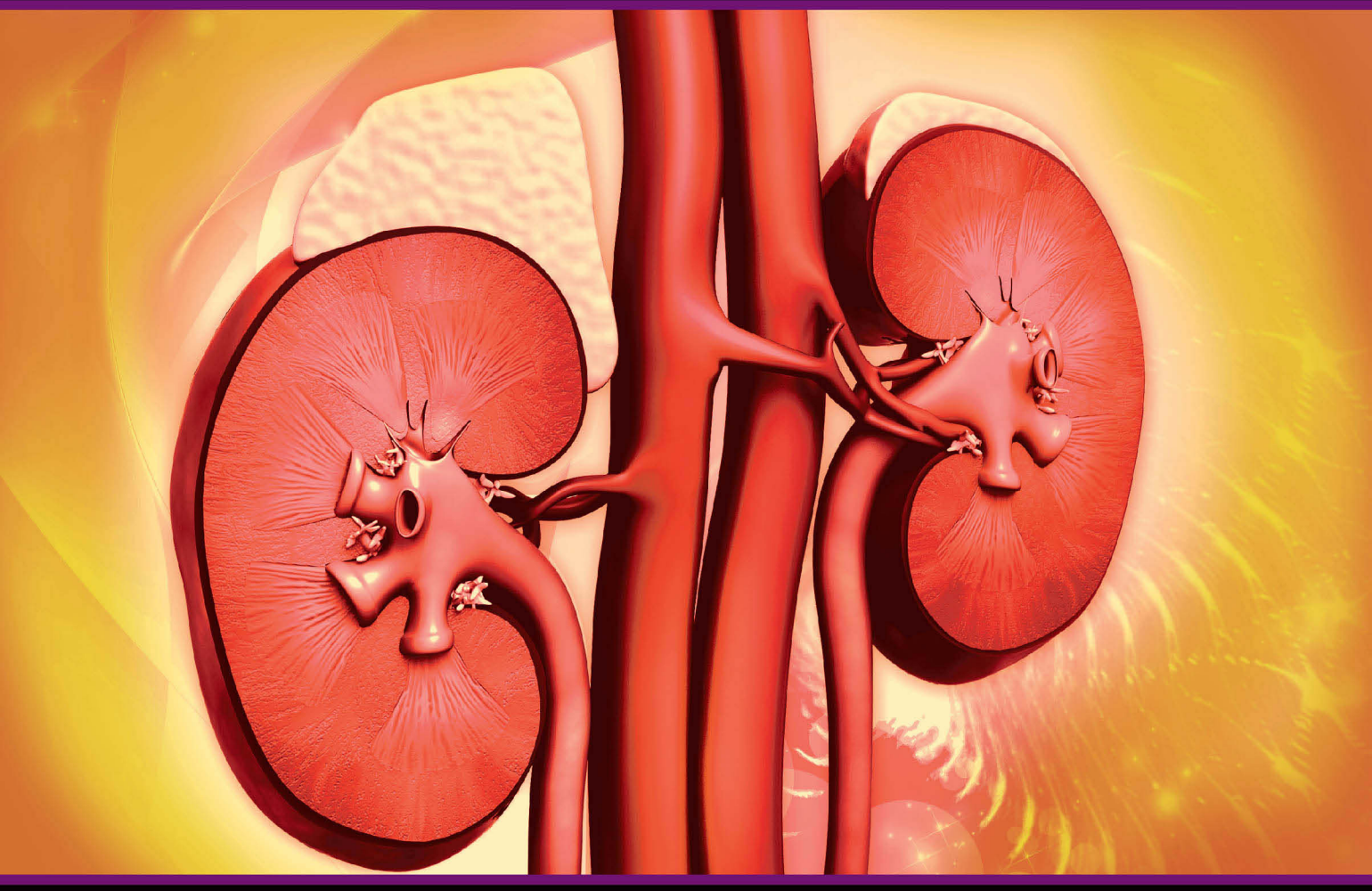
CRC Press
Taylor & Francis Group

WITH VITALSOURCE® EBOOK

Third Edition

Clinical Pediatric Nephrology



Edited by
Kanwal K. Kher • H. William Schnaper
Larry A. Greenbaum

Third Edition

Clinical Pediatric Nephrology

Third Edition

Clinical Pediatric Nephrology

Edited By

Kanwal K. Kher

Chairman, Department of Nephrology
Phoenix Children's Hospital
Phoenix, AZ, USA
and Emeritus Professor of Pediatrics
George Washington University
School of Medicine
Washington, DC, USA

H. William Schnaper

Vice Chair Department of Pediatrics
Northwestern University
Feinberg School of Medicine
and Ann & Robert H. Lurie Children's
Hospital of Chicago
Chicago, IL, USA

Larry A. Greenbaum

Division Director of Pediatric Nephrology
Marcus Professor of Pediatrics
Emory University School of Medicine
and Children's Healthcare of Atlanta
Atlanta, GA, US

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed on acid-free paper
Version Date: 20160808

International Standard Book Number-13: 978-1-4822-1462-8 (Pack - Book and Ebook)

Contents

PART D GLOMERULAR AND TUBULAR DISEASES

PART E KIDNEY IN SYSTEMIC DISEASES

PART F KIDNEY FAILURE

PART G RENAL REPLACEMENT THERAPIES

Preface

This third edition of *Clinical Pediatric Nephrology* continues to have as its main goal providing a primer of pediatric nephrology. Its intended audience includes committed medical students and general trainees, as well as pediatric nephrology fellows and pediatric nephrologists. Our focus remains on the clinical diagnosis and management of pediatric renal disorders.

The book has been thoroughly updated, and each chapter has been rewritten. The number of chapters has expanded from 37 to 53. In part, this represents a degree of specialization, with several chapters divided to focus on specific disorders as their pathogenesis has been clarified. The organization of the book has also been changed with an additional emphasis on the physiology of kidney diseases. Sections now cover kidney anatomy and development, diagnostic evaluation, disorders of homeostasis, glomerular and tubular diseases, systemic diseases and the kidney, acute and chronic kidney disease, renal replacement therapies, hypertension, inherited disorders, urologic disorders, and research tools. We believe that this reorganization and the expansion in the number of chapters better reflects the status of pediatric nephrology today. Each chapter includes "Key Points" boxes to emphasize issues that the authors and editors believe are important take-aways in the section, a set of review questions for the reader, and where appropriate a clinical vignette describing how the information in the chapter could be clinically applied in real life.

An important change has been an evolution in the editorship. Dr. Larry Greenbaum has joined the editorial team. He brings immense experience as an academic nephrologist, clinician and a researcher to the editorial team. His role in shaping the content of the third edition is evident in all sections of the book. We also wish to express our sincerest gratitude and thanks to Dr. Sudesh Makker, who guided the editorial work in the first two editions of this book.

We wish to thank all the contributors who worked diligently with us, under tight time-lines, through several revisions of their texts, and who willingly provided elements for the chapters that are unique to this publication. The outstanding editorial, production, and marketing teams at Taylor and Francis have provided support that has been essential to our reaching fruition. We are especially grateful to Our sincerest thanks to Henry Spilberg, who has managed the publication of the second edition and guided us in the concept design of the third edition of this book at Taylor and Francis. Miranda Bromage, who has taken over the reigns, has been an inspiration to work with. She provided her extraordinary skills in guiding the production of the book. Henry and Miranda were instrumental in advocating for an "all-color" book, which has enhanced its content and visual appeal. We thank both of them from the bottom of our hearts. Amy Blalock and Linda Van Pelt provided their superb expertise in copy editing and composing the galleys. Kyle Meyer, at the Boca Raton office of CRC Press-Taylor and Francis worked patiently with us in the editing of galleys of all chapters. Without his help, publication of this book would not have materialized. Figures in this edition were drawn by a very talented pool of artists. They are the unsung heroes of this work. We wish to thank each one of them for their contributions.

We owe a major debt of gratitude to two groups. Our students, residents, nephrology fellows and colleagues have provided inspiration and frequently challenged us on communication of scientific and educational content, as well as the format of this work. Most importantly, our families, who tolerate our busy and often distracting schedules have provided consistent support and a sense of perspective as we undertook this task. They provide an essential foundation for our lives. It is only with their individual commitments that we have succeeded in completing this task. We owe our heartfelt gratitude and appreciation to each one of them.

Kanwal K. Kher
Larry A. Greenbaum
H. William Schnaper

Contributors

Sun-Young Ahn, MD
Medical director, nephrology inpatient services
Children's National Health System,
The George Washington University
Washington DC, USA

Farah N. Ali, MD, MS
Assistant Professor of Pediatrics
Ann and Robert H. Lurie Children's Hospital of Chicago
Chicago, Illinois

Uri S. Alon, MD
Professor of Pediatrics
University of Missouri, Kansas City School of Medicine
and
Section of Pediatric Nephrology
The Children's Mercy Hospital
Kansas City, Missouri

Mary Andrich, MD
Clinical Associate Professor of Radiology
The George Washington University School of Medicine
Washington, DC

David Askenszi MD, MSPH
Associate Professor of Pediatrics
Medical Director - Pediatric and Infant Center for Acute Nephrology
University of Alabama at Birmingham
Birmingham Alabama , USA

Meredith Atkinson, MD, MHS
Associate Professor of Pediatrics
Division of Pediatric Nephrology
Johns Hopkins University School of Medicine
Baltimore, Maryland, USA

Diego H. Aviles, MD
Division Chief, Pediatric Nephrology
Children's Hospital of New Orleans
Professor of Clinical Pediatrics
Louisiana State University Health Sciences Center New Orleans
New Orleans, Louisiana

Rossana Baracco, MD
Assistant Professor of Pediatrics
Children's Hospital of Michigan
Wayne State University
Detroit, Michigan, USA

Michel Baum, MD
Sara M. and Charles E. Seay Chair in Pediatric Research
Professor of Pediatrics and Internal Medicine
Department of Pediatrics
University of Texas Southwestern Medical Center
Dallas, Texas

Detlef Bockenhauer, PhD
Professor of Paediatric Nephrology
UCL Institute of Child Health and Great Ormond Street Hospital for Children
NHS Foundation Trust
London, United Kingdom

Raed Bou Matar, MD
Assistant Professor of Pediatrics
Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Center for Pediatric Nephrology
Cleveland, Ohio

Dorothy Bulas, MD
Professor of Pediatrics and Radiology
The George Washington University School of Medicine
Children's National Health System
Washington, DC

Timothy E. Bunchman, MD
Director Pediatric Nephrology
Children's Hospital of Richmond
and
Professor of Pediatrics
Virginia Commonwealth University School of Medicine
Richmond, Virginia

Melissa A. Cadnapaphornchai, MD
Associate Professor of Pediatrics and Medicine
University of Colorado Anschutz Medical Campus
The Kidney Center
Children's Hospital Colorado
Aurora, Colorado

Stephen Cha, MD
Clinical Assistant Professor of Pediatrics
Northeast Ohio Medical University
Nephrology & Pediatric Hypertension Center
Akron Children's Hospital
Akron , Ohio

Akash Deep MD, FRCPCH
Honorary Lecturer, King's College
Director, Pediatric Intensive Care Unit
King's College Hospital, London, UK

W. Robert DeFoor, MD, MPH
Associate Professor of Surgery
University of Cincinnati
and
Director Clinical Research
Division of Pediatric Urology
Cincinnati Children's Hospital Medical Center
Cincinnati, Ohio

Georges Deschênes, MD, PhD
Head of Pediatric Nephrology
APHP Robert-Debré, Université Sorbonne-Paris-Cité
Paris, France

Prasad Devarajan, MD
Louise M. Williams Endowed Chair
Professor of Pediatrics and Developmental Biology
Director of Nephrology and Hypertension
Cincinnati Children's Hospital Medical Center
University of Cincinnati School of Medicine
Cincinnati, Ohio

Joseph T. Flynn, MD, MS
Chief, Division of Nephrology
Seattle Children's Hospital
Professor of Pediatrics
University of Washington School of Medicine
Seattle, Washington

John W. Foreman, MD
Chief, Pediatric Nephrology
Department of Pediatrics
Duke University Medical Center
Durham, North Carolina

Debbie S. Gipson, MD, MS
Professor of Pediatrics
University of Michigan School of Medicine
Ann Arbor, Michigan , USA

Caroline Gluck, MD
Pediatric Nephrology Fellow
Children's Hospital of Philadelphia
Philadelphia, Pennsylvania, USA

Brittany Goldberg MD, MS
Adjunct Assistant Professor of Pediatrics
George Washington University School of Medicine
Division of Infectious Diseases
Children's National Health System
Washington, DC

Stuart L. Goldstein, MD, FAAP, FNKF
Director, Center for Acute Care Nephrology
Division of Nephrology and Hypertension
The Heart Institute Cincinnati
Children's Hospital Medical Center
Cincinnati, Ohio, United States of America

Larry Greenbaum, MD, PhD, FAAP
Marcus Professor of Pediatric Nephrology
Chief, Division of Nephrology
Emory University School of Medicine
and
Children's Healthcare of Atlanta
Atlanta, Georgia USA

Lisa M. Guay-Woodford, MD
Hudson Professor of Pediatrics
George Washington University
and
Director, Center for Translational Science
and
Director, Clinical and Translational Institute at Children's National
Children's National Health System
Washington, DC

M. Colleen Hastings, MD, MS
Associate Professor, Pediatrics and Medicine
University of Tennessee Health Science Center
and
Le Bonheur Children's Hospital
and
Children's Foundation Research Institute
Memphis, Tennessee

Danniele Gomes Holanda, MD
Clinical Assistant Professor
Director, Electron Microscopy Laboratory
Department of Pathology
University of Iowa Hospitals and Clinics
Iowa City, Iowa, USA

Christer Holmberg, MD, PhD
Professor of pediatrics
Pediatric Nephrology and Transplantation
Children's Hospital
University of Helsinki
Helsinki
Finland

Franca Iorember, MD, MPH
Associate Professor of Pediatrics
Clinical Division of Nephrology
Children's Hospital of New Orleans
and
Department of Pediatrics
Louisiana State University Health Sciences Center New Orleans
New Orleans, Louisiana

Sherron M. Jackson, MD
Associate Professor, Pediatric Hematology-Oncology
Director of Pediatric Sickle Cell Clinic
Medical University of South Carolina
Charleston, South Carolina USA

Hannu Jalanko, MD
Professor, Head of the Department
Pediatric Nephrology and Transplantation
Children's Hospital, University of Helsinki
Helsinki University Central Hospital
Helsinki, Finland

Barbara Jantausch, MD
Professor of Pediatrics
George Washington University School of Medicine
and
Division of Infectious Diseases
Children's National Medical Center
Washington, DC

Kurt E. Johnson, PhD
Professor of Anatomy and Regenerative Biology
Professor of Obstetrics and Gynecology (Research)
George Washington University School of Medicine and Health Sciences
Washington, DC

Stanley C. Jordan, MD
Director, Nephrology
Director, HLA and Transplant Immunology Laboratory
Comprehensive Transplant Center
Medical Director, Kidney Transplant Program
Cedars-Sinai Medical Center, Los Angeles, California

Gaurav Kapur, MD
Associate Professor, Pediatrics
Director, Pediatric Dialysis
Wayne State University School of Medicine
Children's Hospital of Michigan
Detroit, Michigan, USA

Clifford E. Kashtan, MD
Professor of Pediatrics
Chief, Division of Pediatric Nephrology
University of Minnesota Amplatz Children's Hospital
Minneapolis, Minnesota

Frederick J. Kaskel, MD, PhD
Professor and Vice Chair of Pediatrics
Director, Life Course Research Program
Block Institute for Clinical & Translational Research
Albert Einstein College of Medicine
and
Children's Hospital at Montefiore
Bronx, NY

Kanwal K. Kher, MD
Professor Emeritus of Pediatrics
George Washington University School of Medicine
Washington, DC
and
Division Chief, Nephrology and Hypertension
Phoenix Children's Hospital
Phoenix, AZ

Jeffrey B. Kopp, MD
Kidney Disease Section
National Institute of Diabetes and Digestive and Kidney
Bethesda, Maryland

Martin A. Koyle, MD
Professor Department of Surgery
University of Toronto
Department Head, Urology
The Hospital for Sick Children
Toronto, Canada

Craig B. Langman, MD
The Isaac A Abt MD Professor of Kidney Diseases
Feinberg School of Medicine, Northwestern University
Head, Kidney Diseases
The Ann and Robert H Lurie Children's Hospital of Chicago
Chicago, Illinois USA

Armando J. Lorenzo, MD
Associate Professor
Department of Surgery
University of Toronto
and
Department of Urology.
The Hospital for Sick Children,
Toronto, Canada

John D. Mahan, MD
Professor, Department of Pediatrics
Pediatric Nephrology
The Ohio State University College of Medicine
and
Nationwide Children's Hospital
Columbus Ohio

Massoud Majd
Professor of Pediatrics and Radiology
The George Washington University School of Medicine
and
Children's National Health System
Washington, DC

Robert Mak, MD, PhD
Pediatric Nephrology
University of California San Diego
La Jolla, California

Bruce Markle, MD
Associate Professor of Pediatrics and Radiology
The George Washington University School of Medicine
Children's National Health System
Washington, DC

Tej K. Mattoo, MD, DCH, FRCP (UK)
Professor of Pediatrics
Wayne State University School of Medicine
Chief, Pediatric Nephrology and Hypertension
Children's Hospital of Michigan
Detroit, Michigan

Karen McNiece Redwine, MD, MPH
Associate Professor of Pediatrics
Pediatric Nephrology
University of Arkansas for Medical Sciences/Arkansas Children's Hospital
Little Rock, Arkansas

Michele Mietus-Snyder, MD
Associate Professor of Pediatrics
The George Washington University School of Medicine
and
Children's National Health System
Washington, DC

Eugene Minevich, MD
Professor of Surgery
Division of Pediatric Urology
Cincinnati Children's Hospital Medical Center
Cincinnati, Ohio

Kirtida Mistry, MBBCh, DCH, MRCPCH
Assistant Professor of Pediatrics
Division of Pediatric Nephrology
The George Washington University School of Medicine
and
Medical Director, Dialysis Services
Children's National Medical Center
Washington, DC

Asha Moudgil, MD
Professor of Pediatrics
George Washington University School of Medicine
Acting Chief Division of Nephrology
and
Medical Director, Renal Transplantation
Children's National Medical Center
Washington, DC

Marva Moxey-Mims, MD
KUH Deputy Director for Clinical Research
Director, Pediatric Nephrology and Renal Centers Programs
NIH, NIDDK, DKUH
Bethesda, Maryland

Raj Munshi, MD
Seattle Children's Hospital
Seattle, Washington

Carla M. Nester, MD, MSA
Assistant Professor
Departments of Internal Medicine and Pediatrics
Division of Nephrology
University of Iowa Hospital and Clinics
Iowa City, Iowa

Patrick Niaudet, MD
Pediatric Nephrology
Hôpital Necker-Enfants Malades
Université Paris-Descartes
Paris, France

Hans G. Pohl, MD, FAAP
Associate Professor
Urology and Pediatrics
George Washington University
and
Associate Chief, Department of Urology
Children's National Medical Center
Washington, DC

Raymond Quigley MD
Division of Pediatric Nephrology
University of Texas Southwestern Medical Center
Dallas, Texas

Shreya Raman, MBBS, MRCP, MSc, FRCPath
Specialist Registrar in Histopathology
Royal Victoria Infirmary
Newcastle upon Tyne Hospitals NHS Foundation Trust
Newcastle upon Tyne, United Kingdom

Michelle N. Rheault, MD
Assistant Professor of Pediatrics
and
Medical Director of Dialysis
Division of Pediatric Nephrology
University of Minnesota Amplatz Children's Hospital
Minneapolis, Minnesota

Norman D. Rosenblum MD, FRCPC
Paediatric Nephrologist
Hospital for Sick Children
Professor of Paediatrics, Physiology, and Laboratory Medicine and Pathobiology
Canada Research Chair in Developmental Nephrology
University of Toronto
Peter Gilgan Centre for Research and Learning
Hospital for Sick Children
Toronto, Canada

Isidro B. Salusky, MD
Distinguished Professor of Pediatrics
Chief. Division of Pediatric Nephrology
Director, Clinical and Translational Research Center
Associate Dean for Clinical Research
David Geffen School of Medicine at UCLA
Los Angeles, California, USA

Lisa M. Satlin, MD
Herbert H. Lehman Professor and Chair
Jack and Lucy Clark Department of Pediatrics
Mount Sinai Medical Center
Pediatrician-in-Chief
Kravis Children's Hospital at Mount Sinai
Icahn School of Medicine at Mount Sinai
New York, New York

John Sayer, MBChB, PhD, FRCP
Senior Clinical Lecturer in Nephrology
Institute of Genetic Medicine
International Centre for Life
Newcastle University
Newcastle Upon Tyne, United Kingdom

H. William Schnaper, MD
Irene Heinz Given and John Laporte Given Professorship in Pediatric Research
Vice Chair, Department of Pediatrics
Northwestern University Feinberg School of Medicine
and
Ann & Robert Lurie Children's Hospital of Chicago
Chicago, Illinois

George J. Schwartz, MD
Professor of Pediatrics and Medicine
Chief, Division of Pediatric Nephrology
Department of Pediatrics
University of Rochester School of Medicine
Rochester, New York

Dave Selewski, MD, MS
Assistant Professor
Pediatric Nephrology
University of Michigan
Ann Arbor, Michigan

Eglal Shalaby-Rana, MD
Assistant Professor of Radiology and Pediatrics
George Washington University School of Medicine
and
Children's National Medical Center
Washington, DC

Ibrahim F. Shatat, MD, MS
Associate Professor of Pediatrics
Division of Nephrology and Hypertension
MUSC Children's Hospital
Charleston, South Carolina

William E. Smoyer, MD
C. Robert Kidder Endowed Chair
Vice President Clinical and Translational Research
Director, Center for Clinical and Translational Research
The Research Institute at Nationwide Children's Hospital
Professor of Pediatrics
Columbus, Ohio USA

Shalabh Srivastava, MBBS, MRCP
Specialist Registrar in Nephrology
Institute of Genetic Medicine
Newcastle University
Newcastle upon Tyne, United Kingdom

Tarak Srivastava, MD
Associate Professor of Pediatrics
University of Missouri, Kansas City School of Medicine
and
Director, Nephrology Research Laboratory
Children's Mercy Hospital
Kansas City, Missouri

Mihail M. Subtirelu, MD
Adjunct Associate Professor
University of Missouri, Kansas City
Division of Pediatric Nephrology
and
Children's Mercy Hospital
Kansas City, Missouri

Jordan M. Symons, MD
Professor of Pediatrics
University of Washington School of Medicine
Attending Nephrologist
Seattle Children's Hospital
Seattle, Washington, USA

David B. Thomas, MD
Professor of Pathology
University of Miami Miller School of Medicine
University of Miami Hospital
Miami, Florida

Shamir Tuchman, MD, MPH
Assistant Professor of Pediatrics
George Washington University School of Medicine
and
Division of Pediatric Nephrology
Children's National Medical Center
Washington, DC

Natalie S. Uy, MD
Assistant Professor of Pediatrics
Pediatric Nephrology
Columbia University Medical Center
New York, NY

Rudolph P. Valentini, MD
Chief Medical Officer
Pediatric Nephrology
Children's Hospital of Michigan
Clinical Professor of Pediatrics
Wayne State University School of Medicine
Detroit, Michigan

Vesa Matti Vehaskari, MD, PhD
Professor of Pediatrics
LSU Health Sciences Center
Children's Hospital of New Orleans
New Orleans, Louisiana

Bradley A. Warady, MD
Professor of Pediatrics
University of Missouri, Kansas City School of Medicine
and
Senior Associate Chairman, Department of Pediatrics
Director, Division of Pediatric Nephrology
Children's Mercy Hospital
Kansas City, Missouri

Katherine Wesseling-Perry, MD
Associate Professor of Pediatrics
Division of Pediatric Nephrology
David Geffen School of Medicine at UCLA
University of California, Los Angeles
Los Angeles, California

Robert J. Wyatt, MD, MS
Professor of Pediatrics
University of Tennessee Health Science Center
and
Le Bonheur Children's Hospital
Memphis, Tennessee

Ihor V. Yosypiv, MD
Associate Professor
Department of Pediatrics
Tulane University Health Sciences Center
New Orleans, Louisiana

PART A

Kidney anatomy and development

1

Anatomy and embryology of the urinary tract

KURT E. JOHNSON

Kidneys serve as an important metabolic organ that eliminates nitrogenous waste products, maintains fluid and electrolyte balance, and performs important hormonal functions, such as synthesis of 1-25-dihydroxy vitamin D and erythropoietin. Being paired organs, kidneys have also been used in living organ donation for renal replacement therapy in patients with end-stage renal disease (ESRD). Developmental aspects of kidney have received considerable attention recently and much has now been deciphered about the control of renal and ureteric development. It is also becoming increasingly clear that nephron endowment at birth is an important fetal factor that may determine the development of hypertension and chronic kidney disease (CKD) in adults. This chapter discusses the clinically relevant anatomy and embryogenesis of the kidneys and the urinary tract.

GROSS STRUCTURE AND RELATIONS

In adults, each kidney is reddish-brown, approximately 11 cm long, 5 cm wide, and 3 cm thick, and weighs approximately 130 g. By renal ultrasound measurement, renal length in healthy newborns has been reported to be 4.21 ± 0.45 cm for the right kidney and 4.32 ± 0.46 cm for the left kidney.[1] Kidneys grow with age and achieve adult length by approximately 18 years of age.[2,3] Interestingly, renal length is greater in obese children, possibly reflecting hypertrophy resulting from hyperfiltration.[4] Both kidneys are more or less similar in shape, with a convex lateral surface and a deep recess at the hilum of the kidney on the medial surface that is called the renal sinus. The renal artery and vein and some adipose tissue occupy the renal sinus, along with a funnel-shaped expansion of the ureter known as the renal pelvis. Within the kidney, the renal pelvis is divided into two elongated major calyces and several shorter branches called the minor calyces.

KEY POINT

Newborn kidneys are approximately 4.5 cm long as measured by ultrasound.

The two kidneys lie on the posterior abdominal wall along either side of the vertebral column in the retroperitoneal space, but at slightly different levels. The left kidney has its superior pole at approximately the level of the middle of T11 vertebral body and its inferior pole at approximately the level of the bottom of L2. In contrast, the right kidney lies slightly lower, with its superior pole at the top of T12 and its inferior pole at the top of L3. The superior poles of each kidney are in contact with the diaphragm, and their posterior surfaces are covered by skeletal muscles (from medial to lateral): psoas major, quadratus lumborum, and transversus abdominis.

In a longitudinally cut section, the kidney has a darker cortex, whereas the inner medulla is lighter in color. Each kidney has a collection of triangular renal pyramids, or lobes, with a base bordering the cortex and an apex projecting as renal papillae into the minor calyces (Figure 1.1). Each renal pyramid consists of medullary tissue associated with the corresponding cap of cortical tissue. The distinction

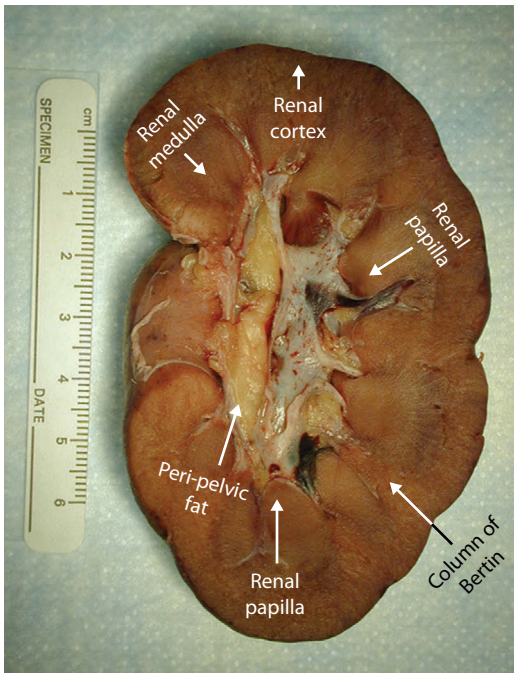


Figure 1.1 Cut surface of normal kidney of a child with anatomic landmarks shown. (Photograph provided by Ronald Przygodzki, MD.)

between cortex and medulla in the kidneys is made difficult by the projection of medullary tissue into the cortex as medullary rays and by the cortical tissue bundled between the renal pyramids, known as the renal columns of Bertin.

The ureters are 25 cm long in an adult but are of variable length in children. The ureter has a thick, fibromuscular wall and a small lumen. Along its descent from the abdominal cavity (upper half) into the lesser pelvis (lower half), the ureter is located retroperitoneally and is closely adherent to the overlying peritoneal lining. Ureters enter the urinary bladder posteriorly. The urinary bladder is a hollow muscular organ located posterior to the symphysis pubis, and its superior surface is covered by a reflection of the peritoneal lining. It is innervated by nerves from the vesical plexus, which has fibers from two distinct sources: (1) sympathetic lumbar nerves through the hypogastric plexus and (2) parasympathetic pelvic splanchnic nerves.

MICROSCOPIC ANATOMY

The renal parenchyma consists of functional units called uriniferous tubules, each with two distinct components: (1) the nephron and (2) the collecting tubules. The nephron consists of the Bowman capsule, proximal convoluted tubules (PCTs), loop of Henle, and distal convoluted tubules (DCTs). Nephrons have highly variable lengths. In general, the superficial (cortical) nephrons are shorter and the deep (juxtamedullary) nephrons are longer, mostly because of the longer loop of Henle. These long nephrons are important in the urinary concentrating mechanism (Figure 1.2). Uriniferous units converge to form the apex of the renal pyramids as they enter the minor calyces. Starting at the bases of the renal pyramids, adjacent to the corticomedullary junction, bundles of tubules and associated blood vessels may project toward the surface of the kidney as radially arranged medullary rays.

KEY POINTS

- Nephrons with glomeruli close to the corticomedullary junction have long loops of Henle. These nephrons participate in the urinary concentration mechanism.
- Superficial or cortical nephrons have shorter loops of Henle.

The most proximal structure of the nephron is the Bowman capsule, a thin-walled, spherical dilation, which is deeply invaginated into a two layered, cup-like structure, with a visceral layer and a parietal layer. The space between these two layers, which is continuous with the lumen of the PCTs, is called the urinary (or Bowman) space. The concave depression in the Bowman capsule is occupied by a network of capillaries known as the glomerulus. The glomerulus and Bowman capsule together constitute a renal corpuscle and comprise the functional filtering units in the kidney.

The PCT receives the glomerular filtrate from the urinary space of Bowman capsule. It is lined by a simple columnar epithelium. Its cells have an abundance of mitochondria that make the cytoplasm intensely acidophilic. Epithelial cells of the PCT have an elaborate apical microvillous brush border, which fills much of the lumen of the PCT, and deep invaginations of the basilar surface membrane. The lateral borders of individual cells are extensively interdigitated, thus giving the individual cells the appearance of a tree stump with elaborate buttressing. These microscopic features play an important role in the reabsorption of tubular water, electrolytes, bicarbonate, phosphate, and amino acids. Proximal tubules also reabsorb and metabolize filtered albumin in proteinuric states and return the amino acids and dipeptides to body's nutrition pool. The PCT leads into a proximal straight tubule, and from there the glomerular filtrate passes into the descending thin limb, the ascending thin limb, and the ascending thick limb of the loop of Henle.

Once the glomerular filtrate has ascended the loop of Henle, it passes into the DCT. A portion of the DCT contacts the vascular pole of the renal corpuscle, where it forms the macula densa of the juxtaglomerular apparatus (JGA). The DCT is lined by simple cuboidal epithelium, although it has a wider lumen than the PCT. There are only scattered apical microvilli in the DCT. The cells in the thin limbs of the loop of Henle are generally flattened and are often frankly

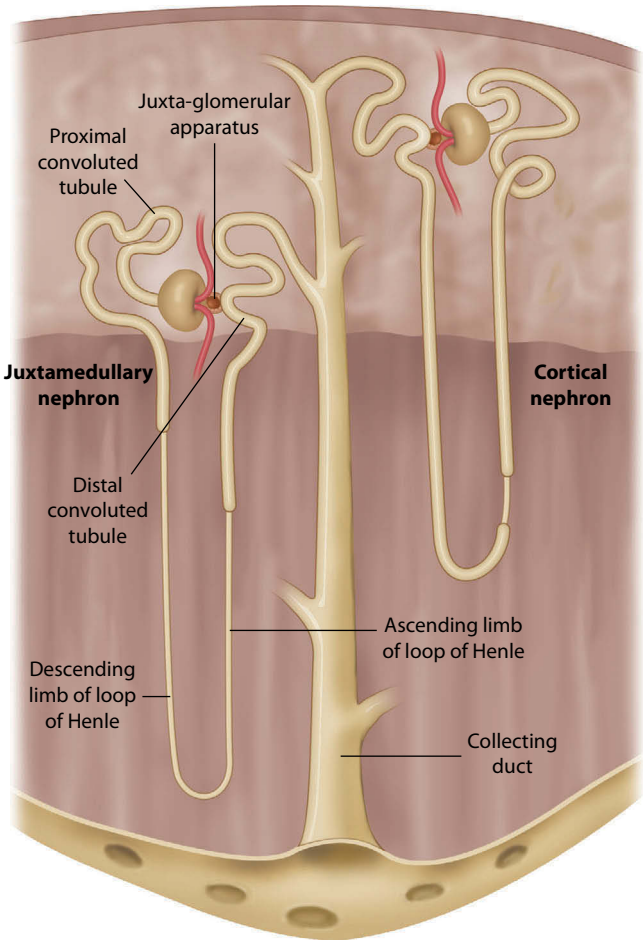


Figure 1.2 Diagrammatic representation of nephrons arranged in the kidney. Juxtaglomerular nephrons have long loops of Henle that dip deep into medulla and are important in the countercurrent urine concentrating mechanism. Cortical nephrons have relatively short loops of Henle. (Figure in the public domain; artwork by Holly Fischer. Reproduced under Creative Commons Attribution 3.0.)

squamous. The collecting tubules, the last component of the nephron, have squamous epithelium, whereas the collecting ducts have taller epithelial cells, starting as cuboidal cells in the cortex but growing taller along the route of descent to the renal papilla, eventually to form tall columnar cells in the walls of the papillary ducts (of Bellini), just before they penetrate the area cribrosa to enter the minor calyx.

RENAL BLOOD SUPPLY

Blood flow through the kidneys is surprisingly extensive (approximately 25% of cardiac output), with approximately 90% flowing through the cortex and the rest flowing through the medulla. This immense blood supply ensures rapid removal of nitrogenous wastes from the blood by the kidneys. The renal artery enters the renal sinus and divides into anterior and posterior branches, which then form segmental arteries. Segmental arteries branch into lobar arteries for each renal pyramid (lobe), which then branch again, passing in the renal columns, between the renal pyramids as interlobar arteries. Once the interlobar arteries have penetrated to the corticomedullary junction, they branch into arcuate arteries, which run parallel to the surface of the kidney. At regular intervals along the arcuate arteries, interlobular arteries project radially toward the most superficial parts of the cortex. As the interlobular arteries pass from the deep cortex to the superficial cortex, they send out branches called afferent arterioles that supply blood to the glomeruli. After leaving the glomerulus, blood enters the efferent arteriole. From here, the blood follows different pathways for cortical nephrons and juxtamedullary nephrons. In cortical nephrons, with short loops of Henle, blood passes through a complex network of capillaries, some surrounding the PCTs and DCTs (as a cortical peritubular capillary network). In contrast, in juxtamedullary nephrons, with long loops of Henle, the efferent arterioles (vasa recta), follow the loop of Henle to its tip. These blood vessels then drain into interlobular veins, arcuate veins, interlobar veins, and eventually the renal vein.

KEY POINTS

- Efferent arterioles of juxtamedullary nephrons develop anatomically distinct, long vascular channels that dip deep into the medulla along the side of their own nephrons and are called vasa recta.
- Vasa recta are low-flow arteries and are prone to desaturation-induced sickling of red blood cells in patients with sickle cell anemia or sickle cell trait.

The spaces between the renal tubules are filled with connective tissue, known as the renal interstitium. It is relatively sparse in the cortex but more abundant in the medulla. The renal interstitium also connects to a distinct connective tissue capsule around the entire kidney, with an inner layer of potentially contractile myofibroblasts and an outer layer of fibroblasts.

THE RENAL CORPUSCLE

The renal corpuscle is a prominent feature of the renal cortex. It consists of three main structures: (1) the glomerulus, a network of capillaries supplied by the afferent arteriole and drained by the efferent arteriole; (2) the Bowman capsule, an invaginated, balloon-like expansion of the PCT; and, (3) mesangial cells (Figure 1.3). Glomerular capillary endothelial cells have numerous 70- to 100-nm fenestrae

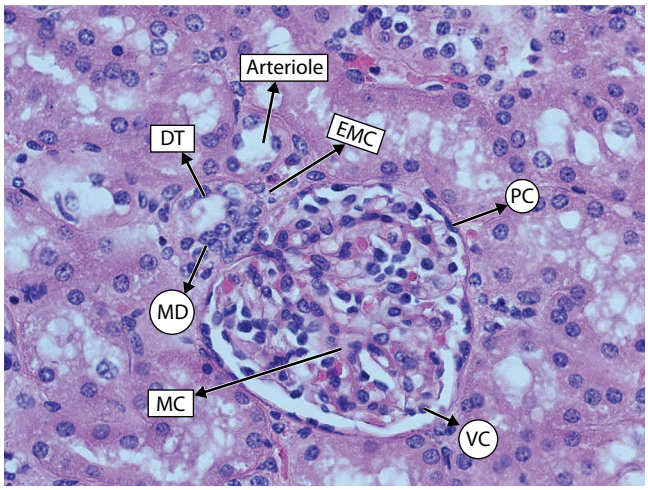


Figure 1.3 Light microscopy showing a glomerulus with the juxtaglomerular apparatus. DT, distal tubule; EMC, extraglomerular mesangial cells MC, mesangial cell with mesangial matrix; MD, macula densa; PC, parietal epithelial cell; and VC, visceral epithelial cells.

(transcellular pores) without diaphragms, and they rest on a basement membrane (Figure 1.4A). The glomerular basement membrane (GBM) is a multilayered structure with a lamina rara interna (adjacent to capillary endothelium), a lamina densa, and a lamina rara externa (adjacent to the podocytes). The laminae rarae are rich in polyanions such as heparan sulfate. These negatively charged macromolecules presumably repel anions that would otherwise cross from the capillary lumen into the urinary space (and vice versa). In addition, the laminae rarae have an abundance of fibronectin, a cell adhesion glycoprotein. Mesangial cells form a supportive complex between the capillary endothelial cells. They can proliferate and produce a basement membrane–like material and appear to have contractile, secretory, and phagocytic activity.

GBM is one of the thickest and most functionally important basement membranes in the body. The GBM is a complex extracellular matrix that is formed by both capillary endothelial cells and podocytes. It contains type IV collagen, laminin, fibronectin, entactin, and proteoglycan complex with polyanionic glycosaminoglycans such as heparan sulfate. The thickness of the GBM varies with age; it is a mean of 373 nm in men and 326 nm in women.[5] In children, however, GBM is thinner at birth, rapidly increases in size by the second year of life, and reaches adult proportions by 9 to 11 years of age.[6,7] Before 1 year, the thickness of GBM has been reported to be 132 to 208 nm, and it reached 244 to 307 nm by 11 years in one study.[6] Thinned GBM is usually seen in Alport syndrome, thin basement membrane nephropathy (TBMN), and occasionally in immunoglobulin A (IgA) nephropathy.

By electron microscopy, the GBM has a thick, central, electron-dense lamina densa (see Figure 1.4A), with less electron-dense layers adjacent to the capillary endothelial cells (lamina rara interna), and podocytes (lamina rara externa). The collagen is thought to function as a scaffold for the attachment of other glycoproteins and proteoglycans that constitute the rest of the GBM. Type IV collagen consists of three intertwined α-chain monomers, each with three distinct domains: the 7S N-terminus, a middle helical collagenous domain, and a C-terminus noncollagenous (NC1) domain.[8] The GBM also allows attachment of epithelial cells (through cell surface integrin receptors for extracellular matrix molecules such as collagen, laminin, and fibronectin) to itself and one another, and it serves as a part

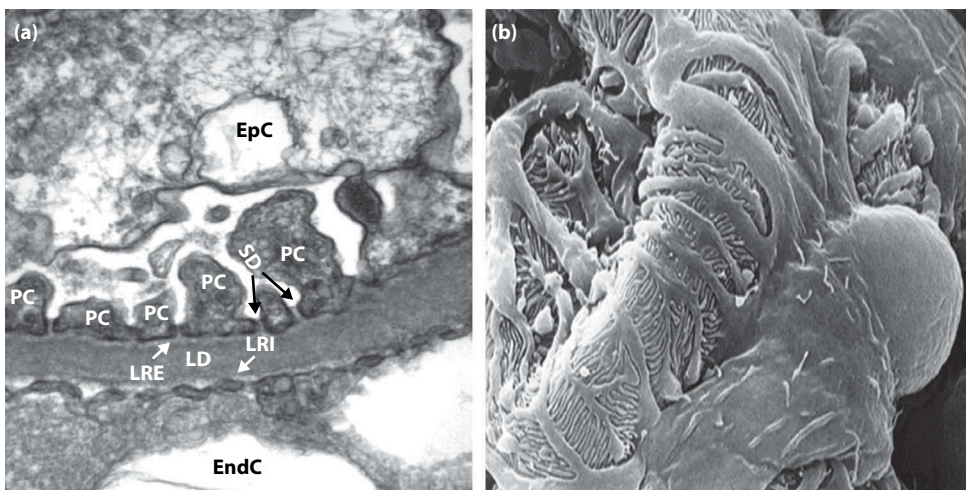


Figure 1.4 **(a)** Electron micrograph of the basement glomerular capillary. Capillary cross section showing an endothelial cell (EndC), the lamina rara interna (LRT), the lamina rara externa (LRE), the lamina densa (LD), the podocyte foot processes (PC), the epithelial cell cytoplasm (EpC), and the slit diaphragm (SD). **(b)** Electron micrograph showing extensive interdigitation of the podocyte foot processes around the basement membrane on the external or urinary side.

of the selective filtration barrier between the lumen of the glomerular capillary (the vascular space) and the lumen of the Bowman capsule (the urinary space).

PODOCYTES

The visceral layer of the Bowman capsule is formed by a sheet of stellate epithelial cells called podocytes, which have a large central cell body containing a nucleus and numerous primary, secondary, and tertiary branches projecting from the cell body. Podocytes have foot processes, which interdigitate extensively and attach the podocytes to the lamina rara externa of the GBM (Figure 1.4B).

The gaps between the adjacent foot processes, known as filtration slits, are covered by a zipper-like slit diaphragm (SD), which forms the second barrier to macromolecular transport after glomerular endothelial and GBM barriers.[8] The structure and function of SDs have been the subject of intense study. Discoveries in this area have provided important insights into the role played by the podocytes in glomerular function. The zipper-like structure of SD is made up of protein molecules from the two adjacent footprocesses that overlie each other in the midline to form the filtration barrier.[9] Of these molecules, nephrin was the first to be identified. Nephrin, which forms the bulk of SD filtration barrier, is a transmembrane protein that is anchored to the cytoplasmic membrane by podocin.[10,11] Neph 1, Neph 2, and Neph3 are other important podocyte-SD proteins that are structurally similar to nephrin and are believed to react with both nephrin and podocin to maintain the cytoskeletal structure and scaffoldings within podocytes.[12–15] The podocyte foot processes are anchored to the GBM by $\alpha_3\beta_1$ integrin.

KEY POINTS

- Epithelial cells and their foot processes are now recognized as among the most functionally relevant cell types in the glomeruli.
- Alterations in podocytes resulting from gene mutations have been linked to the pathogenesis of several types of nephrotic syndrome in children.
- With aging, podocytes are normally shed in urine.
- Injured podocytes do not regenerate.

Mutations in podocytes foot process–associated proteins cause proteinuria and nephrotic syndrome. Nephrin (*NPHS1* gene) mutation in humans result in the Finnish type of congenital nephrotic syndrome, whereas podocin (*NPHS2* gene) mutation also gives rise to steroid-resistant nephrotic syndrome.[15–17] In experimental animals, Neph1-lacking mice develop severe proteinuria that resembles the nephrin mutation in humans.

THE JUXTAGLOMERULAR APPARATUS

The DCT returns to its parent glomerulus and rests close to the vascular pole, in proximity to the afferent arteriole (see Figure 1.3). This specialized structure is the JGA. The JGA consists of three identifiable microscopic structures: (1) specialized cells of the DCT known as the macula densa, (2) juxtaglomerular (JG) cells in the afferent arteriole, and (3) extraglomerular mesangial (EGM) cells. The JGA is a sensor for sodium delivery to the distal nephron and alters the afferent and efferent arterial tone and blood flow through the locally generated renin-angiotensin system (RAS).

KEY POINTS

- The JGA is a specialized structure that is essential for renin-angiotensin hormone generation.
- The JGA controls intraglomerular pressure by regulating the relative diameter of the afferent and efferent arterioles.

Macula densa

The macula densa is the collection of columnar epithelial cells in the wall of the DCT in intimate contact with the vascular pole of the Bowman capsule and the JG cells. Here, the nuclei of the tubular epithelial cells are more crowded together than in the rest of the DCT. The apical surfaces of cells in the macula densa have numerous microvilli and a single cilium.

Juxtaglomerular cells

JG cells are modified smooth muscle like cells in the wall of the afferent arteriole. They are also sometimes found in smaller numbers in the wall of the efferent arteriole. They contain electron-dense granules of renin. As systemic blood pressure falls, the JG cells release their renin granules into the systemic circulation. Renin then cleaves angiotensinogen into the decapeptide angiotensin I. Angiotensin I is converted in pulmonary circulation into angiotensin II by the angiotensin-converting enzyme (ACE) in endothelial cells of alveolar capillaries. Angiotensin II, a potent vasoconstrictor, helps restore blood pressure to the normal range.

Extraglomerular mesangial cells

EGM cells look like fibroblasts. They provide a communicating connection between the macula densa and the JG cells. Ultrastructural studies reveal that EGM cells have long, thin processes that contact both other elements of the JGA. In addition, there are gap junctions at the tips of these processes where the EGM cells contact other cells, probably thus allowing communication among the three cell types of the JGA.

RENAL EMBRYOLOGY

Kidney and ureters develop from the intermediate mesoderm, a bulbous ridge of tissue that lies in the intraembryonic mesoderm between the somites and the lateral plate mesoderm. In contrast, bladder and the urethra develop from the urogenital sinus. Renal development occurs in three phases in a cranial to caudal sequence, starting in the fourth week. The first two stages, pronephros and mesonephros, are transitional renal structures, whereas the third stage, the metanephros, forms the definitive kidney (Figure 1.5).

The pronephros is the first renal structure, which arises as few solid or vesicular tissue segments in the cervical region at the beginning of the fourth week. It degenerates by the end of the fourth week and leaves no adult remnants. The mesonephric kidney and its associated mesonephric duct form in the urogenital ridge of the intermediate mesoderm from the upper thoracic to the upper lumbar segments. The mesonephric kidney forms rudimentary nephrons and probably functions in humans to produce some dilute urine. Most of the mesonephros degenerates by the end of the eighth week. The mesonephric duct persists in boys and men as the epididymis, the vas deferens, and the seminal vesicle, structures that drain the testis. In girls and women, the mesonephric duct forms vestigial structures such as the epoöphoron and Gartner cysts.

The metanephric kidney is the definitive kidney and the last to develop. It begins to form in the fifth week and is located in the caudal portion of the urogenital ridge. An evagination of the mesonephric duct, called the ureteric bud, grows into the surrounding mesenchyme of the urogenital ridge, called the metanephric blastema. By a reciprocal inductive interaction, the metanephric blastema triggers branching of the ureteric bud, thus ultimately forming the renal pelvis, major calyces, minor calyces, and collecting tubules. Meanwhile, the ureteric bud stimulates formation of blood vessels and nephrons in the metanephric blastema. Transformation of the metanephric blastema into a nephron undergoes the developmental stages of renal vesicle, comma-shaped body, and S-shaped body (Figure 1.6). The proximal portion of the S-shaped body invaginates to form the Bowman capsule with a cup-like recess for the development of glomerular capillaries. The distal potion connects to the branching ureteric bud to form the collecting duct.

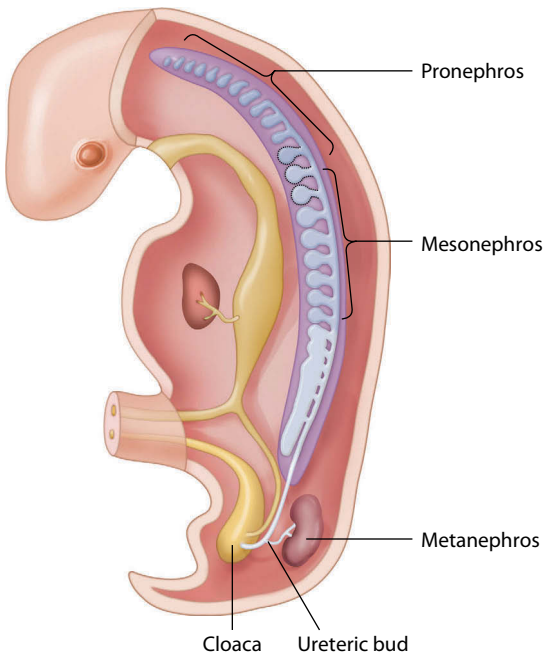


Figure 1.5 A diagrammatic view showing various phases of renal development in the embryo. The pronephros and mesonephros degenerate, whereas the metanephros formed at 5 weeks of gestation gives rise to the definitive kidney.

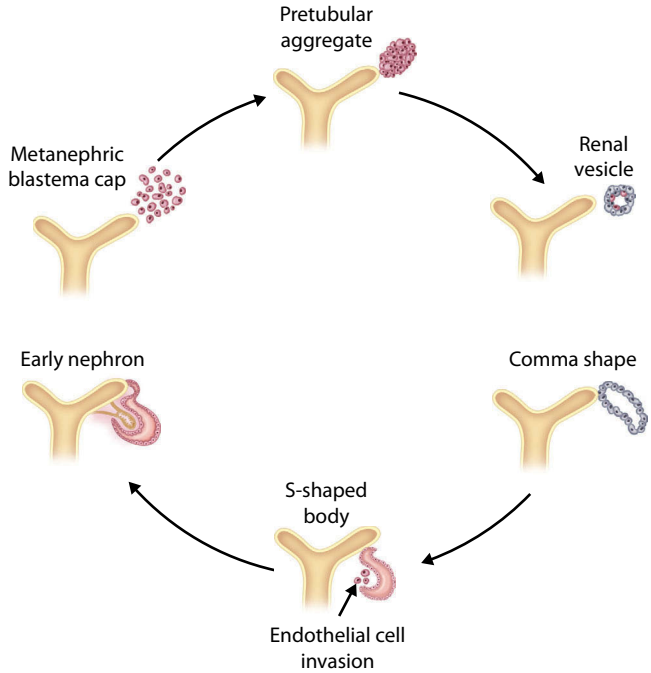


Figure 1.6 Developmental stages of the kidney in the fetus. After the ureteric bud branching, the mesenchymal cells aggregate around the tip of the ureteric bud to form cap-mesenchyme. Next, these cells evolve through the stages of renal vesicle, comma-shaped body, and then S-shaped body. The end of S-shaped body that is closest to the ureteric bud fuses with it and becomes part of the distal nephron, whereas the opposite end invaginates to give rise to the glomerular structure. Endothelial cells invade the glomerulus to form glomerular capillaries.

ASCENT OF THE METANEPHROS

The metanephric kidneys originate in the pelvis (S1 to S2 level), but following decurvature of the body axis and lumbar and sacral body growth, the definitive kidneys ascend to the lumbar area (T12 to L3). The metanephric kidney ascends between 6 and 9 weeks of gestation. When the caudal poles

of the metanephroi fuse, the ascent of a horseshoe kidney is halted by the inferior mesenteric artery, a branch from the aorta that supplies hindgut derivatives. Initially, the metanephroi are supplied by sacral branches of the aorta, but as they ascend cranially, they take new branches from the dorsal aorta that eventually develop into the renal arteries. The caudal branches usually degenerate, but supernumerary renal arteries sometimes persist, often caudal to the main renal artery. During ascent, the fetal kidneys also rotate medially by 90 degrees, so that the renal hilum, with the ureter and renal vessels, faces medially. In arrested ascent, metanephric kidneys may be formed in the pelvis, either unilaterally or bilaterally, resulting in ectopic or pelvic kidneys.

CLOACA AND FORMATION OF THE UROGENITAL SINUS

The most caudal portion of the early hindgut is the cloaca. The hindgut precursor and cloaca have a diverticulum, called the allantois, which projects far into the umbilical cord. By the fifth week, the proximal part of the cloaca becomes slightly expanded into a precursor of the urinary bladder. During the fifth week and onward, the urorectal septum divides the cloaca into a posterior anal canal and an anterior urogenital sinus. The urogenital sinus eventually gives rise to major portion of urinary bladder, and the pelvic part that becomes the membranous and prostatic urethra, and phallus. Each ureter enters the urinary bladder more lateral to the midline of the bladder, to form a roughly triangular structure called the trigone of the bladder, which is defined by the two ureteric inlets superiorly and the urethral outlet inferiorly. The proximal ends of the ureters become incorporated into the wall of the urinary bladder, so that it is lined transiently by mesodermally derived epithelium in the trigone. Later, the trigone is overgrown by endodermally derived epithelium from the rest of the bladder.

The distal portion of the allantois eventually degenerates, but the proximal portion persists as a urachal diverticulum that still projects into the umbilical cord. Eventually, the proximal urachal diverticulum forms the median umbilical ligament. Rarely, however, persistent urachus can lead to urachal fistula, wherein urine leaks from the bladder into the umbilicus or urachal cysts in the median umbilical ligament.

INTRAUTERINE RENAL FUNCTION

In humans, the pronephros is a rudimentary structure. It does not form functional nephrons and involutes in a short time. The human mesonephric kidney forms rudimentary renal corpuscles with a Bowman capsule and glomerulus and associated tubules, but there is little substantiation of its function. Mesonephric nephrons have short loops of Henle, a structure suggesting that the small volume of urine produced in the mesonephros is likely to be dilute. By the 16th week of gestation, the mesonephric kidney loses its functional capacity, and the metanephric kidney develops into a urine-producing structure, with renal corpuscles and well-differentiated PCTs with microvillous brush borders at the lumen. However, because the loops of Henle are not fully developed, fetal urine is hypotonic with respect to plasma and is slightly acidic. At term, the human fetus voids approximately 450 mL/day into the amniotic fluid. The urethra is patent in the fetus at approximately 8 weeks.

KEY POINT

The urethra is patent by the eighth week of gestation, and urine begins to form a part of amniotic fluid at around that time.

NEPHRON ENDOWMENT AND FUTURE HEALTH RISKS

Each normal adult kidney has approximately 1 million nephrons, but this estimate is quite variable. Using mature adult kidneys obtained at autopsy, Nyengaard and Bendtsen[18] noted the mean nephron number to be 617,000. A more recent US-Australian, multiethnic autopsy study[19] in adults revealed a significant variation in nephron number, ranging from 210,332 to 1,825,380 in each kidney, with a mean of 870,582 ± 31,062.

Nephron number in human fetuses increases throughout gestation, from a mean of 15,000 at 15 weeks to 740,000 in each kidney by 40 weeks.[20] This number appears to plateau at approximately 36 weeks.[20] Nephrogenesis in full-term fetuses concludes by approximately 34 to 36 weeks of gestation, and new nephrons are not formed after birth.[20,21] Premature infants, conversely, have fewer nephrons, and nephronogenesis may continue after birth for as long as 3 months.[22,23] The clinical impact of low endowed nephron number in premature infants is likely to last a lifetime and is referred to as fetal programming. Indeed, renal length and volume by ultrasonography are known to be significantly lower in young adults who were born prematurely (at less than 32 weeks), a finding suggesting long term adverse renal consequences and risks in these persons.[24] An increased risk for hypertension and CKD associated with prematurity, low birth weight, and intrauterine growth retardation was suggested by Brenner et al.,[25–27] starting in the 1980s. In an Australian study,[28] 18-year-old survivors of extreme prematurity (gestational age less than 28 weeks) had higher

KEY POINT

Poor nephron endowment at birth (fetal programming) affects blood pressure, development of microalbuminuria, and can lead to ESRD in adults.

ambulatory blood pressure readings, thus lending weight to the hypotheses proposed by Brenner and others. A meta-analysis of studies relating prematurity and blood pressure also demonstrated a higher blood pressure in persons who were born prematurely (range, 28.8 to 34.1 weeks).[29]

The link between poor nephron endowment and CKD is plausible, but less well established. Microalbuminuria noted in otherwise healthy nondiabetic adults (46 to 54 years old) has been correlated with low birth weight as a risk factor.[30] Increased prevalence of ESRD in adults born with a birth weight of less than 2.5 kg was noted in one study in the United States, and the odds ratio for ESRD was 1.4 (95% confidence interval [CI], 1.1 to 1.8), as compared with a birth weight of 3 to 3.5 kg.[31] Carmody and Charlton[32] suggested that apart from low nephron endowment in premature infants, other factors that may enhance the risk for CKD include neonatal acute kidney injury, use of nephrotoxic antibiotics, poor nutrition, and hypoxia in early life.

KEY POINTS

Renal risks in premature infants are compounded by:

- Acute kidney injury
- Hypoxia
- Use of nephrotoxic drugs
- Poor nutrition

CONGENITAL ABNORMALITIES OF THE KIDNEY AND URINARY TRACT

The term *congenital abnormalities of the kidney and urinary tract* (CAKUT) denotes developmental urologic and renal abnormalities encountered in children.[32,33] CAKUT, as a term, also emphasizes the shared developmental destiny of the kidney and the urinary tract (Figure 1.7). The CAKUT spectrum encompasses diverse clinical disorders, such as unilateral or bilateral renal agenesis, renal hypodysplasia, multicystic dysplastic kidney (MCDK), ureteropelvic junction obstruction, posterior urethral valves, vesicoureteral reflux (VUR), duplex renal collecting system, ectopic ureters, and megaureter.

KEY POINT

CAKUT, as a concept, reflects a common origin of renal and urologic anomalies in utero.

CAKUT occurs in approximately 1 in 500 live births, but severe abnormalities resulting in neonatal death occur less frequently, in approximately 1 in 2000 births.[33,34] By using prenatal ultrasound as the screening method in 709,030 live births, stillbirths, and induced abortions, 1130 infants

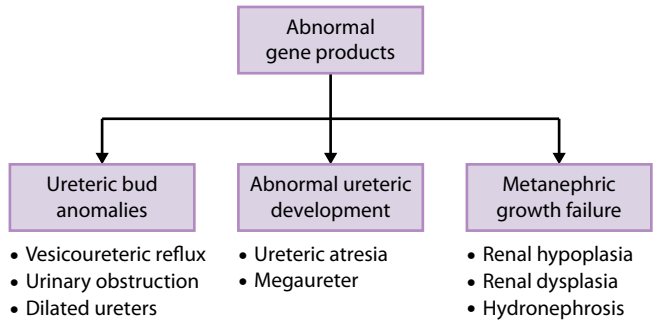


Figure 1.7 Pathogenesis of congenital abnormalities of the kidney and urinary tract. This unifying hypothesis breaks from previously believed concepts that developmental renal defects arise from urinary obstruction. It is now believed that genetic defects disrupt critical signaling processes between the ureteric bud and nephrogenic mesenchyme, eventually leading to uronephrologic developmental disorders. (Based on Ichikawa I, Kuwayama F, Pope JC, 4th, et al. Paradigm shift from classic anatomic theories to contemporary cell biological views of CAKUT. Kidney Int. 2002 61:889–98.)

and fetuses were diagnosed with at least one renal or urologic abnormality, amounting to an incidence of 1.6 in 1000 pregnancies.[35] CAKUT is often encountered as an isolated or sporadic anomaly, but it can also be a clinical feature of numerous syndromes. Additionally, an increased risk of urinary tract anomalies has been reported in the close family members of these patients.[36,37] Some of the common CAKUT malformations are discussed here, as well as in Chapter 2. VUR is discussed in Chapter 48.

Renal agenesis

Bilateral renal agenesis is an uncommon disorder. Potter described 20 cases of bilateral renal agenesis in 5000 autopsies performed in children and estimated the incidence to be 1 in 3000 to 1 in 4000 births.[38,39] Approximately 25% to 40% of fetuses with bilateral renal agenesis are stillborn. Bilateral renal agenesis can result in the clinical features of Potter sequence as a result of prolonged lack of intrauterine urine and oligohydramnios.[40–42] Potter sequence (or Potter syndrome) is characterized by: bilateral renal agenesis (or severe fetal renal disease), oligohydramnios, pulmonary

KEY POINTS

Potter sequence includes:

- Renal agenesis or severe dysplasia
- Oligohydramnios
- Pulmonary hypoplasia
- Low-set ears
- Clubfeet
- Pointed nose

hypoplasia, clubfeet, micrognathia, a pointed nose, and low-set malformed ears.[40] Potter facies refers to the typical facial features seen in Potter sequence. Potter sequence can result from any fetal disorder that leads to prolonged oligohydramnios. There has been an ongoing speculation that bilateral renal agenesis may be an inherited disorder, primarily because of a significantly higher incidence of occult renal defects in close relatives of index cases.[43] Recessive mutations in the integrin α_8-encoding gene *ITGA8* have been described in families with fetal loss secondary to bilateral renal agenesis.[44] Such findings are likely to stimulate investigative pathways to genetic pathogenesis of bilateral renal agenesis.

Accurate information of the incidence of unilateral renal agenesis is difficult to obtain because many of these cases go undetected as a result of compensatory hypertrophy of the contralateral kidney, as well as normal intrauterine and postnatal renal function. Using renal ultrasound screening in healthy school-age children (6 to 15 years), Sheih et al.[45] reported the occurrence of unilateral renal agenesis to be 1 in 290 in the general population of children. European data also suggest the incidence of unilateral renal agenesis to be 1 in 2000 births.[46]

Unilateral renal agenesis is often associated with ipsilateral defects in other genital duct derivatives such as the ductus deferens in boys and the uterine tubes and uterus in girls. VUR is common (approximately 25%) in these patients, and extrarenal malformations affecting the gastrointestinal, cardiac, and musculoskeletal systems are seen in approximately 30% of patients.[46] An increased incidence of renal anomalies in the children and siblings of patients with unilateral renal agenesis has been noted in some studies.[36]

Pelvic and horseshoe kidneys

Pelvic kidney and horseshoe kidney are related renal malformations that result from failed ascent of the kidneys. In horseshoe kidney, the caudal poles of the metanephric kidney are fused. Horseshoe kidney is one of the most common congenital anomalies of the kidney. On the basis of abdominal CT scan data in 15,320 patients, the incidence of horseshoe kidney was noted to be 1 in 666.[47] Approximately half of the patients diagnosed with horseshoe kidney are asymptomatic; the remainder present for renal stones and ascending urinary tract infections.[48] The Rovsing sign, consisting of nausea, vomiting, and abdominal pain that is accentuated on hyperextension, is seen in a minority of patients with horseshoe kidney.[48] Horseshoe kidney can occur as an isolated congenital birth defect but is more frequent in patients with Turner syndrome and trisomy 18 (Edwards syndrome).[48,49]

Multicystic dysplastic kidneys

MCDKs are developmental, nonfunctional cystic masses caused by abnormalities in metanephric differentiation. These kidneys are characterized by abnormal and

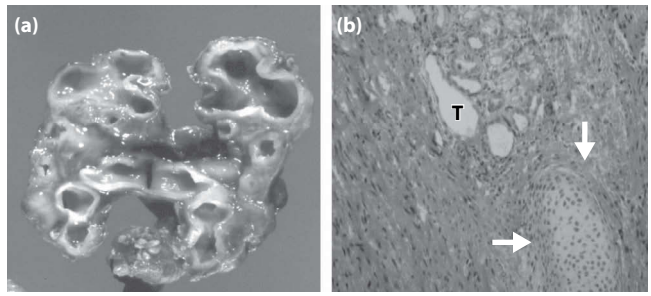


Figure 1.8 **(a)** Cut section of a kidney from a neonate shows cystic dysplasia. Renal architecture is poorly defined, numerous cysts are present, and the corticomedullary differentiation is absent. **(b)** Microscopic section of a kidney with renal dysplasia. Arrows shows the presence of cartilage. Tubulointerstitial tissue is poorly organized, and a large dilated renal tubule (T) is shown. (Photomicrograph courtesy of Arthur Cohen, MD.)

noncommunicating dilated cysts of variable size, with little identifiable renal structure or stroma (Figures 1.8 and 1.9). Renal arterial flow and excretory functions, as demonstrable by mercaptoacetyltriglycine (MAG3) renal scan, are absent.

MCDK is commonly identified by the presence of a unilateral cystic kidney in the fetus during prenatal ultrasound evaluation. Cystic kidneys may involute during gestation in some cases, and the infant is born with a solitary kidney. Other modes of presentation include an abdominal mass noted by the parents or during a clinical examination within few months of birth. In a study of 97 infants with the diagnosis of MCDK, 85% of the cases were detected by prenatal ultrasound evaluation, 4% by the presence of a mass postnatally, and 11% by a postnatal ultrasound examination

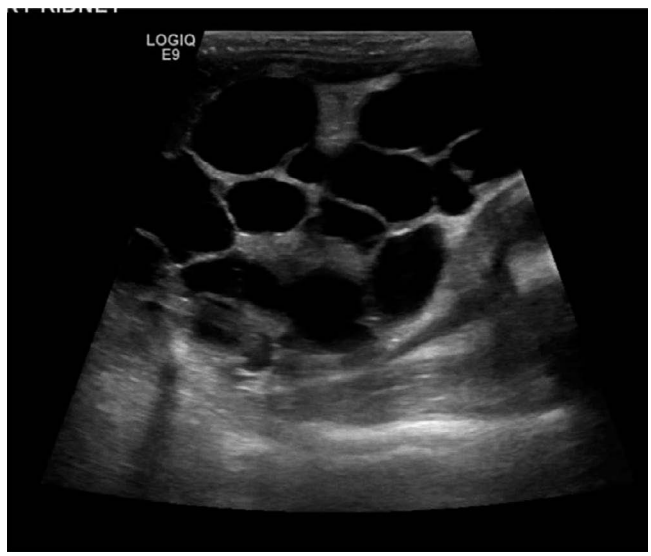


Figure 1.9 Renal ultrasound scan showing multiple large cysts in an infant with multicystic dysplastic kidney disease. Nuclear scan demonstrated no blood flow or excretory function in this kidney.

performed for an unrelated diagnosis.[50] MCDK is slightly more common on the left side in some studies, whereas other investigators have noted a right-sided predominance; these findings suggest that both sides can be equally involved.[38,39] Contralateral kidneys show abnormalities in approximately 40% of cases that include renal agenesis, renal dysplasia or hypoplasia, VUR, hydroureter or pyelectasis, duplex collecting system, and ectopic ureters.[39] In a meta-analysis of the abnormalities in the contralateral system, Schreuder et al.[51] reported VUR in approximately 20% cases: 15% in the contralateral system, ipsilateral VUR in 3.3%, and bilateral VUR in 2.4% cases. Ureteropelvic junction obstruction was the next most common anomaly in the contralateral renal system, accounting for 4.8% cases in this meta-analysis.

Involution of the cystic mass occurs usually occurs over several years, but the rate of involution is variable. Some patients have complete involution of the MCDK in utero and are thus born with a solitary kidney and unilateral agenesis. Complete involution of the MCDK was noted in 34% at 2 years (165 patients), 47% at 5 years (117 patients), and 59% at 10 years (43 patients) in a study from the United Kingdom.[52] The contralateral normal kidney usually undergoes compensatory hypertrophy.

Hypertension has been well documented in patients with MCDK and is believed to be mediated by the RAS.[53] More recent studies, however, point out that the risk of hypertension in MCDK is low. In a review of 29 studies, Narchi et al.[54] reported hypertension to be present in only 6 of the 1115 cases, a finding suggesting the mean probability of developing hypertension in MCDK to be 5.4 in 1000 cases (estimated 95% CI, 1.9 to 11.7 per 1000).

A high risk for Wilms tumor and renal cell carcinoma in the MCDK was suggested in older studies. However, the risk of tumors was very low in several more recent, comprehensive, well-conducted studies.[55,56] Surgical resection of the MCDK, which was often practiced until the late 1980s for the prevention of hypertension and to protect against development of malignant disease, appears to be difficult to justify with the evidence-based results.[54–57]

WAGR syndrome

Wilms tumor, aniridia, genitourinary abnormalities, and mental retardation (WAGR) was first reported as a distinct clinical disorder by Miller et al.[58] in 1964 (Online Mendelian Inheritance in Man [OMIM] number 194072). It is now well established that the disorder results from deletion in distal band of 11p13 in such a way that *WT1* and *PAX6* genes, which are adjacent to each other, are affected by the deletion.[59] The *WT1* gene deletion results in renal malformations and risk for Wilms tumor; whereas the *PAX6* gene deletion results in aniridia and brain malformations.

The *WT1* gene encodes for Wilms tumor suppressor protein (WT1), a transcription factor containing a DNA binding domain, with four zinc fingers. WT1 transcription factor is essential for the development of the nephron, as well as the gonads, and is thought to be involved in mesenchymal-epithelial transition in the renal and germ cell lines.[60] With mutations in the *WT1* gene, the development of glomeruli and of the proximal and distal tubules is adversely affected, leading to renal malformations. A propensity for Wilms tumor in WAGR and other syndromes with the *WT1* gene mutation, such as Denys-Drash syndrome, results from the presence of undifferentiated or poorly differentiated mesenchymal cells in the kidneys.[60,61]

The genital malformations seen in WAGR syndrome include cryptorchidism, ambiguous genitalia, hypospadias, streaked ovaries, and hypoplastic uterus. Renal and urinary tract anomalies encountered in WGAR syndrome include hypoplastic kidneys, unilateral renal agenesis, duplicated ureters,, development of focal segmental glomerulosclerosis, nephrogenic rests, nephroblastomatosis, and renal cysts.[62] Aniridia can be partial or complete and results in severe visual impairment. Apart from risk of CKD and ESRD imposed by the development of Wilms tumor, patients with WAGR syndrome are inherently at an increased risk for development of ESRD. The National Wilms Tumor Study Group reports the incidence of ESRD to be 53% in patients with WAGR syndrome.[63,64] Monitoring of kidneys by ultrasound every 3 months until 6 years of age has been recommended in some studies for Wilms tumor surveillance in patients with WAGR syndrome.[62]

Bladder exstrophy

Bladder exstrophy is an uncommon developmental anomaly seen in newborn infants. It is caused by a ventral body wall, an anterior bladder wall defect, and eversion of the bladder wall mucosa. The term exstrophy, which is derived from the Greek word *ekstriphein*, means "turned inside out." In the newborn infant, the exposed bladder mucosa is bright red and has a raw appearance. The exposed mucosa sometimes undergoes metaplasia, forming colonic epithelium rather than transitional epithelium. Bladder exstrophy is also associated with other congenital abnormalities of the external genitalia such as bifid penis, epispadias, and abnormal scrotal development

Using the Healthcare Cost and Utilization Project Nationwide Inpatient Sample database, Nelson et al.[65] estimated the incidence of bladder exstrophy to be 2.15 in 100,000 live births (or approximately 1 in 40,000 births). The male-to-female ratio was equal in this study, and the congenital malformation appeared to be more common in whites than in nonwhites. Some genetic analyses have demonstrated a higher prevalence of duplication of 22q11.21 in patients with bladder exstrophy.[66]

Surgical repair of bladder exstrophy has evolved since the 1990s. Both early closure and delayed closure of the defect are acceptable surgical options and are generally dictated by technical preference of the surgical team.[67] Postrepair attention to incontinence, recurrent urinary tract infections secondary to associated VUR, and correction of epispadias are common concerns to be addressed during childhood. Many patients require continent urinary diversion procedures,

as well as bladder augmentation by cystoplasty. However, lower urinary tract symptoms persist in 80% of patients with exstrophy as they grow into adulthood.[68] Sexual dysfunction is also a concern in adulthood.[69] In addition, adenocarcinomas occur with higher frequency in the exposed bladder mucosa. Surgical intervention and repair can extend the patient's life and ensure normal renal function.

Angiotensin-converting enzyme fetopathy

Experimental observations indicate that RAS exerts its influence on renal development by promoting the ureteric bud branching process and therefore plays a key role in nephrogenesis.[70,71] The RAS may affect ureteric bud branching and nephrogenesis through its influence on GDNF, the *WT1* gene, and the *PAX2* gene. Disruption of the RAS during nephrogenesis by ACE inhibitors (ACEIs) is well known to cause fetopathy.[70,72] ACEI fetopathy risk is low if the drug is consumed in the first trimester.[72–74] Clinical characteristics of ACEI fetopathy are: oligohydramnios, renal tubular dysgenesis, neonatal anuria, skull defects, intrauterine growth retardation, and patent ductus arteriosus.

KEY POINTS

- The RAS plays a crucial role in nephrogenesis.
- Maternal ACEI use after the first trimester is associated with fetopathy that includes renal dysgenesis.

SUMMARY

A significant advance in our understanding of the signal pathways involved during development of the kidneys and the urinary tract in fetal life has occurred since the 1990s. What has also become obvious is that the renal development is supported by the development of the rest of the urinary tract, especially the ureteric bud. A disruption in any of these developmental processes can lead to abnormalities of renal development and CAKUT. Nephron endowment at birth is beginning to be recognized as an important predictor of CKD and hypertension in adults. Attention to prenatal health and prevention of poor nephron endowment may be ways that kidney disease and hypertension can be prevented in adults.

REFERENCES

1. Scott JE, Hunter EW, Lee RE, et al. Ultrasound measurement of renal size in newborn infants. Arch Dis Child. 1990;65:361–4.
2. Pantoja Zuzuárregui JR1, Mallios R, Murphy J. The effect of obesity on kidney length in a healthy pediatric population. Pediatr Nephrol. 2009;24:2023–7.
3. Alev Kadioglu. Renal measurements, including length, parenchymal thickness, and medullary pyramid thickness, in healthy children: What are the normative ultrasound values? AJR Am J Roentgenol. 2010;194:509–15.
4. Rosenbaum DM, Korngold E, Teele RL. Sonographic assessment of renal length in normal children. AJR Am J Roentgenol. 1984;142:467–9.
5. Steffes MW, Barbosa J, Basgen JM, et al. Quantitative glomerular morphology of the normal human kidney. Lab Invest. 1983;49:82–6.
6. Vogler C, McAdams AJ, Homan SM. Glomerular basement membrane and lamina densa in infants and children: An ultrastructural evaluation. Pediatr Pathol. 1987;7:527–34.
7. Morita M, White RH, Raafat F, et al. Glomerular basement membrane thickness in children: A morphometric study. Pediatr Nephrol. 1988;2:190–5.
8. Miner JH. Glomerular basement membrane composition and the filtration barrier. Pediatr Nephrol. 2011;26:1413–7.
9. Rodewald R, Karnovsky MJ. Porous substructure of the glomerular slit diaphragm in the rat and mouse. J Cell Biol. 1974;60:423–33.
10. Kestilä M1, Lenkkeri U, Männikkö M, et al. Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. Mol Cell. 1998;1:575–82.
11. Wartiovaara J, Ofverstedt LG, Khoshnoodi J, et al. Nephrin strands contribute to a porous slit diaphragm scaffold as revealed by electron tomography. J Clin Invest. 2004;114:1475–83.
12. Sellin L, Huber TB, Gerke P, et al. NEPH1 defines a novel family of podocin interacting proteins. FASEB J. 2003;17:115–7.
13. Liu G, Kaw B, Kurfis J, et al. Neph1 and nephrin interaction in the slit diaphragm is an important determinant of glomerular permeability. J Clin Invest. 2003;112:209–21.
14. Garg P, Verma R, Nihalani D, et al. Neph1 cooperates with nephrin to transduce a signal that induces actin polymerization. Mol Cell Biol. 2007;27:8698–712.
15. Grahammer F, Schell C, Huber TB. The podocyte slit diaphragm: From a thin grey line to a complex signalling hub. Nat Rev Nephrol. 2013;9:587–98.
16. Heeringa SF, Vlangos CN, Chernin G, et al. Thirteen novel NPHS1 mutations in a large cohort of children with congenital nephrotic syndrome. Nephrol Dial Transplant. 2008;23:3527–33.
17. Machuca E, Benoit G, Nevo F, Tête MJ, et al. Genotype-phenotype correlations in non-Finnish congenital nephrotic syndrome. J Am Soc Nephrol. 2010;21:1209–17.

18. Nyengaard JR, Bendtsen TF. Glomerular number and size in relation to age, kidney weight, and body surface in normal man. Anat Rec. 1992;232,194–201.
19. Hoy WE, Douglas-Denton RN, Hughson MD, et al. A stereological study of glomerular number and volume: Preliminary findings in a multiracial study of kidneys at autopsy. Kidney Int Suppl. 2003;63, S31–7.
20. Hinchliffe SA, Sargent PH, Howard CV, et al. Human intrauterine renal growth expressed in absolute number of glomeruli assessed by the dissector method and Cavalieri principle. Lab Invest. 1991;64:777–84.
21. Moritz KM, Caruana G, Wintour EM. Development of kidneys and urinary tract. In Rodeck CH, Whittle MF, editors. Fetal Medicine: Basic Science and Clinical Practice, 2nd ed. Philadelphia: Churchill Livingstone; 2009. p. 147.
22. Hinchliffe SA, Lynch MR, Sargent PH, et al. The effect of intrauterine growth retardation on the development of renal nephrons. Br J Obstet Gynaecol. 1992;99:296–301.
23. Mañalich R1, Reyes L, Herrera M, et al. Relationship between weight at birth and the number and size of renal glomeruli in humans: A histomorphometric study. Kidney Int. 2000;58:770–3.
24. Keijzer-Veen MG1, Devos AS, Meradji M, et al. Reduced renal length and volume 20 years after very preterm birth. Pediatr Nephrol. 2010;25:499–507.
25. Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure: Less of one, more of the other? Am J Hypertens. 1988;1:335–47.
26. Brenner BM, Mackenzie HS. Nephron mass as a risk factor for progression of renal disease. Kidney Int Suppl. 1997;63 S124–7.
27. Luyckx VA, Bertram JF, Brenner BM, et al. Effect of fetal and child health on kidney development and long-term risk of hypertension and kidney disease. Lancet. 2013 20;382:273–83.
28. Roberts G1, Lee KJ, Cheong JL, et al. Higher ambulatory blood pressure at 18 years in adolescents born less than 28 weeks' gestation in the 1990s compared with term controls. J Hypertens. 2014;32:620–6.
29. de Jong F, Monuteaux MC, van Elburg RM, et al. Systematic review and metaanalysis of preterm birth and later systolic blood pressure. Hypertension. 2012;59:226–34.
30. Yudkin JS, Phillips DI, Stanner S. Proteinuria and progressive renal disease: Birth weight and microalbuminuria. Nephrol Dial Transplant. 1997;12(Suppl 2):10–3.
31. Lackland DT, Bendall HE, Osmond C, et al. Low birth weights contribute to high rates of early-onset chronic renal failure in the Southeastern United States. Arch Intern Med. 2000;160:1472–6.
32. Carmody JB, Charlton JR. Short-term gestation, long-term risk: Prematurity and chronic kidney disease. Pediatrics. 2013;131:1168–79.
33. Ichikawa I, Kuwayama F, Pope JC, 4th, et al. Paradigm shift from classic anatomic theories to contemporary cell biological views of CAKUT. Kidney Int. 2002;61:889–98.
34. Pope JC 4th, Brock JW, 3rd, Adams MC, et al. How they begin and how they end: Classic and new theories for the development and deterioration of congenital anomalies of the kidney and urinary tract, CAKUT. J Am Soc Nephrol. 1999;10:2018–28.
35. Wiesel A, Queisser-Luft A, Clementi M, et al. Prenatal detection of congenital renal malformations by fetal ultrasonographic examination: An analysis of 709,030 births in 12 European countries. Eur J Med Genet. 2005;48:131–44.
36. McPherson E. Renal anomalies in families of individuals with congenital solitary kidney. Genet Med. 2007;9:298–302.
37. Bulum B, Ozçakar ZB, Ustüner E, et al. High frequency of kidney and urinary tract anomalies in asymptomatic first-degree relatives of patients with CAKUT. Pediatr Nephrol. 2013;28:2143–7.
38. Mansoor O, Chandar J, Rodriguez MM, et al. Long-term risk of chronic kidney disease in unilateral multicystic dysplastic kidney. Pediatr Nephrol. 2011;26:597–603.
39. Damen-Elias HA, Stoutenbeek PH, Visser GH, et al. Concomitant anomalies in 100 children with unilateral multicystic kidney. Ultrasound Obstet Gynecol. 2005;25:384–8.
40. Potter EL. Bilateral renal agenesis. J Pediatr. 1946;29:68–76.
41. Potter EL. Normal and Abnormal Development of the Kidney. Chicago: Year Book Medical Publishers; 1972. p. 1–305.
42. Wilson RD, Baird PA. Renal agenesis in British Columbia. Am J Med Genet. 1985;21:153–69.
43. Roodhooft AM, Birnholz JC, Holmes LB. Familial nature of congenital absence and severe dysgenesis of both kidneys. N Engl J Med. 1984;310:1341–5.
44. Humbert C, Silbermann F, Morar B, et al. Integrin alpha 8 recessive mutations are responsible for bilateral renal agenesis in humans. Am J Hum Genet. 2014;94:288–94.
45. Sheih CP, Liu MB, Hung CS, et al. Renal abnormalities in schoolchildren. Pediatrics. 1989;84:1086–90.
46. Westland R, Schreuder MF, Ket JC, et al. Unilateral renal agenesis: A systematic review on associated anomalies and renal injury. Nephrol Dial Transplant. 2013;28:1844–55.
47. Weizer AZ, Silverstein AD, Auge BK, et al. Determining the incidence of horseshoe kidney from radiographic data at a single institution. J Urol. 2003;170:1722–6.
48. Glenn JF. Analysis of 51 patients with horseshoe kidney. N Engl J Med. 1959;261:684–7.
49. Bilge I, Kayserili H, Emre S, et al. Frequency of renal malformations in Turner syndrome: Analysis of 82 Turkish children. Pediatr Nephrol. 2000;14:1111–4.

50. Kuwertz-Broeking E, Brinkmann OA, Von Lengerke HJ, et al. Unilateral multicystic dysplastic kidney: Experience in children. BJU Int. 2004;93:388–92.
51. Schreuder MF, Westland R, van Wijk JA. Unilateral multicystic dysplastic kidney: A metaanalysis of observational studies on the incidence, associated urinary tract malformations and the contralateral kidney. Nephrol Dial Transplant. 2009;24:1810–8.
52. Aslam M, Watson AR, Trent, and Anglia MCDK Study Group. Unilateral multicystic dysplastic kidney: Long term outcomes. Arch Dis Child. 2006;91:820–3.
53. Snodgrass WT. Hypertension associated with multicystic dysplastic kidney in children. J Urol. 2000;164:472–3.
54. Narchi H. Risk of hypertension with multicystic kidney disease: A systematic review. Arch Dis Child. 2005;90:921–4.
55. Narchi H. Risk of Wilms' tumour with multicystic kidney disease: A systematic review. Arch Dis Child. 2005;90:147–9.
56. Eickmeyer AB, Casanova NF, He C, et al. The natural history of the multicystic dysplastic kidney: Is limited follow-up warranted? J Pediatr Urol. 2014;10:655–61.
57. Tilemis S, Savanelli A, Baltogiannis D, et al. Is the risk of hypertension an indication for prophylactic nephrectomy in patients with unilateral multicystic dysplastic kidney? Scand J Urol Nephrol. 2003;37:429–32.
58. Miller RW, Fraumeni JF Jr, Manning MD. Association of Wilms' tumor with aniridia, hemihypertophy and other congenital malformations. N Engl J Med. 1964;270:922–7.
59. Francke U, Holmes LB, Atkins L, Riccardi VM. Aniridia-Wilms' tumor associations: Evidence for specific deletion of 11p13. Cytogenet Cell Genet. 1979;24:185–92.
60. Rauscher FJ. The WT1 Wilms tumor gene product: A developmentally regulated transcription factor in the kidney that functions as a tumor suppressor. FASEB J. 1993;7:896–903.
61. Morrison AA, Viney RL, Saleem MA, et al. New insights into the function of the Wilms tumor suppressor gene *WT1* in podocytes. Am J Physiol Renal Physiol. 2008;295:F12–7.
62. Fischbach BV, Trout KL, Lewis J, et al. WAGR syndrome: A clinical review of 54 cases. Pediatrics. 2005;116:984–8.
63. Breslow NE, Norris R, Norkool P, et al. Characteristics and outcomes of children with the Wilms' tumor-aniridia syndrome: A report from the National Wilms' Tumor Study Group. J Clin Oncol. 2003;24:4579–85.
64. Breslow NE, Collins AJ, Ritchey ML, et al. End stage renal disease in patients with Wilms tumor: Results from the National Wilms Tumor Study Group and the United States Renal Data System. J Urol. 2005;174:1972–5.
65. Nelson CP, Dunn RL, Wei JT. Contemporary epidemiology of bladder exstrophy in the United States. J Urol. 2005;173:1728–31.
66. Draaken M, Baudisch F, Timmermann B, et al. Classic bladder exstrophy: Frequent 22q11.21 duplications and definition of a 414 kb phenocritical region. Birth Defects Res A Clin Mol Teratol. 2014;100:512–7.
67. Dickson AP. The management of bladder exstrophy: The Manchester experience. J Pediatr Surg. 2014;49:244–50.
68. Taskinen S, Suominen JS. Lower urinary tract symptoms (LUTS) in patients in adulthood with bladder exstrophy and epispadias. BJU Int. 2013;111:1124–9.
69. Gupta AD, Goel SK, Woodhouse CR, et al. Examining long-term outcomes of bladder exstrophy: A 20-year follow-up. BJU Int. 2014;113:137–41.
70. Yosypiv IV. Renin-angiotensin system in ureteric bud branching morphogenesis: Insights into the mechanisms. Pediatr Nephrol. 2011;26:1499–512.
71. Song R, Spera M, Garrett C, et al. Angiotensin II AT2 receptor regulates ureteric bud morphogenesis. Am J Physiol Renal Physiol. 2010;298:F807–17.
72. Pryde PG, Sedman AB, Nugent CE, Barr M. Angiotensin-converting enzyme inhibitor fetopathy. J Am Soc Nephrol. 1993;3:1575–82.
73. Buttar HS. An overview of the influence of ACE inhibitors on fetal-placental circulation and perinatal development. Mol Cell Biochem. 1997;176:61–71.
74. Anonymous. Post marketing surveillance for angiotensin-converting enzyme inhibitor use during the first trimester of pregnancy: United States, Canada, and Israel, 1987–1995. MMWR Morb Mortal Wkly Rep. 1997;46:240–2.

REVIEW QUESTIONS

1. The juxtamedullary nephrons have long loops of Henle that participate in:
   a. Sodium reabsorption
   b. Chloride transport
   c. Urine concentrating mechanism
   d. None of the above
2. Nephrin constitutes a structural component of:
   a. Endothelial cytoskeleton
   b. Podocyte cytoskeleton
   c. Slit diaphragm
   d. Bowman capsule
3. Which of the following combinations are transitional embryonic kidneys?
   a. Metanephros and mesonephros
   b. Mesonephros and pronephros
   c. Pronephros and metanephros
   d. None of the above

4. In a premature infant, nephrogenesis can continue up to 3 months after birth:
   a. True
   b. False
5. Characteristic finding on MAG3 renal scan in a patient with multicystic dysplastic kidney (MCDK) is:
   a. Photopenic areas in the affected kidney suggesting cysts
   b. Arterial flow from the surrounding tissue
   c. Adequate blood flow but no excretory function
   d. No demonstrable renal blood flow and no excretory function
6. Vesicoureteral reflux on the same side or contralateral side of MCDK occurs in:
   a. Fewer than 5% cases
   b. 20% cases
   c. 50% cases
   d. Almost always in all patients
7. Wilms tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR) is caused by:
   a. Mutation in WT1 gene
   b. Mutation in PAX6 gene
   c. Mutation in PAX6 and WT1
   d. Mutation in *WT1* and GDNF
8. ACEI-induced fetapathy is more common if the drug exposure occurs in:
   a. First 2 weeks of pregnancy
   b. Six to 12 weeks of pregnancy
   c. Last week of pregnancy
   d. After first trimester of pregnancy
9. Horseshoe kidneys are common in:
   a. Down syndrome
   b. Turner syndrome
   c. WAGR syndrome
   d. Renal coloboma syndrome
10. Low nephron number at birth is associated with:
    a. Future risk of diabetes mellitus
    b. Future risk of cardiac disease
    c. Future risk of hypertension
    d. No associated risk of disease

ANSWER KEY

1. c
2. c
3. c
4. a
5. d
6. b
7. c
8. d
9. b
10. c

2

Molecular basis of developmental renal disease

NORMAN D. ROSENBLUM

Developmental abnormalities of the urinary tract are common and account for 30% to 50% cases of end-stage renal disease in children.[1] Formation of the kidney consists of a complex series of morphogenetic events during intrauterine development that are complete by approximately 34 weeks of gestation. However, growth of kidney cells continues after birth, as does functional development of the kidneys. Renal development is incomplete at birth, even in full-term infants and to a greater extent in preterm infants. Mature levels of renal function are not achieved until approximately 2 years of age. The objectives of this chapter are to supplement the broad concepts of renal embryologic development discussed in Chapter 1 and to explore the molecular events involved in nephrogenesis.

EARLY FETAL KIDNEY DEVELOPMENT

In mammals, the kidneys develop in three stages, from rostral (head end) to caudal (rump end): the pronephros, the mesonephros, and the metanephros (permanent kidney). The pronephros is rudimentary and nonfunctional. The mesonephros functions briefly and then involutes toward the end of the first trimester. The metanephros does not involute and becomes the permanent kidneys. Metanephric development begins during the fifth week of gestation, and urine excretion is initiated at approximately the 10th week of gestation.

During fetal life, the kidneys are lobulated, but a lobular appearance is present even at birth. Thereafter, the external kidney surface becomes smooth as the kidney grows. It is becoming increasingly apparent that the number of nephrons endowed at birth has an important bearing on the susceptibility to hypertension and chronic kidney disease as adults.[2] Minimizing disruptions in fetal renal development may, indeed, be central to preventing kidney disease in adults.

Initially, the kidneys lie adjacent to each other in the pelvis, and the hilum of each faces ventrally (toward the anterior abdominal wall). As the trunk grows, the kidneys come to lie higher in the abdomen and farther apart. In addition, the hilum rotates almost 90 degrees. By 9 weeks of gestation, the kidneys attain their adult positions. Malrotation and ectopic kidney location are caused by abnormal rotation and ascent, respectively. Failure of the kidneys to migrate upward from the pelvis results in the formation of pelvic kidneys. These kidneys are positioned close to each other and may fuse in some cases to give rise to a *pancake kidney*. In approximately 1 in 500 persons, the inferior poles fuse before ascent, thus generating a *horseshoe kidney*.

KEY POINTS

- The met anephros develops at approximately 5 weeks.
- Urine excretion starts at approximately 10 weeks.

KEY POINTS

- Failure to migrate up (cephalad) from the pelvic location in ectopic kidneys.
- Pancake kidney and horseshoe kidney result from fusion of the fetal kidneys.

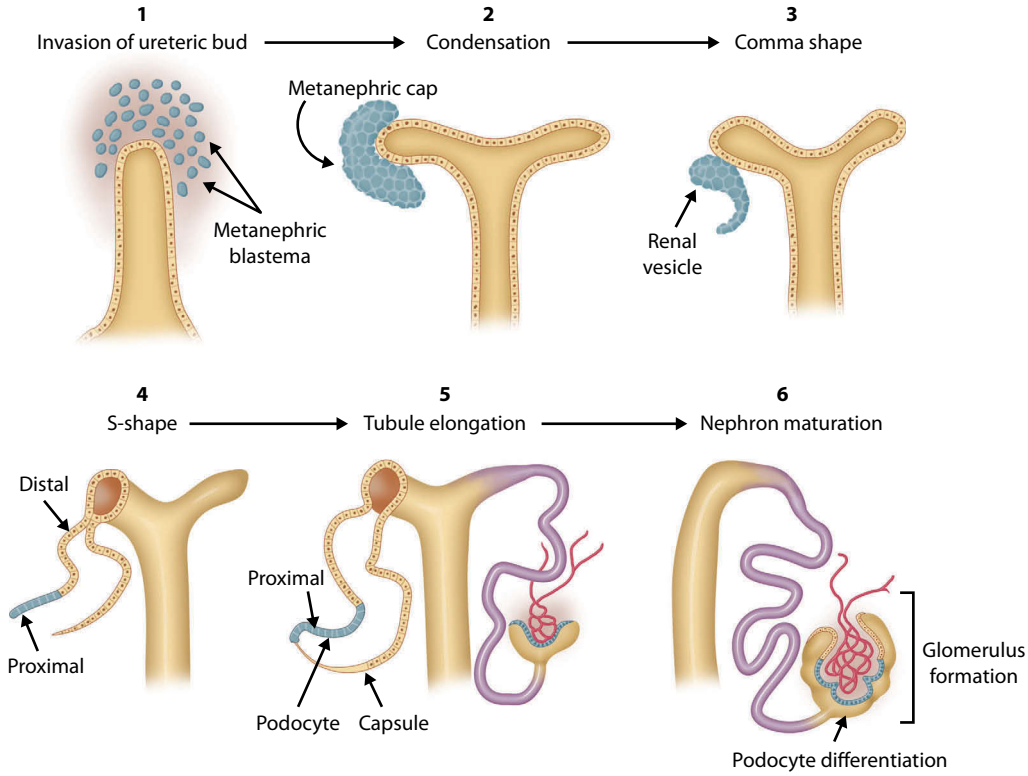


Figure 2.1 Stages of renal morphogenesis. Ureteric bud outgrowth from the wolffian duct is modulated by factors secreted by the metanephric blastema and the mesoderm surrounding the duct (1). Morphologic intermediates formed during nephrogenesis consist of condensation of the metanephric cap around the ureteric bud branch (2) renal vesicle comma shape (3), S shape (4), elongation of the tubule (5), invasion of blood vessels into the glomeruli, and formation of the glomerular corpuscle (6). (Modified from Chau YY, Hastie ND. The role of Wt1 in regulating mesenchyme in cancer, development, and tissue homeostasis. Trends Genet. 2012;28:515–24.)

At the earliest stage of kidney formation, the renal arteries are derived as branches of the common iliac arteries. As the metanephroi ascend, they receive branches from the distal aorta, then from the abdominal aorta. Normally, the distal branches disappear, and the abdominal branches become the permanent renal arteries. Variations in the arterial supply are common and reflect the changing nature of the arterial supply during fetal life. Although most persons have a single renal artery, approximately 25% have two to four.[3]

METANEPHRIC DEVELOPMENT

Metanephric induction occurs at 5 weeks of gestation, at a time when the ureteric bud is induced to grow out from the wolffian (mesonephric) duct and invade the metanephric blastema. The blastema is composed of a heterogeneous population of cells, including mesenchymal cells that eventually transform into epithelial, glomerular, tubular progenitors, and stromal cells that support the formation of glomerular and tubular elements.

Under the direction of growth factor–mediated signals elaborated by the metanephric mesenchyme, the ureteric bud undergoes repetitive growth and branching events, a process termed branching morphogenesis. In general, each branch divides to form two daughter branches that create generations of ureteric bud branches. In reciprocal fashion, the ureteric bud induces the mesenchyme adjacent to each bud tip to develop through a stereotypic sequence of structures consisting of mesenchymal aggregates (known as cap mesenchyme), renal vesicles, and comma-shaped and S-shaped bodies (Figure 2.1). At the distal end of the S-shaped body, a layer of epithelial cells gives rise to future podocytes. The basal aspect of these cells rests on the future glomerular basement membrane. A cleft between the podocytes becomes the glomerulus and the proximal tubule.

KEY POINTS

- Under the influence of growth factors from metanephric mesenchyme, the ureteric bud undergoes programmed and repetitive divisions or branching morphogenesis.
- Ureteric branching provides the biologic architecture for nephrogenesis.

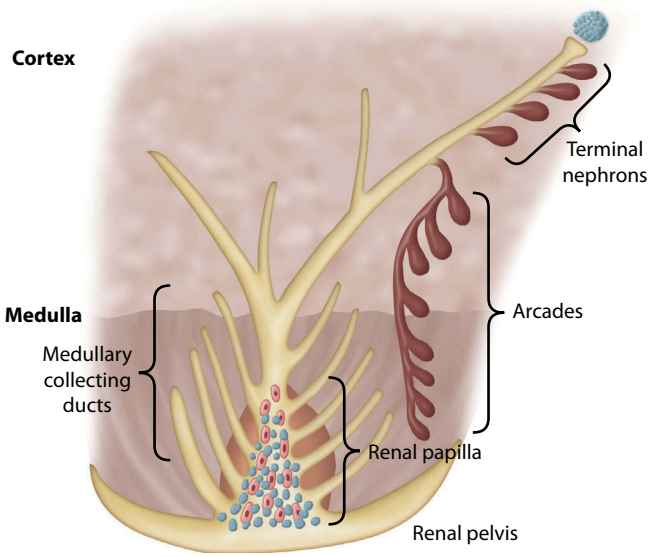


Figure 2.2 Patterning of the kidney into a cortex and medulla. The cortex consists of nephrons with short and long tubular (Henle) loops and collecting ducts that connect to the distal tubules. The medulla consists of the tubules from long loops of Henle and collecting ducts that terminate in the papillae.

Endothelial and mesangial cells migrate into this cleft. Each branch of the ureteric bud and its daughter collecting ducts induce formation of one nephron.

The formation of 15 generations of ureteric buds and collecting ducts induces an identical number of nephrons. The remaining nephrons are formed by induction of approximately 10 nephrons around the stem of an elongating ureteric bud and collecting duct branch that are initially formed. The connecting tubules of each of these nephrons then attach to the stem of the collecting duct branch in series to form an arcade (Figure 2.2). After formation of arcades, the terminal branch of the 15th generation begins to elongate and to develop a succession of ampullae that also induce nephrons on each side of the terminal branch. During the latter stages of kidney development, tubular segments formed from the first five generations of ureteric bud branching undergo remodeling to form the pelvis and calyces.

SEQUENCING MOLECULAR EVENTS

During embryogenesis, formation of tissues is controlled by one or more morphogenetic pathways that consist of a hierarchy of control elements integrated within a circuit. An ever-expanding body of knowledge of embryonic renal development has been generated by the study of experimental models, most notably in the mouse, which has kidney development closely resembling that in humans.

Nephron development is initiated by the activity of one or more genes that control the behavior of target cells. These target cells are either those in which the genes are themselves expressed or the neighboring cells. After receiving appropriate signals, the target cells are instructed to engage in a repertoire of activities that include proliferation, programmed cell death (apoptosis), movement, shape change, or alteration in their interactions with extracellular matrices. One or more of these changes in cell behavior will influence the manner in which a particular three-dimensional structure (e.g., a collecting duct) is constructed. In turn, changes in cell behavior and structural architecture affect inducing gene expression, thereby creating a feedback mechanism.

URETERIC BUD OUTGROWTH

Outgrowth of the ureteric bud from the wolffian duct performs a critical role in nephrogenesis. Development of the ureteric bud from the wolffian duct is controlled by genes expressed in both in the wolffian duct and the metanephric blastema.[4] These genes function within a morphogenetic pathway and tightly regulate the pathways that promote or inhibit ureteric bud development and eventual nephrogenesis.

KEY POINTS

Key genes regulating ureteric bud growth are:

- *Pax2:* transcription factor
- *Eya1:* transcription factor
- *GDNF:* growth factor
- *RET:* the GDNF receptor

Genes expressed in the metanephric blastema that are required for ureteric bud outgrowth include the transcription factors *Pax2* and *Eya1,* the secreted growth factor glia-derived neurotrophic factor (GDNF), and RET, which is the GNDF cell surface receptor. Genes that function upstream of *Gdnf* either limit or promote its expression, thereby precisely controlling ureteric bud outgrowth. *Pax2* and *Eya1* are positive regulators of *Gdnf* and promote its expression.[5,6] Homozygous deficiency of *Pax2, Eya1, Gdnf,* or *Ret* in mice causes failure of ureteric bud outgrowth and results in bilateral renal agenesis or severe renal dysgenesis, depending on the gene involved. Identical phenotypes have also been observed in mice deficient in heparan sulfate 2-sulfotransferase, a finding demonstrating a critical role for heparan sulfate in mediating interactions between the ureteric bud and the metanephric blastema.[7]

Foxc1 (also known as *Mf1*), a forkhead/winged helix transcription factor, is expressed during embryonic development in a metanephric domain similar to that of *Gdnf.* Homozygous *Foxc1*-null mutant mice exhibit renal abnormalities consisting of ureteric duplication, hydroureter, and ectopic ureteric buds, findings suggesting that *Foxc1* negatively controls the domain of *Gdnf* expression.[8] BMP4 (bone

KEY POINTS

- BMP4, expressed in the mesenchymal cells surrounding the wolffian duct, exerts an inhibitory control by regulating the site from which the ureteric bud would emerge.
- BMP4 prevents development of ectopic ureteric bud sites from emerging.

morphogenetic protein 4) is another inhibitory gene that is expressed in the mesenchymal cells surrounding the wolffian duct. By inhibiting ectopic ureteric budding, *BMP4* regulates the site in the wolffian duct from where the ureteric bud emerges.[9] *Bmp4* heterozygous null mutant mice develop renal and ureteric abnormalities, such as hypodysplastic or dysplastic kidneys, hydroureter, ectopic ureterovesical junction, and double collecting system.[9]

During kidney development, the site of ureteric bud outgrowth is invariant, precisely positioned, and the number of outgrowths is limited to one. It is believed that outgrowth of a single ureteric bud at the appropriate position is controlled by mesenchymal factors that restrict the location of ureteric bud outgrowth (Figure 2.3). Furthermore, the site of ureteric bud outgrowth from the wolffian duct determines the final site of the ureter orifice in the bladder. A more caudal or cranial budding from the wolffian duct can lead to a defective ureterovesical valve, urinary outflow obstruction, and aberrant insertion of the ureteric bud into the metanephric mesenchyme that can result in renal dysplasia.

COLLECTING DUCT BRANCHING AND ELONGATION

The relatively fixed number and spatial pattern of collecting ducts in the mature kidney suggest that ureteric bud branching morphogenesis is tightly regulated. In mice,

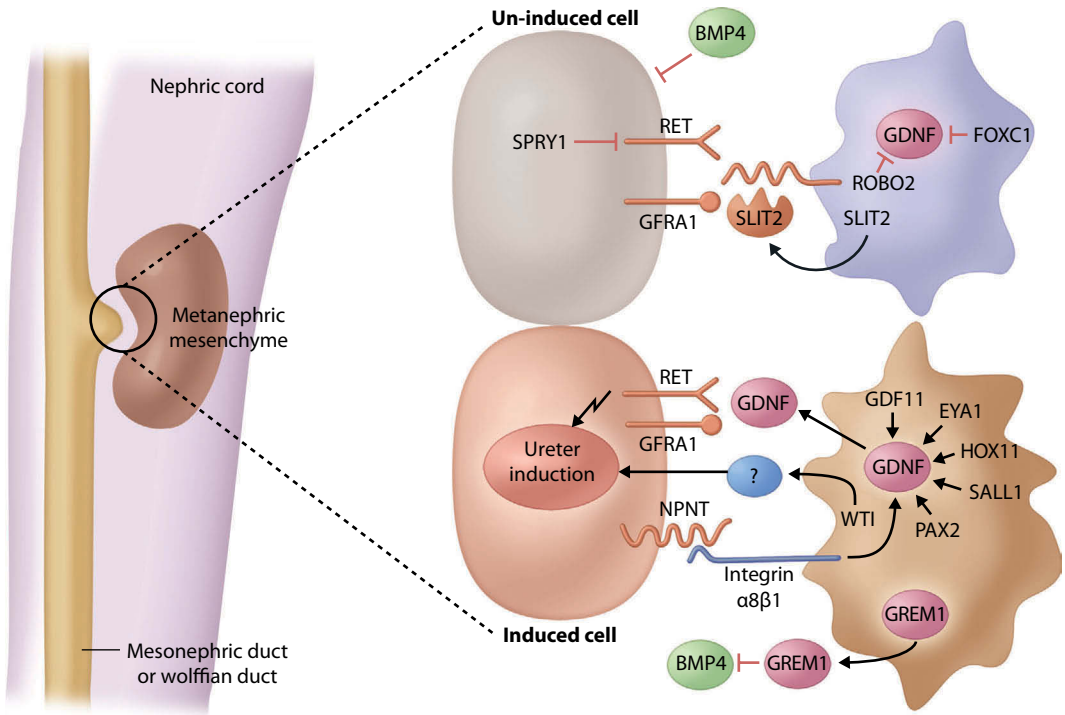


Figure 2.3 Gene products that control cellular events and induction of the ureteric bud in the wolffian duct. Mesenchymal cells at the caudal end of the nephrogenic cord (light blue cell) express various factors that activate expression of glial-derived neurotrophic factor (GDNF). In addition, mesenchymal cells release gremlin-1 (GREM1), an inhibitor of bone morphogenetic protein (BMP) signaling, and other still unidentified factors. Released GDNF binds to RET and GDNF-family receptor α_1 (GFRA1) receptors that are on the epithelial cells of the mesonephric duct (wolffian duct). The combination of these signals induces ureteric budding. Mesenchymal cells at a more rostral level (dark blue cell) express forkhead box protein C1 (FOXC1), slit homologue 2 (SLIT2), and its receptor roundabout homologue 2 (ROBO2), leading to a repression of GDNF. In epithelial cells of the mesonephric duct, the tyrosine kinase inhibitor sprouty 1 *(Spry1)* suppresses RET activation. Finally, BMP4 also inhibits ureter outgrowth. EYA1, eyes-absent homologue 1; GDF11, growth differentiation factor-11; HOX11, homeobox protein 11; NPNT, nephronectin; WT1, Wilms tumor transcription factor. (From Schedl A. Renal abnormalities and their developmental origin. Nat Rev Genet. 2007;8:791–802.)

fewer ureteric bud branches are formed in the posterior than in the anterior portion of the kidney. This asymmetry is probably controlled, in part, by *Hox* genes, originally described as regulators of body segmentation in fruit flies.[10] In addition to their critical roles during ureteric bud outgrowth, GDNF and its cognate receptors stimulate ureteric bud branching. In mice, genetic deficiency of *Gdnf* and *Ret* causes decreased ureteric bud branching. RET expression is controlled by members of the retinoic acid receptor (RAR) family of transcription factors. These members, including RAR α and RAR β_2, are expressed in stromal cells surrounding *Ret*-expressing ureteric bud branch tips.[11,12] Mice deficient in these receptors exhibit a decreased number of ureteric bud branches and diminished expression of *Ret*.

KEY POINTS

- GDNF plays a critical role in ureteric bud formation, as well as branching of the bud.
- Inhibitory signals and positive gene signals are crucial for programmed ureteric bud branching and renal morphogenesis.

Two members of the fibroblast growth factor (FGF) family of signaling peptides stimulate collecting duct morphogenesis in mice. Homozygous null mutations in the *Fgf7* gene result in a reduced number of ureteric bud branches and underdevelopment of the renal papilla.[13] Mice with a homozygous null mutation in *Fgf10* also have kidneys that are smaller than those in wild-type mice and exhibit a decreased number of medullary collecting ducts, medullary dysplasia, and dilatation of the renal pelvis.[14] Thus, a repertoire of signaling pathways promotes renal branching morphogenesis.

Renal branching morphogenesis is also regulated by inhibitory signaling pathways. In mice, BMP signaling through their activin-like kinase (ALK) receptors inhibits branching morphogenesis. Targeted overexpression of ALK3 in the ureteric bud lineage decreases branching morphogenesis and is associated with decreased nephron formation.[15] Deficiency of BMPs and their signaling intermediates is associated with increased branching.[16] Thus, integration of signals from these diverse and opposing pathways by ureteric bud and collecting duct cells controls branching behavior.

Elongation of collecting ducts is noted during later stages of renal development. This is accomplished by cell divisions that are aligned with the long axis of the duct, a process termed oriented cell division. Members of the WNT family of secreted proteins, specifically WNT9b and WNT7b, are required for collecting duct elongation and formation of the medulla and papillae.[17,18] Collecting ducts deficient in WNT9b dilate during embryonic development because of loss of alignment of the mitotic spindle with the long axis of the tubule, and they develop into large cysts postnatally. Similar abnormalities in the medulla are observed in mice lacking WNT7b.

FORMATION OF THE CALYCES AND PELVIS

Patterning of the collecting system to form the calyces and pelvis is controlled by sonic hedgehog (SHH), by members of the BMP family, and by angiotensin and its cell surface receptors. SHH is a secreted growth factor that controls cell determination and proliferation in many developmental contexts. In mice, *Shh* deficiency interferes with formation of the smooth muscle layer surrounding the upper ureter and causes dilatation of the pelvis.[19] Loss of *Bmp4* expression appears to be a pathogenetic mechanism in the genesis of hydronephrosis in these mice. Consistent with these observations, a subset of mice with spontaneous and engineered mutations in *Bmp4* and *Bmp5* demonstrates dilatation of the ureters and collecting system (ureterohydronephrosis), and ureteral bifurcation.[20,21] Mutations in the genes encoding components of the renin-angiotensin axis, best known for their role in controlling renal hemodynamics, also cause abnormalities in the development of the renal calyces and pelvis.

KEY POINT

Sonic hedgehog (SHH) and members of the BMP gene family control the patterning of the renal collecting system: the calyces and the pelvis.

Mice that are homozygous null for angiotensin receptor-1 *(Agtr1)* demonstrate atrophy of the papillae and underlying medulla.[9] The underlying defect appears to be a decrease in proliferation of the smooth muscle cell layer lining the pelvis that results in decreased thickness of this layer in the proximal ureter. Mutational inactivation of *Agtr2* results in a range of anomalies including vesicoureteral reflux (VUR), a duplex kidney, renal ectopia, ureteropelvic junction stenosis, ureterovesical junction stenosis, renal dysplasia, renal hypoplasia, multicystic dysplastic kidney (MCDK), or renal agenesis.[22] Null mice demonstrate a decreased rate of apoptosis of the cells around the ureter, a finding suggesting that *Agtr2* plays a role in modeling of the ureter. Together, these studies highlight the role of smooth muscle patterning in the formation of the pelvic-ureteric junction.

FORMATION OF GLOMERULAR AND TUBULAR PRECURSORS

The development of metanephric derivatives begins when the blastema is rescued from apoptosis and is induced to

proliferate coincident with the invasion of the ureteric bud. Expression of the Wilms Tumor 1 *(Wt1)* gene product, a transcription factor, is critical in maintaining viability of the metanephric blastema at this early stage of development.[23] With the invasion of the ureteric bud, the blastemal cells differentiate along distinct pathways. Cells adjacent to the ureteric bud tips aggregate and begin to display morphologic and molecular features characteristic of nephron epithelial cells, a process termed mesenchymal to epithelial transformation (MET). Other metanephric mesenchyme cells, which are stromal cells, give rise to endothelial and mesangial cells and interstitial fibroblasts.

KEY POINT

Morphologic and molecular transformation of mesenchymal cells adjacent to the ureteric bud into various epithelial components of the glomeruli is termed mesenchymal-to-epithelial transformation (MET).

MAINTAINING PROGENITOR CELL POOL

A pool of self-renewing progenitor cells gives rise to nephrons and controls transformation of metanephric mesenchyme cells into cells with specific differentiated characteristics. The molecular mechanisms that control maintenance of this pool are being defined at an ever-increasing level of detail. Before and after invasion of the metanephric mesenchyme by the ureteric bud cells, expression of genes including *Wt1, Fgf8* and its cognate receptors, and *Bmp7* is required to maintain cell viability.[23–25] In the absence of these gene products, metanephric mesenchyme cells undergo apoptosis, and very few, if any, nephrons form.

The cells that can take part in the formation of any nephron progenitor structure—glomerulus, proximal tubule, and distal tubule—are marked by expression of *Six2,* a transcription factor. SIX2-positive cells are then further specified to become differentiated cells in proximal or distal nephron segments. Analysis of gene expression in these segments has identified a large number of genes, the expression of which is restricted to either segment.[26,27] Some of these genes are functionally required to define the identity of these segments. For example, distal segments require the expression of *Brn1,* a transcription factor.[28] In contrast, expression of *Lhx1* and NOTCH family members is required for establishment of the proximal tubule.[29,30]

PODOCYTE AND ENDOTHELIAL DIFFERENTIATION

Several classes of genes are required for formatting podocytes and for directing the migration of endothelial cells into the glomerulus. As nephrogenesis proceeds, *Wt1* expression becomes restricted to the podocyte lineage. Transcription of *Wt1* results in the formation of multiple isoforms generated by alternative splicing. Mutations in *Wt1* that prevent the generation of certain splice forms result in formation of abnormal glomeruli, thereby implicating *Wt1* in glomerulogenesis.[31] *Lmx1b* is a transcription factor mutated in patients with nail-patella syndrome and is expressed in podocytes.[32] Mutational inactivation in mice decreases formation of foot processes and decreases expression of the α_3 and α_4 chains of type IV collagen. *Pod1* is a basic helix-loop-helix class transcription factor that is expressed in podocytes in S-shaped bodies. *Pod1* deficiency in mice results in arrest at the single capillary loop stage of glomerular development.[33]

KEY POINTS

- Podocyte and vascular endothelium formation in the glomeruli is controlled by multiple genes.
- The *Wt1* gene plays an important role in glomerulogenesis.

Kreisler (MafB), a leucine zipper class transcription factor, is expressed in podocytes. *Kreisler* deficiency in mice results in failure of foot process attachment to the basement membrane.[34] The α_3 chain of $\alpha_3\beta_1$ integrin is required for formation of foot processes in mice.[35] Podocalyxin is a sulfated cell surface sialomucin that is expressed on the surface of podocytes. In a podocalyxin-deficient state, foot process and slit diaphragm assembly is abrogated.[36] Podocyte-derived vascular endothelial growth factor A (VEGF-A) and Notch 2 play central roles in directing endothelial cell migration into glomeruli. Inactivation of VEGF-A in podocytes by genetic means in mice disrupts glomerular capillary formation.[37] Similarly, inactivation of *Notch2,* a member of a family of cell determination genes, results in a similar phenotype.[38]

GENE FUNCTIONS AND DEVELOPMENTAL RENAL DISORDERS

The human and mouse genome projects have been complementary in generating a rapid expansion of our knowledge of human developmental biology. Although the diversity of human phenotypes projects existence of more than 80 loci associated with renal dysplasia, to date mutations in a much smaller number of genes have been implicated in pathogenesis.[39] The functions of a subset of these genes have been elucidated in genetic mouse models that provide critical insights into the molecular control of normal and abnormal renal development (Table 2.1). Some of the disorders described here are also discussed in Chapters 1 and 46.

Table 2.1 Human gene mutations exhibiting defects in renal morphogenesis

Primary disease	Gene	Kidney phenotype	References
Alagille syndrome	*JAGGED1*	Cystic dysplasia	49
Apert syndrome	*FGFR2*	Hydronephrosis	50
Beckwith-Wiedemann syndrome	*p57KIP2*	Medullary dysplasia	51
Branchio-oto-renal (BOR) syndrome	*EYA1*	Unilateral or bilateral agenesis or dysplasia, hypoplasia, collecting system abnormalities	52
Campomelic dysplasia	*SOX9*	Dysplasia, hydronephrosis	53
Fraser syndrome	*FRAS1*	Agenesis, dysplasia	54
Hyoparathyroidism, sensorineural deafness, and renal anomalies (HDR) syndrome	*GATA3*	Dysplasia	55
Kallmann syndrome	*KAL1*	Agenesis	56
Mammary-ulnar syndrome	*TBX3*	Dysplasia	57
Meckel Gruber syndrome	*MKS1* *MKS3 NPHP6 NPHP8*	Cystic dysplasia	58
Nephronophthisis	*CEP290, GLIS2, RPGRIP1L,NEK8, SDCCAG8,TMEM67, TTC21B*	Cystic dysplasia	59
Okihiro syndrome	*SALL4*	Unilateral agenesis, VUR, malrotation, cross fused ectopia	60
Pallister Hall syndrome	*GLI3*	Agenesis, dysplasia, hydronephrosis	61
Renal coloboma syndrome	*PAX2*	Hypoplasia, VUR	62
Renal hypodysplasia, isolated	*TCF2, PAX2, EYA1, SIX1, SIX2, SALL1, RET, BMP4, DSTYK*	Hypoplasia, VUR	63–67
Renal tubular dysgenesis	Renin, angiotensinogen, ACE, AT1 receptor	Tubular dysgenesis	68
Rubinstein-Taybi syndrome	*CREBBP*	Agenesis, hypoplasia	69
Simpson-Golabi-Behmel syndrome	*GPC3*	Medullary dysplasia	70
Smith-Lemli-Opitz syndrome	*DHCR7*	Hypoplasia, cysts, aplasia	71
Townes-Brock syndrome	*SALL1*	Hypodysplasia, VUR	72
Zellweger syndrome	*PEX1*	Cystic dysplasia	73

ACE, angiotensin-converting enzyme; AT1 receptor, angiotensin I receptor; VUR, vesicoureteral reflux.

PAX2

Heterozygous mutations in *PAX2* are found in patients with the renal coloboma syndrome (Online Mendelian Inheritance in Man [OMIM] 120330) that is characterized by renal hypoplasia and VUR. Heterozygous *Pax2* mutations in mice result in a similar phenotype.[40] Investigation of *Pax2* suggests that it functions in the ureteric bud to promote cell proliferation and inhibit apoptosis.[41] These results support a model that proposes that *Pax2* controls the number of ureteric bud branches, thereby determining the number of nephrons formed.

EYA1

EYA1, a transcription factor, is mutated in patients with branchio-oto-renal (BOR) syndrome (OMIM 113650) and with unilateral or bilateral renal agenesis, or dysplasia.[42] In mice, the spatial pattern of *Eya1* expression overlaps that of *Gdnf* at the time of ureteric bud outgrowth. Because biallelic inactivation of *Eya1* causes renal agenesis and abrogates *Gdnf* expression, *Eya1* is thought to function upstream of *Gdnf* to control ureteric bud outgrowth.

SALL1

SALL1, a transcription factor, is expressed in the metanephric mesenchyme at the time of induction by the ureteric bud. Mutations in *SALL1* exist in patients with Townes-Brock syndrome (OMIM 107480). In *Sall1*-deficient mice, ureteric bud outgrowth occurs, but the bud fails to invade the metanephric blastema, with resulting renal agenesis. This failure

of invasion appears to be caused by a *Sall1*-dependent signal rather than the competence of the metanephric blastema to undergo induction.[43]

GLI3

The gene encoding GLI3 is mutated in patients with Pallister-Hall syndrome (OMIM 146510) and renal dysplasia. GLI3 is one member among a family of GLI proteins that control gene transcription. Their actions are controlled by SHH. All *GLI3* mutations identified to date result in the expression of a truncated protein that functions as a transcriptional repressor. Investigations in mice provide insight into the biologic significance of this repressor form of GLI3. During inductive stages of kidney development, GLI3 repressor represses the transcription of GLI1 and GLI2, renal patterning genes including *Pax2* and *Sall1,* and genes that modulate the cell cycle (cyclin D1 and N-Myc), resulting in renal aplasia or severe dysplasia.[44] During later stages of renal development, GLI3 repressor plays cell lineage-specific roles; it is required for ureteric branching but abrogates coordinated contraction of the ureter by deleterious effects on renal pacemaker cells.[45,46]

Glypican-3 and p57^{KIP2}

Investigation of the genes mutant in two human overgrowth syndromes, Simpson–Golabi–Behmel syndrome (OMIM 312870) and Beckwith-Wiedemann syndrome (OMIM 13650), provides novel insight into the pathogenesis of medullary renal dysplasia. Patients with Simpson–Golabi–Behmel syndrome have mutations in glypican-3, a glycosyl-phosphotidylinositol (GPI)–linked cell surface heparan sulfate proteoglycan. The pathogenesis of renal medullary dysplasia in *Gpc3*-deficient mice involves massive medullary collecting duct apoptosis preceded by increased ureteric bud proliferation.[47] Thus, *Gpc3* controls collecting duct cell number and survival. A role for control of the cell cycle in the pathogenesis of medullary renal dysplasia is further supported by the finding of medullary renal dysplasia in mice and humans (Beckwith-Wiedemann syndrome) with inactivating mutations in *p57*^{KIP2}, a cell cycle regulatory gene that encodes a cyclin-dependent kinase inhibitor.[48]

Table 2.1 lists clinical syndromes that feature renal anomalies and genes associated with these syndromes.[49–73] Identification of genes such as these has provided a basis for screening children with sporadic cases of renal hypodysplasia.[63,64,67,74,75] These investigations have identified mutations in genes including *TCF2, PAX2, EYA1, SIX1, SIX2, SALL1, RET, BMP4, and DSTYK*. Remarkably, mutations in genes previously identified in the context of a particular clinical syndrome have also been identified in patients with no evidence of that syndrome, other than the presence of a renal malformation.

CLINICAL ASPECTS OF RENAL MALDEVELOPMENT

Advances in genetics have generated a revolution in our understanding of congenital malformations of the kidney. Although it has been accepted that an association exists among poorly developed kidneys, renal dysfunction, and urologic abnormalities, a generation ago it was widely held that some sort of obstructive process led to maldevelopment. The discovery of these genetic relationships has led to an understanding that maldevelopment results from failure of programmed genetic control, with the likelihood that VUR and urinary tract obstruction stem from the same failure. As a result, we have come to understand that such disorders may demonstrate familial predisposition that can have clinical relevance.

The three categories of developmental abnormalities that can occur, separately or in concert, are renal hypoplasia, renal dysplasia, and abnormal development of the lower urinary tract.

HYPOPLASIA

Renal hypoplasia is characterized by a smaller than normal complement of nephrons in the kidney. The nephron structure and the overall renal architecture are well maintained. Hypoplasia can affect one or both kidneys. In hypoplasia, an abnormality in epithelial-mesenchymal interactions leads to decreased or abnormal branching of the ureter. Unless it is associated with other malformations, renal hypoplasia can be asymptomatic. Hypoplasia is often discovered as an incidental finding during an abdominal sonogram or other imaging studies, in which a smaller than normal kidney is detected. Decreased renal function and chronic kidney disease can be seen in patients with severe cases with bilateral disease. Renal hypoplasia has been reported to be a predisposing condition for hypertension later in life.[2]

MULTICYSTIC DYSPLASTIC KIDNEY

MCDK is reported to be the second most common renal anomaly diagnosed by prenatal ultrasound examination, with a reported prevalence of 1 in 3640 births.[76] MCDK can manifest as a flank mass in newborn infants. Renal ultrasound evaluation shows a large, cystic, nonreniform structure located in the renal fossa. The characteristic and diagnostic finding is absence of any function demonstrated by radionuclide scans. VUR in the contralateral normal kidney is the most common associated urinary tract abnormality and has been reported in approximately 25% of cases.[77] Hypertension can be seen some patients but appears to be less common than previously assumed.[77] Wilms tumor has been reported in patients with MCDK. It has been argued that these cases of apparent malignant degeneration in MCDK may actually result from nephrogenic rests.

Gradual reduction in renal size and eventual resolution of the mass of the MCDK are common. At 2 years, an involution in size by ultrasound examination has been noted in up to 60% of the affected kidneys.[78] Complete disappearance of the MCDK can occur in a minority of patients (3% to 4%) by the time of birth and in 20% to 25% by 2 years. Increase in the size of MCDK can be seen in some cases. The contralateral kidney shows compensatory hypertrophy by ultrasound evaluation.

Management of patients with MCDK has shifted from routine nephrectomy in the past to observation and medical therapy. Because of the risk of associated anomalies in the contralateral kidney, the possibility of VUR should be considered, and clinical follow-up for evolution of hypertension is advised. Renal ultrasound is generally recommended at an interval of 3 months for the first year of life and then every 6 months up to involution of the mass, or at least for up to 5 years. Compensatory hypertrophy of the contralateral kidney is expected and should be followed on ultrasound evaluations. Medical therapy is usually effective in treating hypertension in most patients, but nephrectomy may be curative in resistant cases.

RENAL DYSPLASIA

Renal dysplasia is characterized by the presence of malformed and disorganized tissue elements, a decreased number of nephrons, collecting ducts surrounded by muscular rings (Figure 2.4), and elements of aberrant development, such as cartilage, or even calcified tissue. Often, dysplasia is accompanied by hypoplasia of the kidney as well. Abnormalities of renal function and development of chronic kidney disease should be expected in patients with severe bilateral renal dysplasia or those with additional urinary tract malformations, such as obstruction. Potter syndrome, which is characterized by oligohydramnios, pulmonary hypoplasia, renal failure, low-set ears, and a beaked nose, may be observed in patients with severe cases of renal dysplasia.

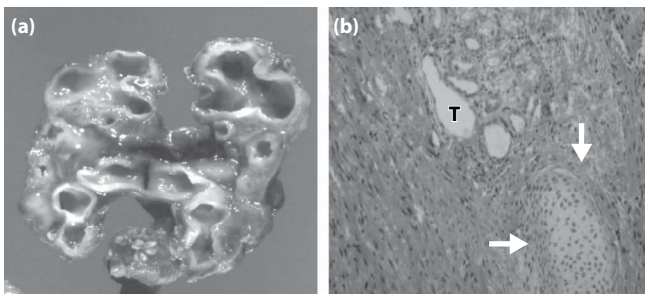


Figure 2.4 Renal dysplasia. **(a)** A cut section of dysplastic kidney with malformed collecting system, lack of well-patterned renal parenchyma, and absence of corticomedullary differentiation. **(b)** Microscopic section of the kidney from the patient demonstrating malformed tubules. Some tubules are widely dilated (T). Arrows show cartilage within the renal parenchyma. (Figure courtesy of Arthur Cohen, MD.)

SUMMARY

This chapter summarizes the major morphologic features of the developing and mature kidney. The concept of morphogenetic pathways is presented as a means to understand how genes control cellular events that, in turn, build three-dimensional structures. Genetic pathways that control normal renal branching morphogenesis are assuming an important role in understanding nephrogenesis and are likely to be explored in detail. An understanding of genetic mutations associated with renal hypoplasia and dysplasia has begun to appear, and it provides a link to the congenital abnormalities of the kidney and urinary tract (CAKUT) malformations complex. An understanding of the genetics of renal development also highlights the close developmental path that the urinary tract and the kidneys take in their mutual organogenesis. All these discoveries provide a window into our understanding of the pathogenic role played by genetic mutations in clinical disorders of the kidney and the urinary tract encountered by pediatric nephrologists.

ACKNOWLEDGMENTS

This work was supported by grants from the Canadian Institutes of Health Research, Kidney Foundation of Canada, and Canada Research Chairs Program (to NDR).

REFERENCES

1. Warady BA, Chadha V. Chronic kidney disease in children: The global perspective. Pediatr Nephrol. 2007;22:1999–2009.
2. Keller G, Zimmer G, Mall G, et al. Nephron number in patients with primary hypertension. N Engl J Med. 2003;348:101–8.
3. Moore KL, Persaud TVN. The Developing Human. 7th ed. Philadelphia: WB Saunders; 2003.
4. Piscione TD, Rosenblum ND. The molecular control of renal branching morphogenesis: Current knowledge and emerging insights. Differentiation. 2002;70:227–46.
5. Brophy PD, Ostrom L, Lang KM, et al. Regulation of ureteric bud outgrowth by Pax2-dependent activation of the glial derived neurotrophic factor gene. Development. 2001;128:4747–56.
6. Xu P-X, Adams J, Peters H, et al. Eya1-deficient mice lack ears and kidneys and show abnormal apoptosis of organ primordia. Nat Genet. 1999;23:113–7.
7. Bullock SL, Fletcher JM, Beddington RSP, et al. Renal agenesis in mice homozygous for a gene trap mutation in the gene encoding heparan sulfate 2-sulfotransferase. Genes Dev. 1998;12:1894–906.

8. Kume T, Deng K, Hogan BLM. Murine forkhead/winged helix genes Foxc1 (Mf1) and Foxc2 (Mfh1) are required for the early organogenesis of the kidney and urinary tract. Development. 2000;127:1387–95.
9. Miyazaki Y, Oshima K, Fogo A, et al. Bone morphogenetic protein 4 regulates the budding site and elongation of the mouse ureter. J Clin Invest. 2000;105:863–73.
10. Patterson LT, Pembaur M, Potter SS. Hoxa11 and Hoxd11 regulate branching morphogenesis of the ureteric bud in the developing kidney. Development. 2001;128:2153–61.
11. Mendelsohn C, Batourina E, Fung S, et al. Stromal cells mediate retinoid-dependent functions essential for renal development. Development. 1999;126:1139–48.
12. Batourina E, Gim S, Bello N, et al. Vitamin A controls epithelial/mesenchymal interactions through Ret expression. Nat Genet. 2001;27:74–8.
13. Qiao J, Uzzo R, Obara-Ishihara T, et al. FGF-7 modulates ureteric bud growth and nephron number in the developing kidney. Development. 1999;126:547–54.
14. Ohuchi H, Kimura S, Watamoto M, et al. Involvement of fibroblast growth factor (FGF)18-FGF8 signaling in specification of left-right asymmetry and brain and limb development of the chick embryo. Mech Dev. 2000;95:55–66.
15. Hu MC, Piscione TD, Rosenblum ND. Elevated Smad1/beta-catenin molecular complexes and renal medullary cystic dysplasia in ALK3 transgenic mice. Development. 2003;130:2753–66.
16. Hartwig S, Hu MC, Cella C, et al. Glypican-3 modulates inhibitory Bmp2-Smad signaling to control renal development in vivo. Mech Dev. 2005;122:928–38.
17. Karner CM, Chirumamilla R, Aoki S, et al. Wnt9b signaling regulates planar cell polarity and kidney tubule morphogenesis. Nat Genet. 2009;41:793–9.
18. Yu J, Carroll TJ, Rajagopal J, et al. A Wnt7b-dependent pathway regulates the orientation of epithelial cell division and establishes the cortico-medullary axis of the mammalian kidney. Development. 2009;136:161–71.
19. Yu J, Carroll TJ, McMahon AP. Sonic hedgehog regulates proliferation and differentiation of mesenchymal cells in the mouse metanephric kidney. Development. 2002;129:5301–12.
20. Miyazaki Y, Oshima K, Fogo A, et al. Bone morphogenetic protein 4 regulates the budding site and elongation of the mouse ureter. J Clin Invest. 2000;105:863–73.
21. Green MC. Mechanism of the pleiotropic effects of the short-ear mutant gene in the mouse. J Exp Zool. 1968;176:129–50.
22. Nishimura H, Yerkes E, Hohenfellner K, et al. Role of the angiotensin type 2 receptor gene in congenital anomalies of the kidney and urinary tract, CAKUT, of mice and men. Mol Cell. 1999;3:1–10.
23. Kreidberg JA, Sariola H, Loring JM, et al. WT-1 is required for early kidney development. Cell. 1993;74:679–91.
24. Grieshammer U, Cebrian C, Ilagan R, et al. FGF8 is required for cell survival at distinct stages of nephrogenesis and for regulation of gene expression in nascent nephrons. Development. 2005;132:3847–57.
25. Dudley AT, Lyons KM, Robertson EJ. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. Genes Dev. 1995;9:2795–807.
26. Kobayashi A, Valerius MT, Mugford JW, et al. Six2 defines and regulates a multipotent self-renewing nephron progenitor population throughout mammalian kidney development. Cell Stem Cell. 2008;3:169–81.
27. Brunskill EW, Aronow BJ, Georgas K, et al. Atlas of gene expression in the developing kidney at microanatomic resolution. Dev Cell. 2008;15:781–91.
28. Nakai S, Sugitani Y, Sato H, et al. Crucial roles of Brn1 in distal tubule formation and function in mouse kidney. Development. 2003;130:4751–9.
29. Kobayashi A, Kwan KM, Carroll TJ, et al. Distinct and sequential tissue-specific activities of the LIM-class homeobox gene Lim1 for tubular morphogenesis during kidney development. Development. 2005;132:2809–23.
30. Cheng HT, Kim M, Valerius MT, et al. Notch2, but not Notch1, is required for proximal fate acquisition in the mammalian nephron. Development. 2007;134:801–11.
31. Hammes A, Guo JK, Lutsch G, et al. Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation. Cell. 2001;106:319–29.
32. Chen H, Lun Y, Ovchinnikov D, et al. Limb and kidney defects in Lmx1B mutant mice suggest an involvement of LMX1B in human nail patella syndrome. Nat Genet. 1998;19:51–5.
33. Quaggin SE, Schwartz L, Cui S, et al. The basic-helix-loop-helix protein pod1 is critically important for kidney and lung organogenesis. Development. 1999;126:5771–83.
34. Sadl V, Jin F, Yu J, et al. The mouse Kreisler (Krml1/MafB) segmentation gene is required for differentiation of glomerular visceral epithelial cells. Dev Biol. 2002;249:16–29.
35. Kreidberg JA, Donovan MJ, Goldstein SL, et al. Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. Development. 1996;122:3537–47.
36. Doyonnas R, Kershaw DB, Duhme C, et al. Anuria, omphalocele, and perinatal lethality in mice lacking the CD34-related protein podocalyxin. J Exp Med. 2001;194:13–27.
37. Eremina V, Sood M, Haigh J, et al. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest. 2003;111:707–16.

38. McCright B, Gao X, Shen L, et al. Defects in development of the kidney, heart and eye vasculature in mice homozygous for a hypomorphic Notch2 mutation. Development. 2001;128:491–502.
39. Reidy KJ, Rosenblum ND. Cell and molecular biology of kidney development. Semin Nephrol. 2009;29:321–37.
40. Porteous S, Torban E, Cho N-P, et al. Primary renal hypoplasia in humans and mice with PAX2 mutations: Evidence of increased apoptosis in fetal kidneys of $Pax2^{1Neu}$ +/- mutant mice. Hum Mol Genet. 2000;9:1–11.
41. Dziarmaga A, Clark P, Stayner C, et al. Ureteric bud apoptosis and renal hypoplasia in transgenic PAX2-Bax fetal mice mimics the renal-coloboma syndrome. J Am Soc Nephrol. 2003;14:2767–74.
42. Abdelhak S, Kalatzis V, Heilig R, et al. A human homologue of the *Drosophila eyes absent* gene underlies branchio-oto-renal (BOR) syndrome and identifies a novel gene family. Nat Genet. 1997;15:157–64.
43. Nishinakamura R, Matsumoto Y, Nakao K, et al. Murine homolog of SALL1 is essential for ureteric bud invasion in kidney development. Development. 2001;128:3105–15.
44. Hu MC, Mo R, Bhella S, et al. GLI3-dependent transcriptional repression of Gli1, Gli2 and kidney patterning genes disrupts renal morphogenesis. Development. 2006;133:569–78.
45. Cain JE, Islam E, Haxho F, et al. GLI3 repressor controls nephron number via regulation of Wnt11 and Ret in ureteric tip cells. PLoS One. 2009;4:e7313–25.
46. Cain JE, Islam E, Haxho F, et al. GLI3 repressor controls functional development of the mouse ureter. J Clin Invest. 2011;121:1199–206.
47. Grisaru S, Cano-Gauci D, Tee J, et al. Glypican-3 modulates BMP- and FGF-mediated effects during renal branching morphogenesis. Dev Biol. 2001;230:31–46.
48. Zhang P, Liégeois NJ, Wong C, et al. Altered cell differentiation and proliferation in mice lacking $p57^{KIP2}$ indicates a role in Beckwith-Wiedemann syndrome. Nature. 1997;387:151–8.
49. Oda T, Elkahloun AG, Pike BL, et al. Mutations in the human Jagged1 gene are responsible for Alagille syndrome. Nat Genet. 1997;16:235–42.
50. Wilkie AOM, Slaney SF, Oldridge M, et al. Apert syndrome results from localised mutations of FGFR2 and is allelic with Crouzon syndrome. Nat Genet. 1996;9:165–72.
51. Hatada I, Ohashi H, Fukushima Y, et al. An imprinted gene $p57^{KIP2}$ is mutated in Beckwith-Wiedemann syndrome. Nat Genetics. 1996;14:171–3.
52. Chang EH, Menezes M, Meyer NC, et al. Branchio-oto-renal syndrome: The mutation spectrum in EYA1 and its phenotypic consequences. Hum Mutat. 2004;23:582–9.
53. Wagner T, Wirth J, Meyer J, et al. Autosomal sex reversal and compomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. Cell. 1994;79:1111–20.
54. McGregor L, Makela V, Darling SM, et al. Fraser syndrome and mouse blebbed phenotype caused by mutations in FRAS1/Fras1 encoding a putative extracellular matrix protein. Nat Genet. 2003;34:203–8.
55. Van Esch H, Groenen P, Nesbit MA, et al. GATA3 haplo-insufficiency causes human HDR syndrome. Nature. 2000;406:419–22.
56. Franco B, Guioli S, Pragliola A, et al. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. Nature. 1991;353:529–36.
57. Bamshad M, Lin RC, Law DJ, et al. Mutations in human TBX3 alter limb, apocrine and genital development in ulnar-mammary syndrome. Nat Genet. 1997;16:311–5.
58. Barker AR, Thomas R, Dawe HR. Meckel-Gruber syndrome and the role of primary cilia in kidney, skeleton and central nervous system development. Organogenesis. 2014;10:96–107.
59. Benzing T, Schermer B. Clinical spectrum and pathogenesis of nephronophthisis. Curr Opin Nephrol Hypertens. 2012;21:272–8.
60. Sakaki-Yumoto M, Kobayashi C, Sato A, et al. The murine homolog of SALL4, a causative gene in Okihiro syndrome, is essential for embryonic stem cell proliferation, and cooperates with Sall1 in anorectal, heart, brain and kidney development. Development. 2006;133:3005–13.
61. Kang S, Graham JM, Jr., Olney AH, et al. GLI3 frameshift mutations cause autosomal dominant Pallister-Hall syndrome. Nat Genet. 1997;15:266–8.
62. Sanyanusin P, Schimmenti LA, McNoe LA, et al. Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. Nat Genet. 1995;9:358–63.
63. Weber S, Moriniere V, Knuppel T, et al. Prevalence of mutations in renal developmental genes in children with renal hypodysplasia: Results of the ESCAPE study. J Am Soc Nephrol. 2006;17:2864–70.
64. Weber S, Taylor JC, Winyard P, et al. SIX2 and BMP4 mutations associate with anomalous kidney development. J Am Soc Nephrol. 2008 ;19:891–903.
65. Saisawat P, Tasic V, Vega-Warner V, et al. Identification of two novel CAKUT-causing genes by massively parallel exon resequencing of candidate genes in patients with unilateral renal agenesis. Kidney Int. 2012 ;81:196–200.
66. Thomas R, Sanna-Cherchi S, Warady BA, et al. HNF1B and PAX2 mutations are a common cause of renal hypodysplasia in the CKiD cohort. Pediatr Nephrol. 2011;26:897–903.
67. Skinner MA, Safford SD, Reeves JG, et al. Renal aplasia in humans is associated with RET mutations. Am J Hum Genet. 2008 ;82:344–51.

68. Gribouval O, Gonzales M, Neuhaus T, et al. Mutations in genes in the renin-angiotensin system are associated with autosomal recessive renal tubular dysgenesis. Nat Genet. 2005;37:964–8.
69. Petrij F, Giles RH, Dauwerse HG, et al. Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature. 1995;376:348–51.
70. Pilia G, Hughes-Benzie RM, MacKenzie A, et al. Mutations in GPC3, a glypican gene, cause the Simpson-Golabi-Behmel overgrowth syndrome. Nat Genet. 1996;12:241–7.
71. Nowaczyk MJ, Irons MB. Smith-Lemli-Opitz syndrome: Phenotype, natural history, and epidemiology. Am J Med Genet C Semin Med Genet. 2012;160C:250–62.
72. Salerno A, Kohlhase J, Kaplan BS. Townes-Brocks syndrome and renal dysplasia: A novel mutation in the SALL1 gene. Pediatr Nephrol. 2000;14:25–8.
73. Shimozawa N, Tsukamoto T, Suzuki Y, et al. A human gene responsible for Zellweger syndrome that affects peroxisome assembly. Science. 1992;255:1132–34.
74. Vivante A, Mark-Danieli M, Davidovits M, et al. Renal hypodysplasia associates with a WNT4 variant that causes aberrant canonical WNT signaling. J Am Soc Nephrol. 2013;24:550–8.
75. Sanna-Cherchi S, Sampogna RV, Papeta N, et al. Mutations in DSTYK and dominant urinary tract malformations. N Engl J Med. 2013;369:621–9.
76. James CA, Watson AR, Twining P, et al. Antenatally detected urinary tract abnormalities: Changing incidence and management. Eur J Pediatr. 1998;157:508–11.
77. Sukthankar S, Watson AR. Unilateral multicystic dysplastic kidney disease: Defining the natural history. Anglia Paediatric Nephrourology Group. Acta Paediatr. 2000;89:811–3.
78. Heymans C, Breysem L, Proesmans W. Multicystic kidney dysplasia: A prospective study on the natural history of the affected and the contralateral kidney. Eur J Pediatr. 1998;157:673–5.

REVIEW QUESTIONS

1. Metanephric induction begins at:
   a. Week 7 of gestation
   b. Week 10 of gestation
   c. Week 5 of gestation
   d. Week 20 of gestation
2. Renal dysplasia is characterized by a decreased number of nephrons, the presence of collecting ducts surrounded by muscular rings, and elements of aberrant development, such as cartilage, or even calcified tissue.
   a. True
   b. False
3. Branchio-oto-renal (BOR) syndrome results from mutation of:
   a. *PAX2*
   b. *PEX1*
   c. *SOX9*
   d. *EYA1*
4. The most common urinary tract abnormality associated with multicystic dysplastic kidney (MCDK) is:
   a. Duplication of the collecting system
   b. Vesicoureteric reflux
   c. Posterior urethral valves
   d. Wilms tumor
5. During renal development, the *WTI* gene is important in:
   a. Glomerulogenesis
   b. Tubulogenesis
   c. Formation of the renal pelvis
   d. Renal ascent
6. BMP4, expressed in the mesenchymal cells surrounding the wolffian duct, exerts a positive control in directing the site from which ureteric bud would emerge.
   a. True
   b. False
7. Patterning of the renal collecting system (renal pelvis and calyces) is controlled by:
   a. Sonic hedgehog (SHH) and BMP gene family
   b. Renin, angiotensinogen genes
   c. *JAGGED1* gene
   d. *SIX1* and *SIX2* genes
8. The characteristic renal phenotype in Alagille syndrome consists of:
   a. Renal dysplasia
   b. Renal aplasia
   c. Multicystic dysplastic kidney
   d. Hypoplasia

ANSWER KEY

1. c
2. b
3. d
4. b
5. a
6. b
7. a
8. a

PART B

Diagnostic evaluation of kidney diseases

3

Urinalysis

GEORGE J. SCHWARTZ

Urinalysis provides a window into the function and some inflammatory pathologic processes in the kidneys. A carefully conducted urinalysis can reveal underlying renal and systemic disorders. Evaluation of urine has been recorded since ancient times and was commonly practiced by Roman and Greek physicians.[1] Uroscopy (also known as urinoscopy), or visual examination of urine, eventually evolved as a formal science among English and Italian physicians during the Renaissance. The next major contribution to the technique of urinalysis was made by Thomas Addis, who developed the method for quantifying urinary excretion of red blood cells (RBCs) in the diagnosis and prognosis of Bright disease.[2] The science of urinalysis has further evolved in the last several decades, especially with the advent of the dipstick technology, wherein a fairly precise chemical analysis of urine can be ascertained within a few minutes by the bedside or in the clinic facility. This chapter will discuss the urinalysis procedure and its diagnostic utility in nephrology.

URINE SAMPLE COLLECTION

In general, the most reproducible urine specimen for urinalysis is the one obtained on waking from sleep, because it is not influenced by exercise and can assess urinary concentration after withholding fluid overnight. It is often recommended that parents bring in the first morning urine from home when a child has difficulty urinating on command. In absence of toilet training, younger children should have urine collected in a plastic bag device attached to the perineum. A patient identifier label should be affixed to the urine container before being handed over to the patient for collection. The container for urinalysis need not be sterile, but it should be clean. In adults, second morning urine is sometimes preferable to the first morning urine sample because of convenience and also because of concern that cellular elements in the urine may undergo autolysis in the bladder overnight.[3]

PRINCIPLES OF EVALUATION

Collected urine should be evaluated quickly, no later than 2 hours after collection. Routine urinalysis consists of the following three evaluation steps: (1) appearance of the collected urine, (2) chemical analysis, and (3) microscopy of the urine sediment.[4]

KEY POINTS

- Only a freshly collected urine sample should be used for urinalysis.
- Automated dipstick-based methodologies for chemical analysis have increased the range of tests that can be conducted with a few drops of urine.

In most clinical laboratories the chemical analysis of urine is done with the help of urine test strips, also known as dipsticks. Early versions of this technology were limited to detection of urine glucose and albumin, but current versions are able to test for urine pH, specific gravity, protein, blood, glucose, ketones, urobilinogen, nitrite, and leukocyte esterase. Change of color of the individual test strips after they have been moistened by the urine is used to determine the test results. To automate the urinalysis and standardize the results, several devices are available for clinical use, especially for point-of-care testing.

APPEARANCE AND CONSTITUENTS

Urine samples should be grossly examined and then routinely tested for pH, specific gravity, protein, blood, glucose, and nitrites using commercially available dipsticks.

APPEARANCE

Fresh urine generally ranges from pale yellow to deep amber. The color of urine may provide clues to some of the underlying renal and nonrenal disorders (Table 3.1). Red discoloration may be caused by the presence of blood (hematuria), hemoglobin (hemoglobinuria), or myoglobin (myoglobinuria). RBCs are seen only in the urinary sediment of patients with hematuria. Urinary bleeding from the bladder or other parts of the collecting system (urologic hematuria) tends to color the urine pink or red, whereas glomerular bleeding appears rusty brown or the color of cola or tea (Figure 3.1). Red urine also may be seen in patients with porphyrias, as well as after intake of beets, certain food additives, or some drugs.

The urine is usually clear but may be turbid because of the precipitation of phosphates in alkaline urine or uric acid in acid urine, especially on chilling. The presence of leukocytes in the urine also can render the urine cloudy.

ODOR

The normal odor of urine is mildly aromatic. Bacterial infection may lead to a fetid or ammonia odor. Some disorders of metabolism cause particular odors in the urine. In maple syrup urine, the urine smells like burnt sugar or maple syrup; in phenylketonuria the urine smells musty;

Table 3.1 Causes of abnormal urine color

Pathologic Causes	Physiologic, foods, drugs
Red-Burgundy-Pink • Menstrual contamination • Gross hematuria • Papillary necrosis (often with clots) • Hemoglobinuria, hemolysis • Myoglobinuria • Porphyria • *Serratia marcescens* infection	**Red-Burgundy-Pink** • Beets • Blackberries • Urates • Pyridium • Phenolphthalein • Anthocyanin • Rhodamine B • Aminopyrine • Phenytoin sodium • Azo dyes
Dark Brown or Black • Homogentisic aciduria • Alkaptonuria • Methemoglobinemia • Melanin • Tyrosinosis	**Dark Brown or Black** • Senna • Cascara • Aniline • Hydroxyquinone • Resorcinol • Thymol
Blue-Green • Obstructive jaundice • Hepatitis • Blue diaper syndrome (intestinal tryptophan transport defect) • *Pseudomonas* infections • Phenol poisoning	**Blue-Green** • Carotene • Chlorophyll • Riboflavin • Methylene blue • Indigo-carmine • Resorcinol • Tetrahydronaphthalene • Methocarbamol
Cloudy-Milky • Urinary tract infection • Urates, uric acid (acid pH) • Calculi, "gravel" (phosphates, oxalates) • Chyluria • Nephrotic syndrome • Radiographic dye (acid pH	

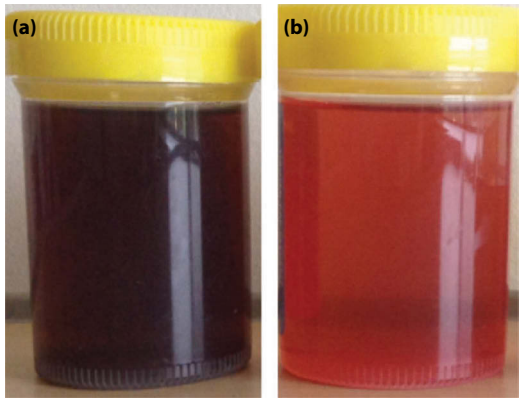


Figure 3.1 **(a)** Visible hematuria in a patient with post-streptococcal glomerulonephritis. The urine is dark coke-colored (glomerular hematuria). **(b)** Visible hematuria in a patient during passage of renal stone showing light pinkish urine color (urologic hematuria).

cystinuria and homocystinuria have sulfur-like odor; and tyrosinemia has an odor similar to that of fish or cabbage.

PH

Urine pH normally ranges from 4.5 to 8, depending on the acid-base balance, metabolic state, and dietary habits. In general, vegetarians may have a more alkaline urine pH and a high-protein meal may cause the urine pH to be more acidic. Freshly voided urine should be examined for determining pH, because loss of carbon dioxide to air will falsely alkalinize the pH. Bacterial contamination also may change the baseline pH, depending on the metabolism of the particular organism. This dipstick determination of pH is adequate for routine testing, but if a precise urine pH value is to be obtained, it can be done using a pH meter in the laboratory.

SPECIFIC GRAVITY AND OSMOLALITY

Urine osmolality is the key indicator of urinary concentration and is maximal after an overnight thirst (greater than 870 mOsm/kg in children younger than 2 years).[5] Urine osmolality is a function of the concentration of solutes present in the urine. Determination of the urine specific gravity and osmolality in a urine sample obtained after overnight thirsting can be a useful screening test for the diagnosis of diabetes insipidus. Apart from central and nephrogenic diabetes insipidus, urine specific gravity and osmolality may be decreased in renal insufficiency. Presence of protein, glucose, and osmotic contrast agents may increase urine specific gravity. In these cases, osmolality is the preferred measurement to assess urinary concentration ability.

Urine osmolality is not routinely measured in most clinical laboratories, but it can be approximated from the specific gravity according to the following formula[5]:

$$\text{Osmolality} = (\text{Specific gravity} - 1.000) \times 40{,}000$$

PROTEIN

Proteinuria is an important indicator of renal disease. Normally, the glomerulus restricts the filtration of proteins based on size and charge. Most proteins that are filtered are reabsorbed by endocytosis of the proximal tubule, and less than 10 mg/dL is found in normal urine. Most of these are low-molecular-weight proteins filtered at the glomerulus. Tamm-Horsfall protein (uromodulin) is a tubular glycoprotein normally secreted by the thick ascending limb of the loop of Henle and forms the matrix of urinary casts.[6]

Quantification of proteinuria

DIPSTICK TEST

The urinary dipstick examination for proteinuria is a convenient method for detection of proteinuria. This method is able to provide a semiquantitative estimate of the degree of proteinuria. The test is affected by the urinary concentration and pH level. Concentrated urine may give a positive reading even when the daily protein excretion is normal, whereas a dilute urine may result in a negative or only slightly positive reading, even in the presence of elevated daily protein excretion. False-positive results also may occur if the urine is highly alkaline; false-negative results can be seen in the presence of ingestion of large doses of ascorbic acid.

KEY POINTS

- First morning urine sample is preferred to quantify baseline proteinuria and establish the diagnosis of orthostatic proteinuria in children.
- Dipsticks are more specific for urinary albumin and are unable to detect globulin or Bence-Jones proteinuria.
- Very alkaline urine can give a false-positive dipstick test for protein.

SULFOSALICYLIC ACID TEST

Proteinuria also can be detected by using 10% sulfosalicylic acid, which precipitates urinary protein (Table 3.2). This semiquantitative assessment of the urine's turbidity correlates well with total urinary protein, including albumin. The sulfosalicylic acid test is not affected by urine pH or the presence of ascorbic acid.

KEY POINTS

Proteinuria can be quantified by:

- Dipstick (semiquantitative)
- 24-Hour urine collection
- Ratio of urine protein to urine creatinine

Table 3.2 Semiquantitative estimation of proteinuria using the sulfosalicylic acid precipitation test and the dipsticks

Sulfosalicylic acid			
Degree of turbidity		**Protein (mg/dL)**	**Dipstick equivalent**
No turbidity	Negative	No protein	Negative
Slight turbidity	Trace	1–20	Trace
Turbid (newsprint visible)	1+	30	1+
White cloud (heavy lines visible)	2+	100	2+
Fine precipitate (heavy lines invisible)	3+	300	3+
Flocculent precipitate (like yogurt)	4+	>500	4+ (2000 mg/dL)

Note: Sulfosalicylic acid test is conducted by adding 10 drops of 10% sulfosalicylic acid to 10 mL of urine. Sulfosalicylic acid detects all urinary proteins, including albumin. A dipstick test is more specific for albumin and does not detect globulins or tubular proteins.

Timed collection

The gold standard of urinary protein quantitation is the quantitative measurement of protein in a carefully timed urine collection, usually over 24 hours. Normal value for 24-hour urine protein excretion is less than 100 mg/m^2/day, and nephrotic range proteinuria exceeds 1000 mg/m^2/day, or 40 mg/m^2/h.[7] Calculation of the creatinine excretion index in the collected urine is often employed by nephrologists to judge completeness of the collected 24-hour urine sample. The creatinine excretion index, or creatinine excretion per kilogram of body weight (total urinary creatinine ÷ patient's weight), should be 15 to 20 mg/kg/24 h for the urine collection to be considered adequate.

Protein-to-creatinine ratio

Because of the difficulty in collecting timed urine samples in children, spot urine protein and creatinine estimation is preferred in children and also has been endorsed by the Kidney Disease Outcomes Quality Initiative (KDOQI) clinical practice guidelines.[8] The urine protein-to-creatinine (mg/mg) ratio correlates well with the 24-hour urine protein excretion.[9-13] Normal value for urinary protein-to-creatinine ratio is less than 0.2, but is slightly higher in younger children (Table 3.3). Proteinuria is considered to be of nephrotic range if the protein-to-creatinine ratio exceeds 2.0.[9,10] An approximate value of 24-hour urine protein excretion can be calculated from urine protein and creatinine obtained in a spot sample and using the following formula[11]:

24-Hour urinary protein excretion = Urine protein/urine creatinine × 0.63 (mg/m^2/day)

Table 3.3 Urinary protein-to-creatinine ratios showing age-related 95th percentiles

Age (years)	Protein (mg/mg creatinine
<2	0.492
2–13	0.178
>13	0.178

Source: Data from Houser MJ. Assessment of proteinuria using random urine samples. J Pediatr. 1984;104:845.

MICROALBUMINURIA

Microalbuminuria (also known as albuminuria), is used to screen children with diabetes of 5 years or longer of duration and is usually expressed as an albumin-to-creatinine ratio (ACR). Normal ACR is less than 30 mg/g creatinine.[14,15] Urine collections of 24 hours are not necessary in most children, and a spot urine sample estimation is sufficient for screening purposes. Orthostatic proteinuria in children can affect the quantification of ACR; therefore, estimation using the first morning urine sample is recommended.

KEY POINTS

- In patients with diabetes, urine ACR estimation is used to detect early manifestations of diabetic nephropathy.
- First morning urine should be used for measuring the ACR ratio in children with diabetes.
- Normal ACR is less than 30 mg/g of creatinine.

BLOOD

Dipsticks detect the presence of hemoglobin in the urine. The reaction relies on the peroxidase-like activity of hemoglobin to catalyze the reaction of a hydroperoxide with tetramethylbenzidine to give a green-blue color. Myoglobin also will give a positive reaction with the dipsticks. Therefore, a positive dipstick test for blood may be the result of hematuria, hemoglobinuria (intravascular hemolysis), or myoglobinuria (muscle injury or disease). Identification of RBCs on urine microscopy establishes the diagnosis of hematuria, and lack of RBCs suggests hemoglobinuria or myoglobinuria. Differentiation between hemoglobinuria and myoglobinuria can be accomplished

using immunochemical methods. A negative dipstick test rules out hematuria.

KEY POINTS

A positive test for blood may result from:

- Hematuria
- Myoglobinuria
- Hemoglobinuria

GLUCOSE

Dipstick detection of glucose is based on the oxidation of glucose by glucose oxidase. False-negative results occur if there are large quantities of reducing agents, such as vitamin C, tetracyclines, or homogentisic acid in the urine. False-positive results have not been reported. Glycosuria is seen in patients with diabetes mellitus and those with proximal tubular disorders, such as Fanconi syndrome. Renal glycosuria is an isolated proximal tubular defect of glucose reabsorption and is distinguished from diabetes mellitus by a normal simultaneously obtained serum glucose level and a normal hemoglobin A1C level. Abnormalities in SGLT2, a critical tubular glucose transporter located in the S1 segment of the proximal tubule, has been demonstrated to be associated with familial renal glycosuria.[16]

KEY POINTS

Glucosuria with a normal simultaneously drawn serum glucose level can be the result of:

- Renal glycosuria
- Fanconi syndrome

NITRITE

More than 90% of common urinary pathogens are nitrite-forming bacteria that convert urinary nitrate to nitrite with the help of the enzyme nitrate reductase. Nitrite can be detected in urine using a dipstick containing *p*-arsanilic acid, which reacts with nitrite to generate a diazonium compound that is then converted to 3-hydroxy-1,2,3,4-tetrahydrobenzo-quinolin-3-ol to produce a pink azo dye. Bacteria must be in contact with urine for 2 to 4 hours to allow nitrate conversion to nitrite and result in a positive test. Patients who have urinary frequency associated with the urinary tract infection (UTI) may not allow sufficient bacterial contact time, resulting in a false-negative urine test for nitrite. It is also important to point out that the nitrite test is not able to detect UTIs secondary to *Staphylococcus saprophyticus, Pseudomonas* species or enterococci, all of which lack the enzyme nitrate reductase and are unable to convert nitrate to nitrite.[17] A false-positive reading will occur if bacterial overgrowth is allowed to occur during inappropriate transport of the urine to the laboratory. False-negative results occur in the presence of ascorbic acid. The nitrite test for UTI is highly specific, but its sensitivity is low. Meta-analysis of pediatric studies demonstrated that the specificity of nitrite test was 98% (96% to 99%) and sensitivity was 49% (41% to 57%).[18]

KEY POINTS

- The nitrite test cannot detect infections caused by organisms that do not produce the nitrate reductase.
- Bacterial contact with urine needs to be at least 2 to 4 hours (in the bladder) for the nitrite test to be positive.
- The nitrite test has high specificity (98%) but low sensitivity (50%).

LEUKOCYTE ESTERASE

Leukocyte esterase (LE) has been incorporated into the routine dipstick evaluation of urine for almost a decade. The LE test is a colorimetric test that detects the presence of esterase produced by the polymorphonuclear leukocytes in the urine and is therefore used for the diagnosis of UTI. False-positive results may be seen in contamination of urine with vaginal secretions, tubulointerstitial nephritis, and severe glomerulonephritis. False-negative LE test results can be caused by high urinary protein concentration (nephrotic syndrome) and high urinary ascorbic acid level. Combining the LE and nitrite tests offers a better sensitivity and specificity profile than either of them separately. In a meta-analysis of pediatric studies, the LE test or nitrite-positive dipstick had a specificity of 88% (82% to 91%) and sensitivity of 79% (69% to 87%).[18]

MICROSCOPIC ANALYSIS

Microscopic examination of the urine is used semiquantitatively to confirm the presence of RBCs, cellular casts, crystals, and bacteria. Urine for microscopy should be freshly obtained, because stored samples are prone to developing autolysis of the cells and casts, rendering the evaluation inaccurate.

KEY POINTS

- Freshly obtained urine samples should be used for microscopy.
- Stored or frozen samples result in disruption of cellular elements.

Technique

The Clinical and Laboratory Standards Institute recommends that each laboratory should standardize the urine microscopy procedure. A standard urine volume (10, 12, or 15 mL) should be spun for a standard time of 5 minutes.[19] The centrifuge speed should be such as to achieve a relative centrifugal force (RCF) of approximately 400. To calculate RCF from rotations per minute (RPM) for a specific centrifuge, following formula should be used:

$$RCF\ (g) = 1.18 \times 10^{-5} \times Radius\ (cm) \times RPM^2$$

After centrifugation, the supernatant is decanted, and the pellet resuspended in the remaining 0.25 to 0.5 mL of urine (Figure 3.2). The sediment is gently resuspended in urine by tapping the pellet in the centrifuge tube, and is then examined under low-power (10 × 10 = 100 ×) and high-power (10 × 40 = 400 ×) microscopy. An examination of five random fields at 40 × throughout the slide permits assessment of the number of cells per high-power field (hpf). Urinary microscopy is generally performed in unstained urinary sediment slides. If a detailed analysis and identification of white blood cells (WBCs) is necessary (e.g., identification of eosinophils in interstitial nephritis), the sediment may be stained by Sternheimer-Malbin stain (Sedi-Stain, Becton, Dickinson, Franklin Lakes, NJ).

KEY POINTS

- Urine should be centrifuged at standardized speeds for 5 minutes.
- Unstained specimens provide adequate information for most clinical purposes.
- Staining the urine sediment may be indicated to identify some cellular elements.

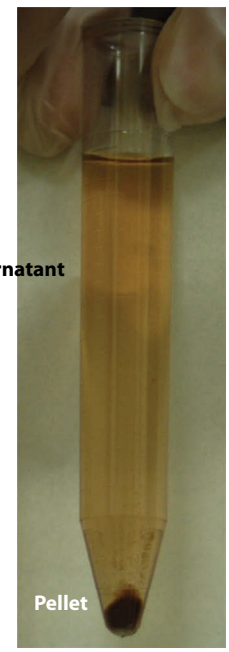


Figure 3.2 Centrifuged urine in a conical tube in a patient with visible hematuria showing the pellet of red blood cells at the bottom and the clear supernatant.

Red blood cells

In healthy children the normal upper limit for the number of RBCs in fresh midstream urine is 2 to 4 per hpf. The morphology of the RBCs should be reviewed in high power to distinguish between glomerular and nonglomerular hematuria. Dysmorphic RBCs with a large variation in size and shape, cell wall blebs, and distribution of hemoglobin content are more likely to be seen with glomerulonephritis (Figure 3.3).[20] The dysmorphic RBCs with a doughnut shape or with one or more blebs are also known as G1 cells. Eumorphic or normal RBCs are observed in nonglomerular urinary bleeding secondary to stones, hypercalciuria, and trauma.[21,22] At times, glomerular hematuria also can have a mixture of eumorphic and dysmorphic RBCs. The value of erythrocyte morphology in the diagnosis of glomerular disease in patients with persistent isolated hematuria was evaluated by Fogazzi et al.[23] In 16 such patients (10 children and 6 adults) classified as having glomerular hematuria, a renal biopsy showed glomerular disease in 14 of the 16 patients (87.5%).

White blood cells

In healthy children, the upper limit of normal for the number of WBCs in fresh midstream urine is 0 to 4 per hpf, with most of these being neutrophils. Neutrophils are recognizable by the presence of a multilobed nucleus and granular cytoplasm. In UTIs, neutrophils are the dominant type of WBCs noted in the urinary sediment and lymphocytes may be present in large numbers in urine during acute renal transplant rejection. Although eosinophils in the urinary sediment (eosinophiluria) can be seen in allergic interstitial nephritis, they are not pathognomonic of this disorder and can be seen in other inflammatory lesions of the kidneys and the urinary tract.[24]

Renal epithelial cells

Epithelial cells found in urine can come from the renal tubules, collecting system, or bladder, each with distinctive morphologic characteristics. Renal tubular epithelial (RTE) cells are only slightly larger than white cells and have a large central nucleus (Figure 3.4). These are normally present in small numbers in the urinary sediment, but an excessive number may be seen in acute kidney injury (AKI) and acute

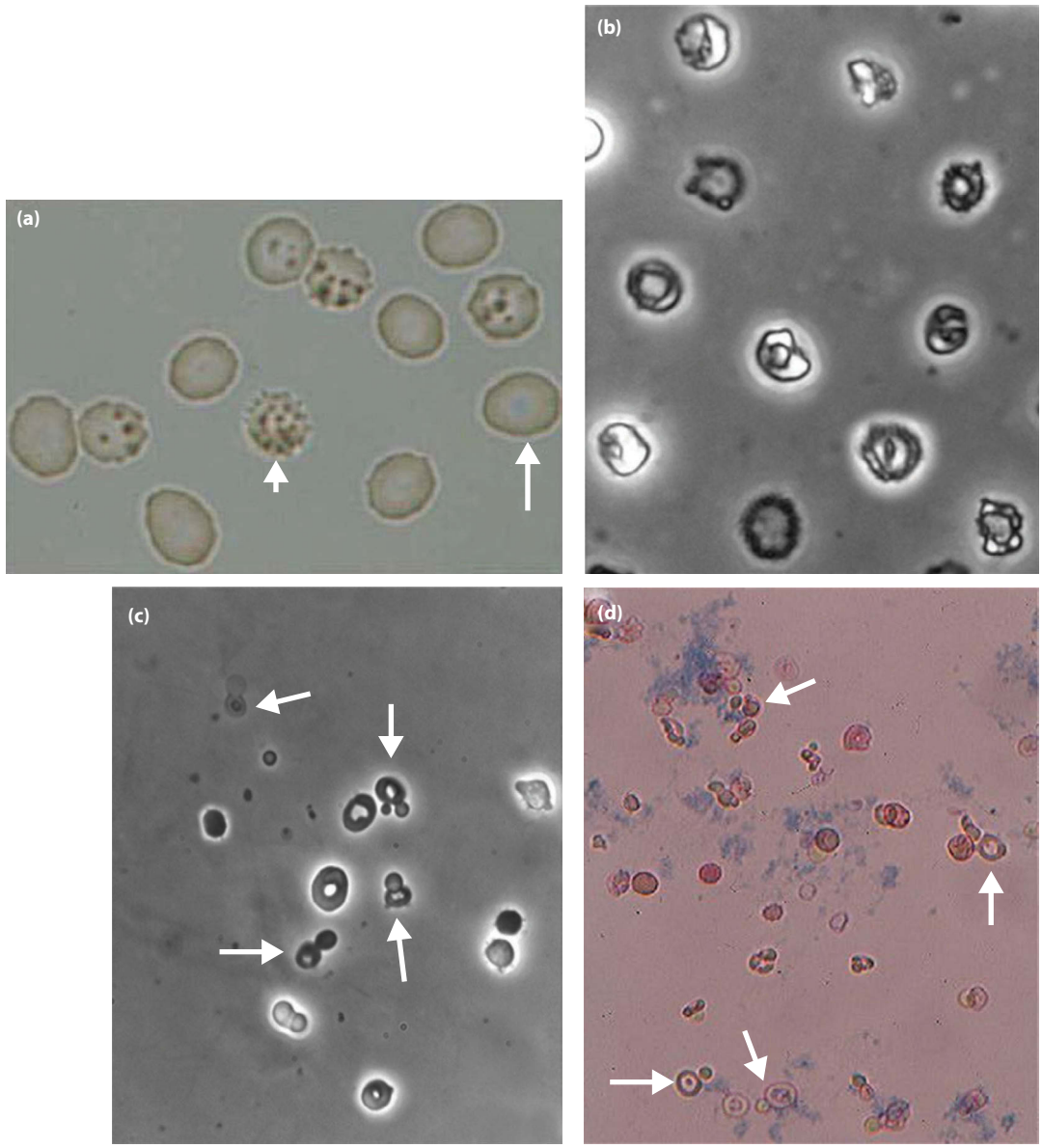


Figure 3.3 **(a)** Crenated red blood cells (RBCs) seen in concentrated (hypertonic) urine. These cells are isomorphic RBCs that are deformed in a hypertonic urine (intracellular dehydration). They do not represent glomerular hematuria. **(b)** Dysmorphic RBCs showing margination of hemoglobin and formation of "doughnut-shaped RBCs." **(c and d)** Acanthocytes or G1 cells (RBCs) in glomerular hematuria with cytoplasmic blebs (Mickey Mouse RBCs). Arrows point to the various types of dysmorphic cells in the urinary sediment. ([a] and [c] reproduced with permission from: Fogazzi GB, Verdesca S, Garigali G. Urinalysis: core curriculum 2008. Am J Kidney Dis. 2008;51:1052.)

renal transplant rejection. In nephrotic syndromes, RTEs may appear granular because of the accumulation of proteins or lipids in cytoplasmic vesicles. "Oval fat bodies" is the term reserved for fat-laden RTE cells seen in patients with nephrotic syndrome (Figure 3.5A). When viewed with a polarized light source, birefringence of lipid droplets in the RTE cells results in the Maltese-cross appearance of these cells (Figure 3.5B).

Bladder epithelial cells are three to four times the size of leukocytes, are thin, may appear folded upon themselves along edges, and have a relatively small nucleus. Although normally present in the urinary sediment, an excessive number may be seen in cystitis and urethritis. Transitional cells originate in the renal pelvis and ureters and are midway between RTE cells and bladder epithelial cells in their cellular characteristics.

Casts

Casts are cylindrical structures formed in the renal tubules by the precipitation of Tamm-Horsfall protein (uromodulin) and are sometimes overlaid with cellular elements. Hyaline casts are derived entirely from Tamm-Horsfall protein and appear as translucent cylindrical

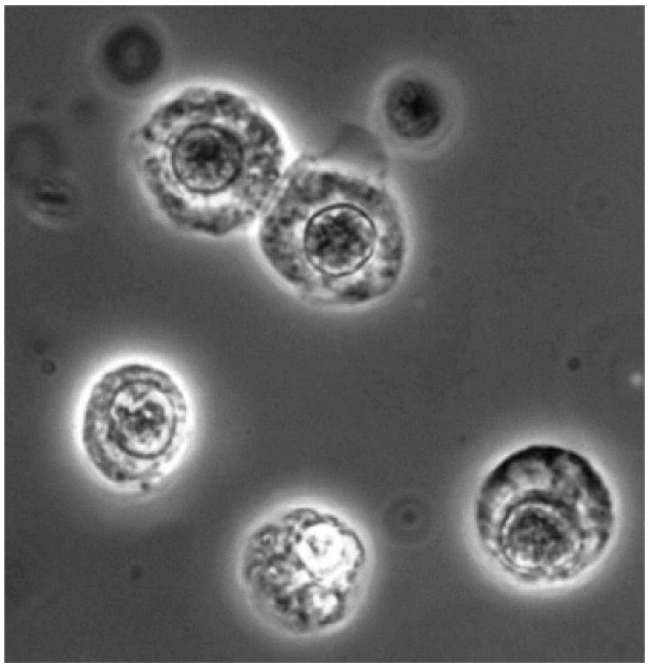


Figure 3.4 Renal tubular cell showing large central nuclei and granular cytoplasm. (Reproduced with permission from: Fogazzi GB, Verdesca S, Garigali G. Urinalysis: core curriculum 2008. Am J Kidney Dis. 51:1052, 2008.)

structures. These may be seen in concentrated urine of normal children, as well as in fever, exercise, dehydration, diuretic use, congestive heart failure, and nephrotic syndrome. Cells may be trapped within the matrix, giving rise to cellular casts. Degeneration of the cellular elements eventually leads to formation of granular casts (Figure 3.6A). Cellular casts are classified according to the dominant cell type included therein (red, white, or epithelial cell). WBC casts can be observed in acute pyelonephritis, glomerular diseases, and transplant rejection. RBC casts appear as round discoid cells embedded in the Tamm-Horsfall matrix and may be further identified by the hemoglobin pigment within the matrix of the cast (Figure 3.6B). RBC casts are pathognomonic of glomerular bleeding, or glomerulonephritis.

Waxy casts are similar to hyaline casts, but are more broad (Figure 3.6C). They are commonly seen in chronic renal diseases. Large waxy casts (broad casts) are often seen in chronic kidney disease (CKD). Broad casts are believed to originate in damaged nephron segments or from the collecting system. Fatty casts result from the incorporation of fat within the Tamm-Horsfall matrix and are common in nephrotic syndrome. Casts made of renal tubular cells can be seen in AKI (Figure 3.6D). A muddy brown cast is a specific type of granular cast seen in AKI that is an intensely brownish color and may be derived from degeneration of tubular epithelial cells. Light brown pigment casts also can be seen in hemoglobinuria and myoglobinuria.

Bacteriuria

Significant bacteriuria usually can be detected using a 40 × objective. From a standardization perspective, centrifuged urine (10 mL) sediment stained with Gram stain provides the most reproducible results. This method has been reported to have 95% sensitivity with 1 bacterium per oil immersion field and 95% specificity for bacteriuria if more than 5 bacteria are visualized.[25] Being cumbersome and time-consuming, the

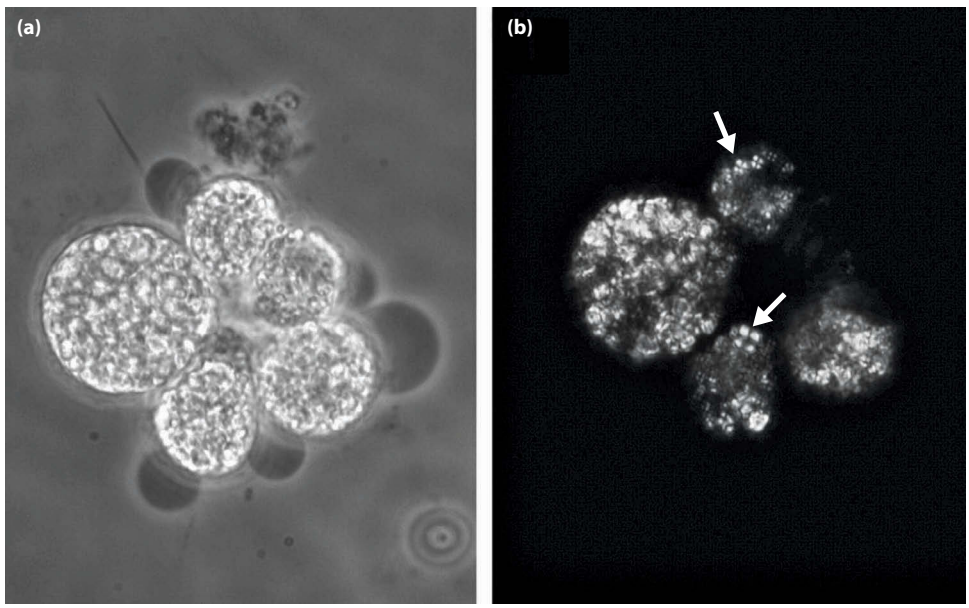


Figure 3.5 **(a)** "Oval fat bodies" or renal tubular cells packed with lipid droplets. **(b)** Same sediment viewed with polarized light showing the Maltese crosses (arrows). (Reproduced with permission from: Fogazzi GB, Verdesca S, Garigali G. Urinalysis: core curriculum 2008. Am J Kidney Dis. 2008;51:1052.)