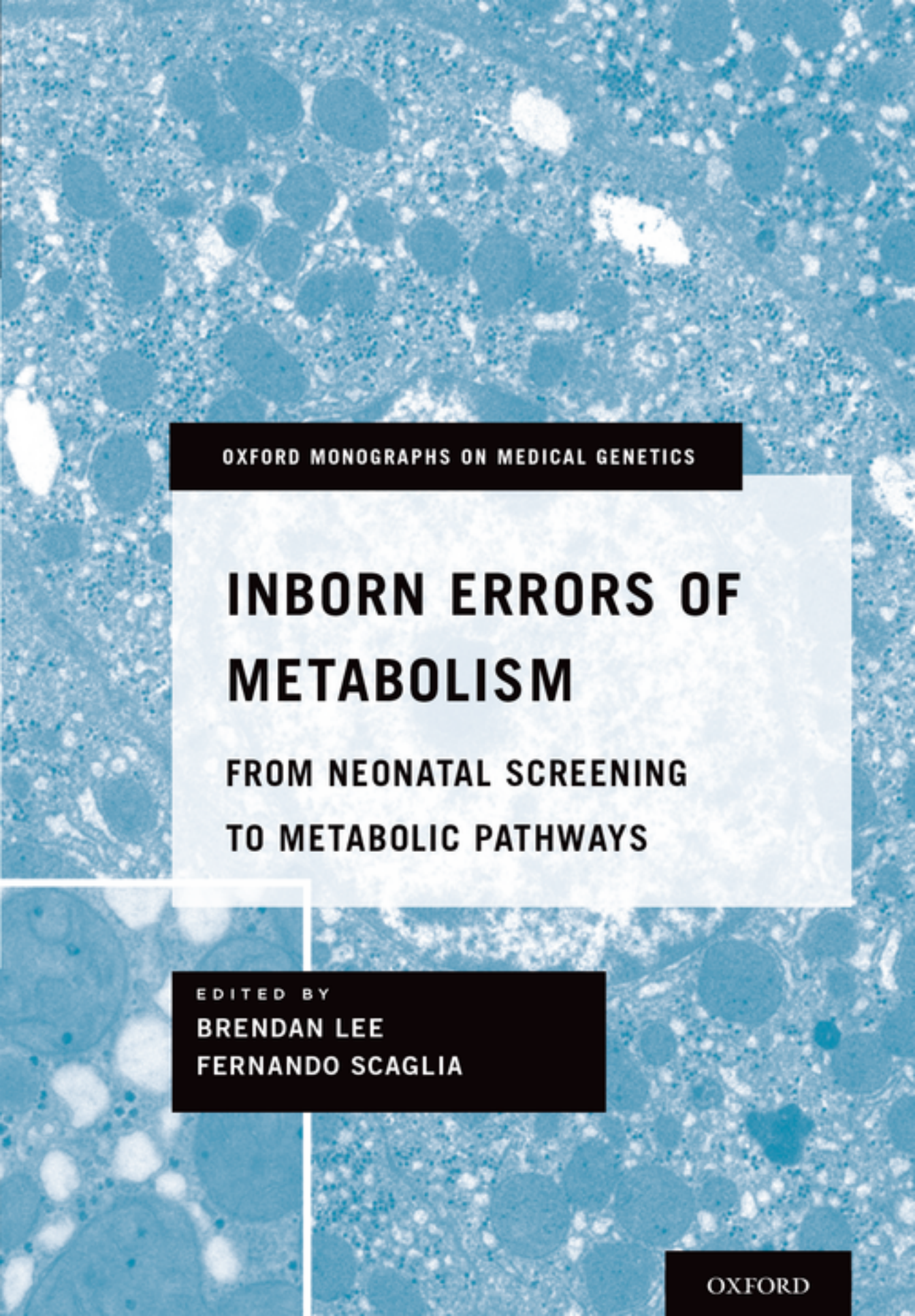
OXFORD MONOGRAPHS ON MEDICAL GENETICS
INBORN ERRORS OF METABOLISM
FROM NEONATAL SCREENING TO METABOLIC PATHWAYS
EDITED BY
BRENDAN LEE
FERNANDO SCAGLIA
OXFORD

Inborn Errors of Metabolism

OXFORD MONOGRAPHS ON MEDICAL GENETICS

1. R.B. McConnell: *The genetics of gastrointestinal disorders*
2. A.C. Kopéc: *The distribution of the blood groups in the United Kingdom*
3. E. Slater and V.A. Cowie: *The genetics of mental disorders*
4. C.O. Carter and T.J. Fairbank: *The genetics of locomotor disorders*
5. A.E. Mourant, A.C. Kopéc, and K. Domaniewska-Sobezak: *The distribution of the human blood groups and other polymorphisms*
6. A.E. Mourant, A.C. Kopéc, and K. Domaniewska-Sobezak: *Blood groups and diseases*
7. A.G. Steinbert and C.E. Cook: *The distribution of the human immunoglobulin allotypes*
8. D. Tills, A.C. Kopéc, and R.E. Tills *The distribution of the human blood groups and other polymorphisms: Supplement I*
10. D.Z. Loesch: *Quantitative dermatoglyphics: classification, genetics, and pathology*
11. D.J. Bond and A.C. Chandley: *Aneuploidy*
12. P.F. Benson and A.H. Fensom: *Genetic biochemical disorders*
13. G.R. Sutherland and F. Hecht: *Fragile sites on human chromosomes*
14. M. d'A. Crawfurd: *The genetics of renal tract disorders*
16. C.R. Scriver and B. Child: *Garrod's inborn factors in disease*
18. M. Baraitser: *The genetics of neurological disorders*
19. R.J. Gorlin, M.M. Cohen, Jr., and L.S. Levin: *Syndromes of the head and neck, third edition*
21. D. Warburton, J. Byrne, and N. Canki: *Chromosome anomalies and prenatal development: an atlas*
22. J.J. Nora, K. Berg, and A.H. Nora: *Cardiovascular disease: genetics, epidemiology, and prevention*
24. A.E.H. Emery: *Duchenne muscular dystrophy, second edition*
25. E.G.D. Tuddenham and D.N. Cooper: *The molecular genetics of haemostasis and its inherited disorders*
26. A. Boué: *Foetal medicine*
27. R.E. Stevenson, J.G. Hall, and R.M. Goodman: *Human malformations*
28. R.J. Gorlin, H.V. Toriello, and M.M. Cohen, Jr.: *Hereditary hearing loss and its syndromes*
29. R.J.M. Gardner and G. R. Sutherland: *Chromosomes abnormalities and genetic counseling, second edition*
30. A.S. Teebi and T.I. Farag: *Genetic disorders among Arab populations*
31. M.M. Cohen, Jr.: *The child with multiple birth defects*
32. W.W. Weber: *Pharmacogenetics*
33. V.P. Sybert: *Genetic skin disorders*
34. M. Baraitser: *Genetics of neurological disorders, third edition*
35. H. Ostrer: *Non-Mendelian genetics in humans*
36. E. Traboulsi: *Genetic factors in human disease*
37. G.L. Semenza: *Transcription factors and human disease*
38. L. Pinsky, R.P. Erickson, and R.N. Schimke: *Genetic disorders of human sexual development*
39. R.E. Stevenson, C.E. Schwartz, and R.J. Schroer: *X-linked mental retardation*
40. M.J. Khoury, W. Burke, and E. Thomson: *Genetics and public health in the 21st century*
41. J. Weil: *Psychosocial genetic counseling*
42. R.J. Gorlin, M.M. Cohen, Jr., and R.C.M. Hennekam: *Syndromes of the head and neck, fourth edition*
43. M.M. Cohen, Jr., G. Neri, and R. Weksberg: *Overgrowth syndromes*
44. R.A. King, J.I. Rotter, and A.G. Motulsky: *The genetic basis of common diseases, second edition*
45. G.P. Bates, P.S. Harper, and L. Jones: *Huntington's disease, third edition*
46. R.J.M. Gardner and G.R. Sutherland: *Chromosome abnormalities and genetic counseling, third edition*
47. I.J. Holt: *Genetics of mitochondrial disease*
48. F. Flinter, E. Maher, and A. Saggar-Malik: *The genetics of renal disease*
49. C.J. Epstein, R.P. Erickson, and A. Wynshaw-Boris: *Inborn errors of development: the molecular basis of clinical disorders of morphogenesis*
50. H.V. Toriello, W. Reardon, and R.J. Gorlin: *Hereditary hearing loss and its syndromes, second edition*
51. P.S. Harper: *Landmarks in medical genetics*
52. R.E. Stevenson and J.G. Hall: *Human malformations and related anomalies, second edition*
53. D. Kumar and S.D. Weatherall: *Genomics and clinical medicine*
54. C.J. Epstein, R.P. Erickson, and A. Wynshaw-Boris: *Inborn errors of development: the molecular basis of clinical disorders of morphogenesis, second edition*
55. W. Weber: *Pharmacogenetics, second edition*
56. P.L. Beales, I.S. Farooqi, and S. O'Rahilly: *The genetics of obesity syndromes*
57. P.S. Harper: *A short history of medical genetics*
58. R.C.M. Hennekam, I.D. Krantz, and J.E. Allanson: *Gorlin's syndromes of the head and neck, fifth edition*
59. D. Kumar and P. Elliot: *Principles and practices of cardiovascular genetics*
60. V.P. Sybert: *Genetic skin disorders, second edition*
61. R.J.M. Gardner, G.R. Sutherland, and L.C. Shaffer: *Chromosome abnormalities and genetic counseling, fourth edition*
62. D. Kumar: *Genomics and health in the developing world*
63. G. Bates, S. Tabrizi, and L. Jones: *Huntington's disease, fourth edition*
64. B. Lee and F. Scaglia: *Inborn errors of metabolism: from neonatal screening to metabolic pathways*

Inborn Errors of Metabolism

From Neonatal Screening to Metabolic Pathways

EDITED BY

Brendan Lee

AND

Fernando Scaglia

OXFORD
UNIVERSITY PRESS

OXFORD
UNIVERSITY PRESS

Oxford University Press is a department of the University of Oxford. It furthers the University's objective of excellence in research, scholarship, and education by publishing worldwide.

Oxford New York
Auckland Cape Town Dar es Salaam Hong Kong Karachi
Kuala Lumpur Madrid Melbourne Mexico City Nairobi
New Delhi Shanghai Taipei Toronto

With offices in
Argentina Austria Brazil Chile Czech Republic France Greece
Guatemala Hungary Italy Japan Poland Portugal Singapore
South Korea Switzerland Thailand Turkey Ukraine Vietnam

Oxford is a registered trademark of Oxford University Press in the UK and certain other countries.

Published in the United States of America by
Oxford University Press
198 Madison Avenue, New York, NY 10016

Library of Congress Cataloging-in-Publication Data

Inborn errors of metabolism (Lee)
Inborn errors of metabolism : from neonatal screening to metabolic pathways / edited by Brendan Lee and Fernando Scaglia.
p. ; cm.
Includes bibliographical references.
ISBN 978–0–19–979758–5 (alk. paper)
I. Lee, Brendan, editor. II. Scaglia, Fernando, editor. III. Title.
[DNLM: 1. Metabolism, Inborn Errors. WD 205]
RC627.8
616.3′9042—dc23
2014010620

1 3 5 7 9 8 6 4 2
Printed in the United States of America
on acid-free paper

Contents

Contributors

Lutfi A. Abu-Elheiga, Ph.D.
Verna and Marrs McLean Department of Biochemistry and Molecular Biology
Baylor College of Medicine
Houston, Texas

Andrea Bordugo, M.D.
Division of Inherited Metabolic Disorders
Department of Pediatrics
University Hospital
Padova, Italy

Nicola Brunetti-Pierri, M.D., F.A.C.M.G.
Telethon Institute of Genetics and Medicine; and
Department of Translational Medical Sciences
Federico II University of Naples
Naples, Italy

Alberto Burlina, M.D.
Division of Inherited Metabolic Disorders
Department of Pediatrics
University Hospital
Padova, Italy

Robert Burnett, B.S.
Medical Genetics Branch
National Human Genome Research Institute
Bethesda, Maryland

Philippe M. Campeau, M.D.
Department of Pediatrics
University of Montreal
Montreal, Quebec

Alexander J. Choi, B.S.
Medical Genetics Branch
National Human Genome Research Institute
Bethesda, Maryland

Erin M. Coffee, Ph.D.
Biology Department
Boston University
Boston, Massachusetts

William J. Craigen, M.D., Ph.D.
Department of Molecular and Human Genetics
Baylor College of Medicine
Houston, Texas

Erik A. Eklund, M.D., Ph.D.
Department of Clinical Sciences
Lund University
Lund, Sweden

Ayman W. El-Hattab, M.D.
Division of Medical Genetics
Department of Pediatrics
The Children's Hospital
King Fahad Medical City
Riyadh, Saudi Arabia
Faculty of Medicine
King Saud bin Abdulaziz University for Health Sciences
Riyadh, Saudi Arabia

Christine M. Eng, M.D.
Professor of Molecular and Human Genetics
Director
DNA Diagnostic Laboratory
Baylor College of Medicine
Houston, TX

Ayelet Erez, M.D., Ph.D.
Incumbent of the Leah Omenn Career Development Chair
Senior Scientist
Department of Biological Regulation
Weizmann Institute of Science
Rehovot, Israel

Yasemen Eroglu, M.D.
Pediatric Gastroenterology and Hepatology
Oregon Health & Science University
Portland, Oregon

Hudson H. Freeze, Ph.D.
Human Genetics Program
Sanford-Burnham Medical Research Institute
La Jolla, California

Areeg El-Gharbawy, M.D.
Division of Medical Genetics, Department of Pediatrics
Children's hospital of Pittsburgh of UPMC-University of Pittsburgh School of Medicine
Durham, North Carolina

Ehud Goldin, Ph.D.
Medical Genetics Branch
National Human Genome Research Institute
Bethesda, Maryland

Brett H. Graham, M.D., Ph.D.
Department of Molecular and Human Genetics
Baylor College of Medicine
Houston, Texas

Georg F. Hoffmann, M.D.
Department of General Pediatrics
University Children's Hospital
Heidelberg, Germany

Dwight Koeberl, M.D., Ph.D.
Division of Medical Genetics, Department of Pediatrics
Duke University Medical Center
Durham, North Carolina

Donna M. Krasnewich, M.D., Ph.D.
National Institute of General Medical Sciences
National Institutes of Health
Bethesda, Maryland

Brendan Lee, M.D., Ph.D.
Robert and Janice Endowed Chair in Molecular and Human Genetics
Department of Molecular and Human Genetics
Baylor College of Medicine
Houston, Texas

Uta Lichter-Konecki, M.D., Ph.D.
Columbia University
Department of Pediatrics
Division of Clinical Genetics
New York, New York

Irini Manoli, M.D., Ph.D.
Organic Acid Research Section
National Human Genome Research Institute
National Institutes of Health
Bethesda, Maryland

Jochen Meyburg, M.D.
Department of General Pediatrics
University Children's Hospital
Heidelberg, Germany

Sandesh C.S. Nagamani, M.D.
Department of Molecular and Human Genetics
Baylor College of Medicine
Houston, Texas

Gregory M. Pastores, M.D.
Consultant
Department of Medicine/National Centre for Inherited Metabolic Disorders
Mater Misericordiae University Hospital
Dublin, Ireland
and
Visiting Professor
Department of Medicine
Yale University School of Medicine
New Haven, CT

Marc C. Patterson, M.D., F.R.A.C.P.
Pediatrics and Medical Genetics
Mayo Clinic College of Medicine
Chair, Division of Child and Adolescent Neurology
Mayo Clinic
Rochester, Minnesota

Jean-Baptiste Roullet, Ph.D.
Department of Pediatrics
Oregon Health & Science University
Portland, Oregon

Fernando Scaglia, M.D.
Department of Molecular and Human Genetics
Baylor College of Medicine
Houston, Texas
Texas Children's Hospital
Houston, Texas

Oleg A. Shchelochkov, M.D., F.A.A.P.
Division of Genetics
Department of Pediatrics
University of Iowa Hospitals and Clinics
Iowa City, Iowa

Marwan S. Shinawi, M.D.
Division of Genetics and Genomic Medicine
Department of Pediatrics
Washington University School of Medicine
St. Louis, Missouri

Ellen Sidransky, M.D.
Medical Genetics Branch
National Human Genome Research Institute
Bethesda, Maryland

Robert D. Steiner, M.D.
Marshfield Clinic Research Foundation
Department of Pediatrics
University of Wisconsin
Madison and Marshfield, Wisconsin

V. Reid Sutton, M.D.
Department of Molecular and Human Genetics
Baylor College of Medicine
Houston, Texas

Dean R. Tolan, Ph.D.
Biology Department
Boston University
Boston, Massachusetts

Luis A. Umaña, M.D.
Department of Molecular and Human Genetics
Baylor College of Medicine
Houston, Texas

Charles P. Venditti, M.D., Ph.D.
Organic Acid Research Section
National Human Genome Research Institute
National Institutes of Health
Bethesda, Maryland

Wei Zheng, M.D., Ph.D.
Therapeutics for Rare and Neglected Disease Program
National Center for Advancing Translational Sciences
National Institutes of Health
Bethesda, Maryland

About the Editors

Brendan Lee, MD, PhD, is Professor and Chair in the Department of Molecular and Human Genetics at Baylor College of Medicine. As a pediatrician and geneticist, Dr. Lee studies structural birth defects and inborn errors of metabolism. In the area of metabolic disease, he is developing new treatments for maple syrup urine disease and urea cycle disorders.

Fernando Scaglia, MD, is a Professor in the Department of Molecular and Human Genetics at Baylor College of Medicine. His primary research interests include the natural history and molecular characterization of mitochondrial cytopathies, clinical trials for Leigh syndrome, and the study of nitric oxide and glucose metabolism in patients with MELAS syndrome.

Introduction

Typically, textbooks on inborn errors of metabolism have focused on presenting the classical biochemical defects, correlating them with different clinical presentations and describing the current therapeutic approaches. However, with the advent of comprehensive newborn screening and improvement in diagnostic methodologies, we are beginning to appreciate the complex natural histories of these disorders. They together underscore that the increasingly diverse disease phenotypes that arise from Mendelian disorders reflect not only the primary effect of the metabolic disturbance, that is, accumulation of toxic metabolites upstream of a biochemical block and deficiency of the product downstream. We and others now appreciate secondary effects of the block as well as new "moonlighting" functions of components of the pathway. Hence, the aim of this textbook focuses on a pathways approach to presenting the phenotypes of inborn errors of metabolism. This textbook covers a myriad of topics from the principles of newborn screening, to presenting the basic underlying biochemical and molecular alterations, to explaining how these basic alterations in pathways may in fact lead to complex secondary and tertiary effects in metabolism that contribute to the complex natural histories of these disorders. The boundaries between Mendelian and complex disorders have become increasingly blurred as we recognize that Mendelian inborn errors of metabolism are indeed complex disorders. An evolving paradigm shift now supported by robust evidence points to complex signaling pathways and networks in inborn errors of metabolism. Thus a new focus on understanding these diseases should be based on studying how their natural histories can inform us about the secondary and tertiary consequences of the primary metabolic defects. The focus on the broad pathway effects of specific metabolic derangements will lead us to a deeper understanding of the mechanisms of pathogenesis. Hence, we hope to extend beyond basic descriptions of the classical biochemistry to prepare future generations of students, clinicians, and scientists in the study of these disorders. We hope that this approach will stimulate new ideas for therapeutic strategies and management.

SECTION 1

Newborn Screening

1

Newborn Screening for Inborn Errors of Metabolism

Introduction and Approaches for Confirmation

V. Reid Sutton and Brett H. Graham

History of Newborn Screening

In the early 1960s, Robert Guthrie and Ada Susi published a method for the detection of phenylketonuria (PKU) in newborns.[1] A small punch from a blood or urine spot on a filter paper card was applied to an agar plate. Elevated phenylalanine (Phe) levels impaired the growth of *Bacillus subtilis* ATCC 6051, and the diameter of clearing of bacterial growth could be correlated with Phe levels in the blood spot. Screening programs were rapidly developed in the industrialized world, and the early detection and treatment of PKU has led to dramatically improved, and sometimes normal, outcomes. This method for screening, known as a bacterial inhibition assay, was employed to detect other inborn errors of metabolism such as galactosemia, maple syrup urine disease (MSUD), homocystinuria, and others, though they were not as widely adopted as screening for PKU. Enzyme assays were subsequently developed for newborn screening (NBS) bloodspots for detection of disorders, including galactosemia in 1964[2] and biotinidase in 1984.[3] In 1975, an immunoassay procedure was published for the detection of neonatal hypothyroidism.[4] and over the next few decades immunoassays were developed for congenital adrenal hyperplasia (21-hydroxylase deficiency) and other disorders. In the 1980s and 1990s, fluorimetric assays replaced bacterial inhibition assays for analyte analysis. In 1990, tandem mass spectroscopy (MS/MS), which had been used clinically to measure urine acylcarnitines, was demonstrated to be amenable to the detection of analytes in NBS bloodspots.[5] The adoption of this methodology by NBS programs in North America and in most industrialized nations rapidly expanded the number of disorders included in NBS programs. This rapid expansion reflected the large number of analytes that could be detected with a single assay; the high level of automation; and the speed of the sample preparation, assay, and analysis. In 2006, the American College

of Medical Genetics (ACMG) proposed a uniform NBS panel[6] that was endorsed by the March of Dimes and has now been widely adopted by NBS programs.

Principles Underlying NBS

The original tenets for NBS were based upon the idea that there is an individual and separate test for every disease screened for. Those original tenets are

- the disorder occurs with significant frequency.
- an inexpensive and reliable method of testing exists.
- an effective treatment/intervention exists.
- if untreated, the baby may die or develop severe mental retardation.
- the affected baby may appear normal at birth.

The advent of MS/MS for NBS has allowed for the interrogation for multiple disorders using a single, multianalyte assay. This has led to a shift in the aforementioned tenets, since adding a test using the same method adds very little expense. For example, even if a disorder were extremely rare, if it could be detected and there were an effective intervention, the minimal cost of adding it to a MS/MS panel might be cost effective. Similarly, if one could add a disorder for which there was no accepted effective treatment, it might be cost effective to add it based upon minimizing diagnostic testing to determine the cause of the phenotype and being able to counsel parents about their reproductive options. The minimum criteria for diseases considered for inclusion in NBS as proposed by the ACMG are

- The disorder can be identified at a phase (24 to 48 hours after birth) at which it would not ordinarily be clinically detected.
- A test with the appropriate sensitivity and specificity is available.
- There are demonstrated benefits of early detection, timely intervention, and efficacious treatment of the condition being tested.[6]

Current Technologies

NBS programs currently employ a variety of methods for initial detection of targeted disorders.

Fluoroimmunoassay

Also known as fluorescence spectroscopy, this method utilizes light (often ultraviolet) to excite the electrons of a particular analyte/antibody interaction that is the product of an enzyme reaction. Readings of sample fluorescence at a specific wavelength or set of wavelengths can be used to determine the concentration of a particular analyte. This method is commonly employed in the measurement of congenital adrenal hypoplasia, congenital

hypothyroidism, cystic fibrosis, biotinidase, and galactose-1-phosphate uridyltransferase enzyme activities. It was also used commonly in the past for PKU but has been widely replaced in the United States by MS/MS.

Radioimmunoassay

In radioimmunoassay, a known quantity of an antigen is made radioactive. This radiolabeled (or "hot") antigen is then mixed with a known amount of antibody for that antigen, and, as a result, the two chemically bind to one another. Then a soluabilized NBS bloodspot punch sample is added that contains natural, unlabled ("cold") antigen. This causes the unlabeled (cold) antigen from the bloodspot sample to compete with the radiolabeled antigen (hot) for antibody binding sites. The higher the concentration of cold antigen in the NBS sample, the more it binds to the antibody, displacing the radiolabeled antigen and thus reducing the ratio of *antibody-bound* radiolabeled antigen to *free* radiolabeled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigen remaining in the supernatant is measured using a gamma counter. Using known standards, a binding curve can then be generated that allows the amount of antigen in the patient's serum to be determined. This method has been used for the detection of congenital hypothyroidism, congenital adrenal hyperplasia (21-hydroxylase deficiency), and cystic fibrosis; however, it has been widely replaced by enzyme-linked immunosorbent assay or fluoroimmunoassay to avoid the use of radioactive substrates.

Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay is another method that may be used for congenital hypothyroidism and congenital adrenal hyperplasia. It uses an antibody linked to an enzyme. When the NBS specimen containing the antigen of interest (e.g., T4) is mixed with the antibody-enzyme complex, it binds to the antibody. A substrate is then added that the enzyme metabolized and produces a signal, such as fluorescence at a particular wavelength, or other signal that can be detected.

Electrophoresis

The use of an electrical current to separate proteins by mass and charge is known as electrophoresis. This is typically performed in a gel-based medium but can also be done on paper. Electrophoresis has been used by NBS programs to separate different hemoglobin chains in the detection of sickle cell disease and other hemoglobinopathies, but this has widely been replaced by isoelectric focusing or high-performance liquid chromatography as these methods better resolve the types and quantities of hemoglobins.

Isoelectric Focusing

Employment of a gel-based medium to separate molecules by charge and pH is used by NBS to test for hemoglobinopathies. It is the most common method currently employed by NBS programs in the United States. High-performance liquid chromatograph is significantly more expensive but is generally viewed to be a superior method as it is more automated and more quantitative.

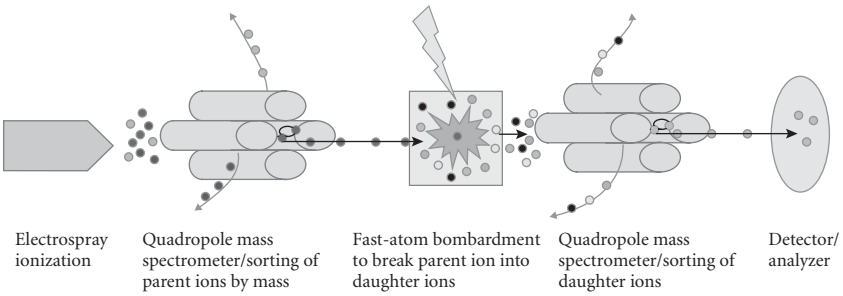

FIGURE 1.1 Tandem mass spectrometry. The specimen is injected into the machine and undergoes electrospray ionization. The first quadrupole uses an electromagnetic field to sort and select the ions of interest (that the scientist has programmed the machine to look for). The ions of interest then undergo fast-atom bombardment to break the parent ion into a host of daughter ions. The daughter ions then enter a second quadrupole that sorts out and selects the daughter ions of interest and the detector then measures the quantity.

Tandem Mass Spectroscopy

MS/MS is presently used in the detection of multiple analytes for the detection of amino acid disorders, organic acidemias, and fatty acid oxidation disorders by NBS. A soluabilized bloodspot sample may be either derivatized (e.g., butylated) or underivatized. In certain cases, derivatization may be more sensitive; underivatized methods may be quicker and allow for the detection of analytes (such as succinylacetone) that cannot be detected using a derivatized method. Most methods employ electrospray technology to ionize the sample and separate the parent ions by mass (this is the first mass spectrometry in MS/MS). The parent ion is then subjected to a force that breaks it into daughter ions, and a second mass spectrometer searches for the mass of a specific daughter ion associated with the analyte of interest (Figure 1.1). For example, propionyl(C3)carnitine is used to detect both methylmalonic and propionic acidemias, and the butylated mass transition is 274; the most commonly used daughter ion in this case is carnitine, which has a mass transition of 85. When the first mass spectrometer detects a mass transition of 274 and the second mass spectrometer detects a mass transition of 85, then the quantity of the parent ion is measured to determine the amount of propionylcarnitine. One problem with this method is that if there is another compound in the specimen that has the same parent ion mass transition and daughter ion mass, it will be assumed to be the analyte of interest, even if it is not. This occurs with pentanoyl(C5)carnitine; pivalic acid, which is a metabolite of ampicillin, has the same mass transition as pentanoylcarnitine and also has a daughter ion that has the same mass as that commonly used by NBS programs.

Selection of Primary and Secondary Analytes

Selection of primary and secondary analytes is dependent on the preference of the individual NBS program. Secondary analytes are typically utilized to improve sensitivity and reduce the false positive rate and tend to vary more from program to program. Commonly accepted primary analytes and the diseases they are able to detect are found in Table 1.1.

Certain NBS programs may reflex to a second-tier test based on an abnormal primary screen result. This is commonly done for cystic fibrosis, whereby individuals with an elevated

TABLE 1.1 **Screening Metabolites and Confirmatory Testing for Inborn Errors of Metabolism Detected by Tandem Mass Spectrometry.**

Disease Category	Screening Analyte [Secondary Analytes]	Primary Metabolic Confirmatory Test(s)	Disorder	Other Metabolic Markers	Additional Confirmatory Test(s)¶
Disorders of amino acid catabolism and transport.	Arginine	PAA[1]	Argininemia (Arginase Deficiency)	Orotic acid	Arginase enzyme activity in RBC[2]s or liver; *ARG1* gene sequencing
	Citrulline	PAA	Argininosuccinic Aciduria (Argininosuccinate Lyase or ASL Deficiency)	Argininosuccinic Acid Orotic acid	ASL enzyme activity in RBCs, SFC[3] or liver; *ASL* gene sequencing
			Citrullinemia, type I (Argininosuccinate Synthetase or ASS Deficiency)	Low Arginine	ASS enzyme activity in SFC or liver; *ASS1* gene sequencing
			Citrullinemia, type II (Citrin Deficiency)	Arginine; Methionine; Threonine; Tyrosine; ↑Threonine/Serine ratio	*SLC25A13* gene sequencing
	Leucine [Valine]	PAA & UOA[4]	Maple Syrup Urine Disease (Branched-Chain Ketoacid Dehydrogenase Deficiency)	Isoleucine; Alloisoleucine	BCKD[5] enzyme activity in LB[6], SFC; *BCKDHA, BCKDHB, DBT* gene sequencing
	Methionine	PAA & total plasma homocysteine	Homocystinuria (CBS Deficiency)	Homocysteine	*CBS* gene sequencing
			Hypermethionemia (MAT[7] I/III, GNMT[8], or S-AdoHcy[9] hydrolase deficiencies)	S-AdoMet[10] (↑ in GNMT or S-AdoHcy hydrolase deficiencies); S-AdoHcy (↑ in S-AdoHcy hydrolase deficiency)	plasma S-AdoMet & S-AdoHcy; *MAT1A* gene sequencing for MAT I/III def.
	Phenylalanine [↑Phe/Tyr ratio]	Plasma Phe	PKU or hyperphenylalaninemia (PAH or Phe hydroxylase deficiency)	Increased urine hydroxyphenylacetic acid, phenylpyruvic acid, phenylacetic acid and phenylacetylglutamine	*PAH* gene sequencing
			Disorders of biopterin metabolism (GTPCH[11], PTPS[12], PCD[13], or DHPR[14] deficiencies)	Biopterin (↓ in all); neopterin (↓ in GTPCH, ↑ or N in others); 5HIAA[15] & HVA[16] in CSF[17] (↓ in all)	urine pterins; CSF neurotransmitters; RBC DHPR enzyme assay; *GCH1, PTS*, or *QDPR* gene sequencing

(continued)

TABLE 1.1 (Continued)

Disease Category	Screening Analyte [Secondary Analytes]	Primary Metabolic Confirmatory Test(s)	Disorder	Other Metabolic Markers	Additional Confirmatory Test(s)[¶]
	Succinylacetone	PAA; succinylacetone in blood or urine	Tyrosinemia, type I (Fumarylacetoacetate Hydrolase Deficiency)	Tyrosine; 4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate, and 4-hydroxyphenylacetate in urine; 5-aminolevulinic acid in urine	*FAH* gene sequencing
	Tyrosine	PAA	Tyrosinemia, type II (Tyrosine Aminotransferase Deficiency)	4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate, and 4-hydroxyphenylacetate in urine	
			Tyrosinemia, type III (4-Hydroxy-phenylpyruvate Dioxygenase Deficiency)	4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate, and 4-hydroxyphenylacetate in urine	
Disorders of Fatty Acid Oxidation	C0 (↓) (Free carnitine)	Total & free plasma carnitine	Carnitine Uptake Defect		carnitine transport assay in SFC; *OCTN2* gene sequencing
	C0/[C16+C18] [↑C0]	Total & free plasma carnitine, ACP[17]	Carnitine Palmitoyl Transferase I (CPT1) Deficiency		CPT assay in SFC; *CPT1A* gene sequencing
	C4 (Butyrylcarnitine)	ACP, UAG[19], UOA	Short-Chain Acyl-CoA Deficiency (SCAD)	Ethylmalonic Acid; Butyrylglycine	*ACADS* gene sequencing
			Ethylmalonic Encephalopathy	C5; Ethylmalonic Acid; Isovalerylglycine	*ETHE1* gene sequencing
			Glutaric Aciduria, Type II or Multiple Acyl-CoA Dehydrogenase Deficiency (MADD)	↑ C6-C18; ethylmalonic acid, isovaleric acid, glutaric acid; isovalerylglycine and hexanoylglycine	ETF/ETF:QO enzyme assays on SFC; *ETFA, ETFB, ETFDH* gene sequencing
	C4-OH (3-Hydroxybutyrylcarnitine)	ACP, UOA	Medium/Short-Chain Hydroxy Acyl-CoA Deficiency (M/SCHAD)	↑ Hydroxy-dicarboxylic acids; elevated insulin	*HADH* gene sequencing
	C8 (Octanoylcarnitine) [C6; C10; C10:1; C8/C10; C8/C2]	ACP, UOA, UAG	Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCAD)	↑ medium chain dicarboxylic acids; hexanoyl-, suberyl-, and 3-phenylpropionylglycines	*ACADM* gene sequencing
	C14:1 (Tetradecenoylcarnitine) [C14; C14:2; C16; C18:1; C14:1/C2]	ACP	Very Long Chain Acyl-CoA Dehydrogenase Deficiency (VLCAD)	↑ medium/long chain dicarboxylic acids	*ACADVL* gene sequencing

	C16 (Hexadecanoylcarnitine) [C18; C18:1; C18:2]	ACP	Carnitine Palmitoyl Transferase II Deficiency (CPT2)		*CPT2* gene sequencing
			Carnitine/ Acylcarnitine Translocase Deficiency (CACT)		*SLC25A20* gene sequencing
	C16-OH (Hydroxyhexadecanoyl-carnitine) [C16:1-OH; C18:1-OH; C18-OH]	ACP	Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD)	↑ 3-hydroxy long chain dicarboxylic acids	*HADHA* gene sequencing
			Trifunctional Protein Deficiency		*HADHA, HADHB* gene sequencing
Organic Acidemias	C3 (Propionylcarnitine) [C3/C2]	ACP, UOA, UAG	Methylmalonic Acidemia (Methylmalonyl-CoA Mutase Deficiency)	Methylmalonic acid, 3-OH propionic acid, methylcitric acid, and tiglylglycine	plasma methylmalonic acid; enzyme assay in SFC; *MUT* gene sequencing
			Propionic Acidemia (Propionyl-CoA Carboxylase Deficiency)	Propionic acid, 3-OH propionic acid, methylcitric acid, and tiglylglycine	enzyme assay in SFC, WBC[20]; *PCCA, PCCB* gene sequencing
			Disorders of Cobalamin Metabolism (Cbl A, B, C, D)	Methylmalonic acid; homocysteine (normal with Cbl A, B; ↑ with Cbl C, D)	plasma methylmalonic acid, plasma homocysteine; Cbl complementation studies in SFC; *MMAA, MMAB*, or *MMACHC* gene sequencing
	C3-DC (Malonylcarnitine)	ACP, UOA	Malonic Aciduria (Malonyl-CoA Decarboxylase Deficiency)	Malonic acid; Methylmalonic acid	*MLYCD* gene sequencing
	C4 (Isobutyrylcarnitine)	ACP, UAG	Isobutyryl-CoA Dehydrogenase Deficiency (Isobutyric Aciduria)	Isobutyrylglycine	*ACAD8* gene sequencing
	C5 (Isovalerylcarnitine or Methylbutyrylcarnitine)	ACP, UOA, UAG	Isovaleric Acidemia (Isovaleryl-CoA Dehydrogenase Deficiency)	Isovalerylglycine; 3-OH-isovaleric acid	*IVD* gene sequencing
			2-Methylbutyrylglycinuria (Short/ Branched-Chain Acyl-CoA Dehydrogenase Deficiency)	2-Methylbutyrylglycine	*ACADSB* gene sequencing
	C5-DC (Glutarylcarnitine)	ACP, UOA	Glutaric Aciduria, Type I (Glutaryl-CoA Dehydrogenase Deficiency)	3-Hydroxyglutaric acid; Glutaric acid	enzyme assay in SFC; *GDCH* gene sequencing

(continued)

TABLE 1.1 (Continued)

Disease Category	Screening Analyte [Secondary Analytes]	Primary Metabolic Confirmatory Test(s)	Disorder	Other Metabolic Markers	Additional Confirmatory Test(s)¶
	C5-OH (3-Hydroxyisovaleryl-carnitine)	ACP, UOA, UAG (Biotinidase enzyme assay should be done if not part of the NBS panel)	Biotinidase Deficiency[21]	3-Hydroxyisovaleric acid; 3-methylcrotonylglycine; propionic acid; lactate	Biotinidase enzyme assay in serum; *BTD* gene sequencing
			Holocarboxylase Deficiency[21]	3-Hydroxyisovaleric acid; 3-methylcrotonylglycine; propionic acid; lactate	multiple carboxylases enzyme testing in SFC, WBC; *HLCS* gene sequencing
			3-Methylcrotonyl Carboxylase Deficiency (3-MCC Deficiency)	3-Hydroxyisovaleric acid; 3-Methylcrotonylglycine	enzyme testing in SFC, WBC; *MCCC1, MCCC2* gene sequencing
			3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency (HMG-CoA Lyase Deficiency)	3-Hydroxyisovaleric acid; 3-Hydroxy-3-methylglutaric acid; 3-Methylglutaconic acid; 3-Methylglutaric acid; 3-Methylcrotonylglycine	*HMGCL* gene sequencing
			3-Methylglutaconic Aciduria, Type I (3-Methylglutaconyl-CoA Hydratase Deficiency)	3-Hydroxyisovaleric acid; 3-Methylglutaconic acid; 3-methylglutaric acid	enzyme testing in SFC; *AUH* gene sequencing
	C5-OH (2-methyl-3-hydroxybutyrylcarnitine)	ACP, UOA	Beta-Ketothiolase Deficiency (Methylacetoacetyl-CoA Thiolase Deficiency)	2-Methyl-3-hydroxbutyric acid; 2-Methylacetoacidic acid; Tiglylglycine	enzyme testing in SFC; *ACAT1* gene sequencing
			2-Methyl-3-Hydroxybutyric Acidemia (2-Methyl-3-Hydroxybutyryl-CoA Dehydrogenase Deficiency)	2-Methyl-3-hydroxbutyric acid; Tiglylglycine	enzyme testing in SFC; *HSD17B10* (*MHBD*) gene sequencing

¶Additional tests to complement metabolic confirmation and to provide molecular and/or biochemical confirmation. [1]PAA = plasma amino acids, [2]RBC = red blood cell, [3]SFC = skin fibroblast cell culture, [4]UOA = urine organic acids, [5]BCKD = branch chain α-keto acid dehydrogenase, [6]LB = transformed lymphoblasts, [7]MAT = methionine S-adenosyltransferase, [8]GNMT = glycine N-methyltransferase deficiency, [9]S-AdoHcy = S-adenosylhomocysteine, [10]S-AdoMet = S-adenosylmethionine, [11]GTPCH = guanosine triphosphate cyclohydrolase I, [12]PTPS = 6-pyruvoyl-tetrahydropterin synthase, [13]PCD = pterin-4α-carbinolamine dehydratase, [14]DHPR = dihydropterin reductase, [15]5HAA = 5-hydroxyindole acetic acid, [16]HVA = homovanelic acid, [17]CSF = cerebrospinal fluid, [18]ACP = acylcarnitine profile, [19]UAG = urine acylglycines, [20]WBC = white blood cell, [21]Multiple Carboxylase Deficiency (MCD) can be caused by either biotinidase or holocarboxylase deficiencies.

immunoreactive trypsinogen receive reflex deoxyribonucleic acid (DNA) testing for cystic fibrosis. Such second-tier reflex testing may use another analyte or another method, or it may be done by targeted DNA mutation analysis or DNA sequencing.

Determination of Analyte Cut-Offs

Each screening program determines where to set cut-off values for each analyte or test, based on their population, the assay, and consideration of the positive predictive value and false negative rate. Programs typically set the cut-off range between the 99th percentile for the normal population and the 5th percentile for affected individuals. When there is overlap between normal and affected individuals, programs typically elect for a higher false positive rate to ensure that false negatives are minimized. Separate cut-off values may be used for low-birthweight infants, for infants receiving parenteral nutrition, and for second or follow-up screens. When secondary analytes are measured, an algorithm may be developed so that, rather than or in addition to a single analyte cut-off, all the data is considered in the determination of whether the screen is positive. Fatty acid oxidation disorders are particularly amenable to such an analysis. In the United States, the Health Resources and Services Administration division of the Department of Health and Human Services funds the Region 4 Genetics Collaborative, which collects data from NBS programs and provides assistance and advice regarding altering cut-offs to maximize sensitivity and specificity.[7,8] Definitions commonly used in discussing NBS cut-off values include

True Positives (TP): The number of individuals who both test positive and actually have the disease.

True Negatives (TN): The number of individuals who both test negative and do not have the disease.

False Positives (FP): The number of individuals who test positive but do *not* have the disease.

False Negatives (FN): The number of individuals who have the disease but have a normal newborn screen result.

Sensitivity: The proportion of individuals with the disorder who have a positive (abnormal) newborn screen = TP ÷ (TP + FN).

Specificity: The proportion of individuals without the disorder who have a normal newborn screen = TN ÷ (FP + TN).

False Positive Rate (FPR): The percentage of healthy (normal) subjects who test positive. FPR = FP ÷ (FP + TN) = 1 – specificity.

False Negative Rate (FNR): The percentage of affected subjects not detected by the newborn screen. FNR = FN ÷ (FN + TP) = 1 – sensitivity.

Presumptive Positive Rate: The percentage of all screened who test positive. TP ÷ (TP + TN + FP + FN).

Positive Predictive Value (PPV): The probability that the patient has the disease when restricted to those patients who test positive. PPV = TP ÷ (TP + FP).

Quality Control for Newborn Screening Programs

Each NBS laboratory and program is responsible for quality control and quality assurance that should monitor preanalytical, analytical, and postanalytical procedures. Assistance with quality assurance is also available through the U.S. Centers for Disease Control and Prevention Newborn Screening Quality Assurance Program. Founded in 1978 to provide assistance with the testing for congenital hypothyroidism, the program now offers quality assurance testing for 44 analytes (and 48 disorders). Nearly 500 NBS laboratories in more than 60 countries participate in this quality assurance program.[9] Accreditation of NBS labs in the United States is through the College of American Pathology program for laboratory accreditation and improvement.

Incidental Detection of Nontargeted Disorders

As discussed previously, MS/MS measures in parallel multiple amino acid and acylcarnitine species from a single sample, allowing for expanded NBS of the ACMG-defined core and secondary metabolic disorders.[6] It is important to realize that there are disorders not included in the ACMG uniform panel that can also present in the newborn period with alterations of amino acid or acylcarnitine markers that are screened by NBS programs and therefore should be considered in the differential diagnosis when confronted with a positive NBS result. Prominent examples include pyruvate carboxylase deficiency, succinyl-CoA ligase deficiency, and hydroxyprolinemia.[10] Pyruvate carboxylase deficiency can be detected through NBS through the presence of elevated citrulline, which results from secondary mitochondrial relative deficiency of oxaloacetate and aspartate. Succinyl-CoA ligase deficiency can be detected through NBS through the presence of mildly elevated methylmalonic acid, which results from the accumulation of succinyl-CoA. Hydroxyprolinemia, a clinically benign biochemical disorder caused by a deficiency of hydroxy-L-proline oxidase, can be detected through NBS because hydroxyproline is indistinguishable from leucine/isoleucine by MS/MS.

Confirmation of Individual Inborn Errors of Metabolism Detected by Tandem Mass Spectroscopy

In this section, individual inborn errors of metabolism targeted for NBS by MS/MS are presented with brief clinical descriptions and the various approaches for biochemical and molecular confirmation. The screening and confirmatory testing for each disorder is summarized in Table 1.1.

Biochemical Versus Molecular Confirmation

When an infant presents with a positive result on the newborn screen, the NBS program typically refers the patient to the local primary care provider or metabolic specialist for confirmation. The initial step usually consists of confirmation of abnormal metabolite pattern(s) through analysis of plasma amino acids (PAAs), urine organic acids (UOAs),

and/or acylcarnitine profile (ACP) analyses to rule out false positives cases from further evaluations. Historically, the next step has been to obtain biochemical confirmation through demonstration of enzymatic deficiency in the appropriate cell or tissue. With the sequencing of the human genome and the dramatic advancement of relatively economical DNA sequencing technologies, the utilization of DNA testing for molecular diagnostic confirmation in lieu of enzymatic confirmation has become an attractive option in many cases. Enzymatic testing can be technically difficult, often requiring invasive tissue biopsies and, given the particular enzymatic test, may be limited to only a few or even a single diagnostic laboratory worldwide. In contrast, testing for pathological mutations in the gene of interest requires only a single blood sample for DNA, which can be obtained in essentially any medical setting. For the more common disorders, these tests are offered by numerous clinical diagnostic laboratories. In addition, molecular confirmation provides an opportunity for prenatal diagnosis in future pregnancies if the parents are so inclined. As with any diagnostic test, DNA sequencing is not 100% specific, with noncoding mutations (except for invariant splice site mutations) typically not detectable or identifiable with current diagnostic approaches. In addition, the detection of novel, unclassified variants can provide interpretive challenges to the physician. In our experience, the combination of metabolic analyses and DNA testing often provides convincing diagnostic confirmation. Also, in the current "postgenomic" age, the ability to recognize rare benign polymorphisms will continue to improve as the number of publicly available sequenced genomes or whole exomes grows from dozens to tens of thousands and beyond in the very near future. In cases where metabolite and/or DNA analyses are equivocal, enzymatic testing for confirmation of a diagnosis should be pursued. Of course, there are examples where enzymatic testing is the preferred option for confirmation, or even screening, as is the case for biotinidase deficiency and galactosemia due to deficiency of galactose-1-phosphate uridyltransferase.

Disorders of Amino Acid Catabolism and Transport

Argininemia (Arginase Deficiency)

Primary Screening Analyte: Arginine

Secondary Screening Analyte(s): None

Other Metabolic Markers: Elevated urine orotic acid

Clinical Summary: Argininemia is a urea cycle disorder due to deficiency of the liver isoform of arginase (ARG1). Argininemia is an autosomal recessive disorder (*ARG1* is encoded on 6q23) with a U.S. incidence of 1:363,000.[11] Arginase deficiency classically presents as spastic diplegia and developmental delay. Rarely, it can present as an infantile acute encephalopathy with mild to moderate hyperammonemia.

Confirmatory Testing: Analysis of PAAs will demonstrate significantly elevated arginine levels (usually greater than 200 μM) and is pathognomonic for arginase deficiency. Enzyme deficiency can be confirmed by measuring arginase enzyme activity in red blood cells (RBCs) or liver tissue. Molecular confirmation is obtained by *ARG1* gene sequencing and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state.

Argininosuccinic Aciduria (Argininosuccinate Lyase Deficiency)

Primary Screening Analyte: Citrulline
Secondary Screening Analyte(s): None
Other Metabolic Markers: Elevated serum argininosuccinic aciduria (ASA), elevated urine orotic acid

Clinical Summary: ASA is a urea cycle disorder due to deficiency of argininosuccinate lyase (ASL). ASA is an autosomal recessive disorder (*ASL* is encoded on 7q11.21) with a U.S. incidence of 1:70,000.[11] ASL deficiency can present as an infantile acute encephalopathy with mild to moderate hyperammonemia or as a chronic encephalopathy with developmental delay/intellectual disability as the predominant feature. Progressive cirrhosis may also be seen.

Confirmatory Testing: Analysis of PAAs will demonstrate mild to moderate elevations of citrulline. In addition, detectable levels of argininosuccinic acid will be present and is pathognomonic for arginase deficiency. Enzyme deficiency can be confirmed by measuring ASL enzyme activity in RBCs, fibroblasts (skin fibroblast cell culture [SFC]), or liver tissue. Molecular confirmation is obtained by *ASL* gene sequencing and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state.

Citrullinemia, Type I (Argininosuccinate Synthetase Deficiency)

Primary Screening Analyte: Citrulline
Secondary Screening Analyte(s): None
Other Metabolic Markers: Low serum arginine, elevated urine orotic acid

Clinical Summary: Citrullinemia, type I is a urea cycle disorder due to deficiency of argininosuccinate synthetase (ASS). Citrullinemia, type I is an autosomal recessive disorder (*ASS1* is encoded on 9q34.11) with a U.S. incidence of 1:57,000.[11] ASS deficiency can present as an infantile acute encephalopathy with hyperammonemia or as later onset progressive cirrhosis.

Confirmatory Testing: Analysis of PAAs will demonstrate significant elevations of citrulline (1 to 3 mM is typical). In addition, low levels of plasma arginine will be observed in the untreated state. Enzyme deficiency can be confirmed by measuring ASS enzyme activity in fibroblasts or liver tissue. Molecular confirmation is obtained by *ASS1* gene sequencing and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state.

Citrullinemia, Type II (Citrin Deficiency)

Primary Screening Analyte: Citrulline
Secondary Screening Analyte(s): None
Other Metabolic Markers: Elevated arginine, methionine, threonine, and tyrosine (Tyr). Increased threonine/serine ratio

Clinical Summary: Citrullinemia, type II is a metabolic disorder due to deficiency of the mitochondrial aspartate-glutamate solute carrier (CITRIN or SLC25A13). Citrullinemia, type II is an autosomal recessive disorder (*SLC25A13* is encoded on 7q21.3)

with incidences in East Asian populations estimated to range between 1:17,000 and 1:50,000[12] but has been observed worldwide at much lower frequencies.[13] ASS deficiency can present as an infantile hepatitis with intrahepatic cholestasis or as an adult encephalopathy with hyperammonemia.

Confirmatory Testing: Analysis of PAAs will demonstrate mild to moderate elevations of citrulline (100 to 600 μM is typical). In addition, plasma arginine, methionine, threonine, and Tyr are typically elevated with the threonine/serine ratio usually elevated (1.5 to 3.0). Molecular confirmation is obtained by *SLC25A13* gene sequencing and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state.

Maple Syrup Urine Disease (Branched-Chain Ketoacid Dehydrogenase Deficiency)

Primary Screening Analyte: Leucine + isoleucine
Secondary Screening Analyte(s): Valine
Other Metabolic Markers: Alloisoleucine

Clinical Summary: MSUD is a metabolic disorder due to deficiency of the branched-chain ketoacid dehydrogenase deficiency (BCKD) complex. MSUD has a worldwide incidence of 1:250,000 but can be as high as 1:150 in certain reproductively isolated populations, such as Old Order Mennonite communities.[14] The BCKD complex consists of three catalytic subcomplexes: E1, a branched chain α-ketoacid decarboxylase that exists as a heterotetrameric subcomplex, composed of two α (E1α) and two β (E1β) subunits; E2, a homo-24-meric dihydrolipoyl transacylase; and E3, a homodimeric dihydrolipoamide dehydrogenase. MSUD can present along a spectrum of disease severity, including a classic severe acute infantile toxic encephalopathy, an intermediate later-onset form with developmental delay, and an intermittent form with acute episodes of encephalopathy often precipitated by illness or other forms of catabolic stress. MSUD is an autosomal recessive disorder that can be caused by mutations in the genes encoding E1α (*BCKDHA* on 19q13.2), E1β (*BCKDHB* on 6q14.1), or E2 (*DBT* on 1p21.2). Since E3 is also a component of the pyruvate dehydrogenase and the α-ketoglutarate dehydrogenase complexes, mutations in the gene encoding E3 (*DLD* on 7q31.1) causing dihydrolipoamide dehydrogenase deficiency presents as multiple dehydrogenase deficiencies with combined features of MSUD and lactic acidemia.

Confirmatory Testing: Analysis of PAAs will demonstrate significantly elevations of branch chain amino acids leucine, valine, and isoleucine, with perturbation of the normal 1:2:3 ratio of isoleucine:leucine:valine. In addition, detection of plasma alloisoleucine is pathognomonic. UOA analysis will demonstrate 2-keto-methylvalerate (which results in the maple syrup smell) as well as other 2-oxo and 2-hydroxy analytes. Enzyme deficiency can be confirmed by measuring BCKD activity from liver biopsy or SFC. Sequencing *BCKDHA, BCKDHB*, or *DBT* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Homocystinuria (Cystathionine β-Synthase Deficiency)

Primary Screening Analyte: Methionine
Secondary Screening Analyte(s): None
Other Metabolic Markers: Homocysteine

Clinical Summary: Homocystinuria is a disorder of sulfur metabolism and has a worldwide estimated prevalence of 1:344,000 based on NBS for elevated blood methionine levels.[15] Homocystinuria is caused be deficiency of cystathionine β-synthase (CBS), which is a pyridoxine-dependent enzyme, and there are both pyridoxine-responsive and pyridoxine-unresponsive forms of the disease. Homocystinuria classically presents in the first or second decade of life with marfanoid habitus, ectopia lentis, myopia, mental retardation, and increased risk for thromboembolic events. Homocystinuria is an autosomal recessive disorder caused by mutations in the gene encoding CBS (*CBS* on 21q22.3).

Confirmatory Testing: Analysis of PAAs will demonstrate elevated methionine and possibly elevated free homocysteine (although total plasma homocysteine level and urine homocysteine should be measured separately to definitively demonstrate elevations). Sequencing *CBS* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Hypermethionemia (Methionine S-Adenosyltransferase, Glycine N-Methyltransferase, or S-Adenosylhomocysteine Hydrolase Deficiencies)

Primary Screening Analyte: Methionine
Secondary Screening Analyte(s): None
Other Metabolic Markers: Elevated plasma S-adenosylmethionine (S-AdoMet) (increased in glycine N-methyltransferase [GNMT] or S-adenosylhomocysteine [S-AdoHcy] hydrolase deficiencies); elevated plasma S-AdoHcy (increased in S-AdoHcy hydrolase deficiency).

Clinical Summary: Hypermethionemia in the absence of significantly elevated homocysteine (isolated hypermethionemia) can be caused by a deficiency in any of the three enzymatic steps of conversion of methionine to homocysteine in the transsulfuration pathway. Methionine is adenosylated to S-AdoMet by methionine S-adenosyltransferase (MAT I/III). S-AdoMet is converted to S-AdoHcy by GNMT, and S-AdoHcy is converted to homocysteine by S-adenosylhomocysteine hydrolase (AHCY). The true incidence of MAT I/III deficiency is unknown but may be between 1:30,000 and 1:100,000.[16] MAT I/III deficiency has identified mostly from NBS, and most individuals are asymptomatic, with only a small subset of patients with neurological findings.[16] The other two deficiencies are quite rare with only a handful of patients described to date demonstrating hepatomegaly and/or neurodevelopmental defects. [17] These disorders are predominantly autosomal recessive and caused by mutations in the genes encoding MAT I/III (*MAT1A* on 10q23.1), GNMT (*GNMT* on 6p21.1), or AHCY (*AHCY* on 20q11.22).

Confirmatory Testing: Analysis of PAAs will demonstrate elevated methionine in all instances. Plasma S-AdoMet will be elevated in both GNMT and AHCY deficiencies, while S-AdoHcy will be elevated in AHCY deficiency. No enzymatic testing is clinically available at this time. Sequencing *MAT1A, GNMT*, or *AHCY* and identifying known or

suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Phenylalanine Hydroxylase Deficiency

Primary Screening Analyte: Phe

Secondary Screening Analyte(s): Phe/Tyr ratio

Other Metabolic Markers: Increased urine phenyl ketoacids (hydroxyphenylacetic acid, phenylpyruvic acid, phenylacetic acid, and phenylacetylglutamine)

Clinical Summary: PKU is caused by deficiency of Phe hydroxylase (PAH). PKU is an autosomal recessive disorder (*PAH* is encoded on 12q23.2) with incidences in Caucasian populations estimated at 1:10,000 but much less frequent in Asian and African populations.[18] Untreated, PAH deficiency will present as developmental delay and intellectual disability.

Confirmatory Testing: Analysis of plasma Phe and Tyr will demonstrate significantly elevated Phe levels (typically between 10 and 30 mg/dL for classical PKU and less for milder forms) and elevated Phe/Tyr ratio (typically greater than 2.5). Analysis of UOA will demonstrate elevations of hydroxyphenylacetic acid, phenylpyruvic acid, phenylacetic acid, and phenylacetylglutamine. Sequencing of *PAH* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Disorders of Biopterin Metabolism (Guanosine Triphosphate Cyclohydrolase I, 6-Pyruvoyl-Tetrahydropterin Synthase, Pterin-4α-Carbinolamine Dehydratase, or Dihydropterin Reductase Deficiencies)

Primary Screening Analyte: Phe

Secondary Screening Analyte(s): Phe/Tyr ratio

Other Metabolic Markers: Biopterin (↓ in all); neopterin (↓ in guanosine triphosphate cyclohydrolase I [GTPCH], ↑ or *N* in others); 5-hydroxyindole acetic acid, and homovanelic acid in cerebral spinal fluid (↓ in all)

Clinical Summary: Approximately 1% to 2% of cases of hyperphenylalanemia are caused by disorders of biopterin metabolism disrupting synthesis or recycling of tetrahydrobiopterin (a cofactor for PAH).[18] Deficiencies of GTPCH, 6-pyruvoyl-tetrahydropterin synthase, pterin-4α-carbinolamine dehydratase, or dihydropterin reductase can result in secondary PAH deficiency and hyperphenalanemia. These disorders are autosomal recessive and due to mutations in genes encoding GTPCH (*GCH1* on 14q22.2), 6-pyruvoyl-tetrahydropterin synthase (*PTS* on 11q23.1), pterin-4α-carbinolamine dehydratase (*PCBD1* on 10q22.1), or dihydropterin reductase (*QDPR* on 4q15.32).

Confirmatory Testing: Analysis of plasma Phe and Tyr will demonstrate significantly elevated Phe levels (typically between 10 and 30 mg/dL) and elevated Phe/Tyr ratio (typically greater than 2.5). Analysis of UOA will demonstrate elevations of hydroxyphenylacetic acid, phenylpyruvic acid, phenylacetic acid, and phenylacetylglutamine. Analysis of urine pterins will demonstrate reduced biopterin in all deficiencies.

Urine neopterin may be elevated in 6-pyruvoyl-tetrahydropterin synthase deficiency and low in GTPCH deficiency. Measurement of neurotransmitters in cerebral spinal fluid will demonstrate reduced levels of 5-hydroxyindolacetic acid and homovanelic acid. Deficiency of dihydropterin reductase can be demonstrated by enzyme assay in RBCs. Sequencing of *GCH1, PTS, PCBD1,* or *QDPR* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Tyrosinemia, Type I (Fumarylacetoacetate Hydrolase Deficiency)

Primary Screening Analyte: Succinylacetone and/or Tyr

Secondary Screening Analyte(s): None

Other Metabolic Markers: Elevated Tyr, Phe, methionine; elevated Tyr metabolites in urine (4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate, and 4-hydroxyphenylacetate); elevated 5-aminolevulinic acid in urine

Clinical Summary: Tyrosinemia type I is an inborn error of Tyr metabolism caused by deficiency of fumarylacetoacetate hydrolase (FAH). FAH deficiency is an autosomal recessive disorder with an overall estimated incidence of 1:100,000 to 1:120,000.[19] Tyrosinemia type I can present as a severe infantile progressive hepatopathy or later in infancy as a renal tubulopathy with rickets, failure to thrive, and hepatopathy. Untreated individuals can display porphyria-like intermittent episodes of acute neurological crises and progress to fatal hepatic failure and/or hepatocellular carcinoma. Tyrosinemia type I is caused by mutations in the gene encoding FAH (*FAH* on 15q25.1).

Confirmatory Testing: Detection of succinylacetone in blood or urine is pathognomonic for FAH deficiency. Analysis of PAAs may reveal normal or modest elevations of Tyr (typically less than 300 μM), Phe, and methionine. The use of Tyr as the primary screening analyte for tyrosinemia type I has generally been abandoned due to unacceptable high false positive rates given that the modest elevations of Tyr as observed in tyrosinemia type I can also be seen in other conditions with relatively high frequency, including hepatic immaturity, transient hypertyrosinemia of the newborn, and other extrinsic causes of hepatic dysfunction (TPN cholestasis, etc). Analysis of UOA will demonstrate elevations of Tyr metabolites (4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate, and 4-hydroxyphenylacetate). Elevations in urine 5-aminolevulinic acid will be present secondary to inhibition of 5-aminolevulinic acid dehydratase (porphobilinogen synthase) in the liver and RBCs by elevated succinylacetone. Sequencing of *FAH* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Tyrosinemia, Type II (Tyr Aminotransferase Deficiency) and Type III (4-Hydroxy-Phenylpyruvate Dioxygenase Deficiency)

Primary Screening Analyte: Tyr

Secondary Screening Analyte(s): None

Other Metabolic Markers: Elevated Tyr metabolites in urine (4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate, and 4-hydroxyphenylacetate)

Clinical Summary: Tyrosinemia type II is an inborn error of Tyr metabolism caused by deficiency of the hepatic cytosolic isoform of Tyr aminotransferase (TAT). It is a rare autosomal recessive disorder also known as oculocutaneous tyrosinemia or Richner-Hanhart Syndrome. Ophthalmologic features include recalcitrant pseudodendritic keratitis and corneal ulcerations, while dermatologic manifestations consist of painful palmoplan`tar keratodermatitis.[20] Tyrosinemia type II is caused by mutations in the gene encoding TAT (*TAT* on 16q22.2). Tyrosinemia type III is an inborn error of Tyr metabolism caused by deficiency of 4-hydroxy-phenylpyruvate dioxygenase. It is a very rare autosomal recessive disorder with only a handful of affected individuals reported; therefore, the phenotype remains poorly defined. Like TAT deficiency, the few patients described with HPD deficiency do not have liver involvement but may have skin, eye, and neurological effects.[21] Tyrosinemia type III is caused by mutations in the gene encoding HPD (*HPD* on 12q24.31).

Confirmatory Testing: Analysis of PAAs will demonstrate elevated Tyr for both conditions. TAT deficiency typically presents with a plasma Tyr greater than 1000 μM while HPD deficiency typically presents with a plasma Tyr between 350 and 650 μM. Analysis of UOA will demonstrate elevations of Tyr metabolites (4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate, and 4-hydroxyphenylacetate). In theory, enzyme deficiency for either condition could be demonstrated on liver biopsy; however, this is not readily available clinically. Sequencing of *TAT* or *HPD* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation for tyrosinemia types II and III, respectively.

Disorders of Fatty Acid Oxidation

Carnitine Uptake Defect (Primary Carnitine Deficiency)

Primary Screening Analyte: Low C0 (free carnitine)

Secondary Screening Analyte(s): None

Other Metabolic Markers: Low total plasma carnitine

Clinical Summary: Primary carnitine deficiency is a defect of the cellular uptake of carnitine due to deficiency of the carnitine transporter OCTN2. OCTN2 deficiency is an autosomal recessive disorder with an estimated incidence of 1:30,000 to 1:60,000.[22,23] Primary carnitine deficiency exhibits prominent variable expressivity, ranging from classic presentations of cardiomyopathy, hepatoencephalopathy, or episodic hypoketotic hypoglycemia to asymptomatic deficient mothers identified through detection of low carnitine via NBS of their unaffected children. Primary carnitine deficiency is caused by mutations in the gene encoding OCTN2 (*SLC22A5* on 5q31.1).

Confirmatory Testing: Analysis of free and total plasma carnitine will demonstrate deficiency. It is important to measure the mother's free and total plasma carnitine levels as well, because newborns of affected mothers will have transient secondary carnitine deficiency. Enzyme deficiency can be shown by carnitine transport assay in SFC. Sequencing of

OCTN2 and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Carnitine Palmitoyl Transferase I Deficiency

Primary Screening Analyte: C0/(C16+C18) ratio
Secondary Screening Analyte(s): C0
Other Metabolic Markers: Generalized decrease of acylcarnitines

Clinical Summary: Carnitine palmitoyl transferase 1 deficiency is a disorder of long-chain fatty acid oxidation that is caused by deficiency of the hepatic isoform of carnitine palmitoyl transferase (CPT1A). It is a rare autosomal recessive disorder with an estimated incidence of 1:750,000 to 1:2,000,000.[24] CPT1A deficiency presents predominantly as episodic hypoketotic hypoglycemia often triggered by catabolic stress. CPT1A deficiency is caused by mutations in the gene encoding CPT1A (*CPT1A* on 11q13.3).

Confirmatory Testing: Analysis of total and free plasma carnitine will demonstrate increased free carnitine. An ACP will exhibit a generalized decrease of acylcarnitines. Enzyme deficiency can be inferred by radiolabeled fatty acid in SFC. Sequencing of *CPT1A* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Short-Chain Acyl-CoA Dehydrogenase Deficiency

Primary Screening Analyte: C4
Secondary Screening Analyte(s): None
Other Metabolic Markers: Ethylmalonic acid and butyrylglycine

Clinical Summary: Short-chain acyl-CoA dehydrogenase (SCAD) deficiency is an inborn error of short-chain fatty acid oxidation. SCAD deficiency is autosomal recessive with an estimated incidence of 1:95,000.[24] While initially thought to be a clinically severe disorder, the identification of numerous asymptomatic individuals through expanded NBS over the past decade has shifted the general consensus toward SCAD deficiency being a biochemical abnormality without clear clinical significance.[25] SCAD deficiency is caused by mutations in the gene encoding SCAD (*ACADS* on 12q24.31).

Confirmatory Testing: Analysis of ACP will demonstrate elevated butyrylcarnitine (C4). Analysis of urine acylglycines (UAG) will demonstrate elevated butyrylglycine. Analysis or UOA will demonstrate elevations of ethylmalonic acid. Sequencing of *ACADS* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Ethylmalonic Encephalopathy

Primary Screening Analyte: C4
Secondary Screening Analyte(s): C5
Other Metabolic Markers: Ethylmalonic acid, isovalerylglycine, lactic acid

Clinical Summary: Ethylmalonic encephalopathy is a rare autosomal recessive disorder due to deficiency of ETHE1, a mitochondrial matrix sulfur dioxygenase. This disorder presents with encephalopathy, chronic diarrhea, and orthostatic acrocyanosis. A mouse

model suggests that the metabolic abnormalities, including elevations of ethylmalonic acid, butyrylcarnitine, and lactic acid, are caused by secondary inhibition of SCAD and cytochrome c oxidase by increased cellular sulfides.[26] Ethylmalonic encephalopathy is caused by mutations in the gene encoding ETHE1 (*ETHE1* on 19q31.13).

Confirmatory Testing: Analysis of ACP will demonstrate elevations in butyrylcarnitine (C4) and isovalerylcarnitine (C5). Evaluation of UOA will show elevations of ethylmalonic acid, isovalerylglycine, and lactic acid. Sequencing of *ETHE1* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Glutaric Aciduria, Type II or Multiple Acyl-CoA Dehydrogenase Deficiency

Primary Screening Analyte: C4

Secondary Screening Analyte(s): C5

Other Metabolic Markers: ↑ C6-C18; ethylmalonic acid, isovaleric acid, glutaric acid; isovalerylglycine and hexanoylglycine

Clinical Summary: Glutaric aciduria, type II (GA II) is a disorder of fatty acid oxidation caused by deficiencies of components of the system that transfers electrons released from β-oxidation to the electron transport chain. This includes the electron-transfer flavoprotein (ETF) and the ETF dehydrogenase (ETF-DH). Because this system is required for proper β-oxidation of all fatty acids, this disorder effectively presents as a deficiency of multiple acyl-CoA dehydrogenases. In addition, this system is required for oxidation of branched chain amino acids, sarcosine, and lysine. GA II is autosomal recessive and genetically heterogeneous with an estimated incidence that ranges from 1:15,000 in Turkey to 1:750,000 to 1:2,000,000 in North America and Europe.[24] In its most severe form, GA II clinically presents as neonatal cardiomyopathy, hypoglycemia, and encephalopathy, while milder forms can present with episodic hypoglycemia induced by catabolic stress. GA II is caused by mutations in the genes encoding components of the electron transfer system, including *ETFA* (15q24.2), *ETFB* (19q13.41), and *ETFDH* (4q32.1).

Confirmatory Testing: Analysis ACP will reveal a generalized elevation of acylcarnitines (C4-C18). Examination of UOA will reveal presence of ethylmalonic acid, isovaleric acid, and glutaric acid and 2-hydroxyglutaric acid. Analysis of UAG will demonstrate presence of isovalerylglycine and hexanoylglycine. Enzyme deficiency can be demonstrated by EFT/EFT:ubiquinone oxidoreductase enzyme assay on SFC. Sequencing of *ETFA, ETFB*, or *ETFDH* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Medium/Short-Chain Hydroxy Acyl-CoA Deficiency

Primary Screening Analyte: C4-OH

Secondary Screening Analyte(s): None

Other Metabolic Markers: ↑ Hydroxydicarboxylic acids; elevated insulin

Clinical Summary: Medium/short-chain hydroxy acyl-CoA (M/SCHAD) deficiency is a rare autosomal recessive disorder involving deficiency of the penultimate step

of β-oxidation. While M/SCHAD deficiency causes a biochemical abnormality (elevated 3-hydroxybutyrylcarnitine), its clinical significance is uncertain, although there are reports of mutations associated with hyperinsulinemic hypoglycemia.[27,28] M/SCHAD deficiency is caused by mutations in the gene encoding M/SCHAD (*HADH* on 4q25).

Confirmatory Testing: Analysis of ACP will demonstrate elevated 3-hydroxybutyrylcarnitine (C4-OH), while analysis of UOA will show elevations of hydroxydicarboxylic acids. Hyperinsulinism in the presence of hypoglycemia may also be present. Sequencing of *HADH* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Medium-Chain Acyl-CoA Dehydrogenase Deficiency

Primary Screening Analyte: C8

Secondary Screening Analyte(s): C6; C10; C10:1; C8/C10; C8/C2

Other Metabolic Markers: ↑ medium-chain dicarboxylic acids; hexanoyl-, suberyl-, and 3-phenylpropionylglycines

Clinical Summary: Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is an autosomal recessive inborn error of β-oxidation. MCAD deficiency has an incidence of 1:15,000 in Caucasian populations.[24] MCAD deficiency presents as a potentially fatal fasting hypoglycemia often induced by catabolic stress. MCAD deficiency is caused by mutations in the gene encoding MCAD (*ACADM* on 1p31.1).

Confirmatory Testing: Analysis ACP will demonstrate elevations in hexanoylcarnitine (C6), octanoylcarnitine (C8), decenoylcarnitine (C10:1), and, to a lesser extent, decanoylcarnitine (C10). Evaluation of UOA will show elevations of medium-chain dicarboxylic acids. Analysis of UAG will demonstrate the presence of hexanoylglycine, suberylglycine, and 3-phenylpropionylglycine. Sequencing of *ACADM* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Very Long-Chain Acyl-CoA Dehydrogenase Deficiency

Primary Screening Analyte: C14:1

Secondary Screening Analyte(s): C14; C14:2; C16; C18:1; C14:1/C2

Other Metabolic Markers: ↑ medium/long-chain dicarboxylic acids

Clinical Summary: Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency is an autosomal recessive inborn error of β-oxidation. VLCAD deficiency has an incidence of 1:85,000 in Caucasian populations.[24] VLCAD deficiency can present along a spectrum of severity that includes neonatal hypoketotic hypoglycemia, cardiomyopathy, or exercise-induced rhabdomyolysis. VLCAD deficiency is caused by mutations in the gene encoding VLCAD (*ACADVL* on 17p13.1).

Confirmatory Testing: Analysis of ACP will demonstrate elevations of long-chain acylcarnitines, with tetradecenoylcarnitine (C14:1) typically most prominent. Evaluation of UOA will reveal elevations of medium- and long-chain dicarboxylic acids. Sequencing of *ACADVL* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Carnitine Palmitoyl Transferase II Deficiency

Primary Screening Analyte: C16
Secondary Screening Analyte(s): C18; C18:1; C18:2
Other Metabolic Markers: None

Clinical Summary: Carnitine palmitoyl transferase II (CPT2) deficiency is a disorder of long-chain fatty acid oxidation that is caused by deficiency of CPT2. It is a rare autosomal recessive disorder with an estimated incidence of 1:750,000 to 1:2,000,000.[24] CPT2 deficiency presents predominantly as episodic hypoketotic hypoglycemia often triggered by catabolic stress. CPT2 deficiency can present along a spectrum of severity that includes neonatal hypoketotic hypoglycemia, cardiomyopathy, or exercise-induced rhabdomyolysis. CPT2 deficiency is caused by mutations in the gene encoding CPT2 (*CPT2* on 1p32.3).

Confirmatory Testing: Analysis of ACP will reveal elevations of long-chain acylcarnitines, in particular hexadecanoylcarnitine (C16). Sequencing of *CPT2* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Carnitine/Acylcarnitine Translocase Deficiency

Primary Screening Analyte: C16
Secondary Screening Analyte(s): C18; C18:1; C18:2
Other Metabolic Markers: None

Clinical Summary: Carnitine/acylcarnitine translocase (CACT) deficiency is a disorder of long-chain fatty acid oxidation that is caused by deficiency of the CACT. It is a rare autosomal recessive disorder with an estimated incidence of 1:750,000 to 1:2,000,000.[24] CACT deficiency presents predominantly as infantile episodic hypoketotic hypoglycemia triggered by fating/catabolic stress with encephalopathy and cardiomyopathy/ventricular arrhythmias. CACT deficiency is caused by mutations in the gene encoding CACT (*SLC25A20* on 3p21.31).

Confirmatory Testing: Analysis of ACP will reveal elevations of long-chain acylcarnitines, in particular hexadecanoylcarnitine (C16). Sequencing of *SLC25A20* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency and Trifunctional Protein Deficiency

Primary Screening Analyte: C16-OH
Secondary Screening Analyte(s): C16:1-OH; C18:1-OH; C18-OH
Other Metabolic Markers: ↑ 3-hydroxy long-chain dicarboxylic acids

Clinical Summary: The mitochondrial trifunctional protein complex, an octamer composed of four α- and four β-subunits, contains three enzymatic activities important for long-chain fatty acid oxidation: a long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) (α), long-chain enoyl-CoA hydratase (α), and 3-ketoacyl-CoA thiolase (β) activities. LCHAD deficiency is a disorder of long-chain fatty acid oxidation that is caused by isolated

deficiency of the α-subunit LCHAD activity of the mitochondrial trifunctional protein complex. It is an autosomal recessive disorder with an overall incidence of 1:250,000.[24] Complete trifunctional protein (TFP) deficiency is caused by deficiency of either the α- or β-subunits leading to deficiencies of all three activities. It is a more rare autosomal recessive disorder with an overall incidence of 1:750,000.[24] These disorders have similar and overlapping clinical features that can present along a spectrum of severity that includes neonatal hypoketotic hypoglycemia, cardiomyopathy, and/or acute rhabdomyolysis. Peripheral neuropathy and retinopathy are long-term complications of these disorders, and a subset of women who present with maternal hemolysis, liver dysfunction, and low platelets (hemolysis, elevated liver enzymes, and low platelets syndrome) or acute fatty liver of pregnancy are carriers with an affected fetus.[29] LCHAD deficiency is caused by mutations in the gene encoding the α-subunit of TFP (*HADHA* on 2p23.3), with homozygosity for the highly prevalent c.1528G>C (p.E510Q) common mutation being the most common genotype. TFP deficiency can be caused by mutations in either the α-subunit of TFP (*HADHA*) or the β-subunit of TFP (*HADHB* on 2p23.3).

Confirmatory Testing: Analysis of ACP will reveal elevations of long-chain hydroxylated acylcarnitines, in particular hydroxyhexadecanoylcarnitine (C16-OH). Evaluation of UOA will reveal elevations of 3-hydroxy long-chain dicarboxylic acids (*C6-C14*).[30,31] Sequencing of *HADHA* (LCHAD or TFP) or *HADHB* (TFP) and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Organic Acidemias

Methylmalonic Acidemia (Methylmalonyl-CoA Mutase Deficiency)

Primary Screening Analyte: C3

Secondary Screening Analyte(s): C3/C2 ratio

Other Metabolic Markers: Methylmalonic acidemia (MMA), 3-OH propionic acid, methylcitric acid, and tiglylglycine

Clinical Summary: Primary MMA is a disorder of amino acid metabolism caused by the deficiency of methylmalonyl-CoA mutase (MUT) affecting the catabolism of isoleucine, valine, methionine, and threonine. It is an autosomal recessive disorder with an estimated incidence of 1:50,000 to 1:100,000.[32] MMA classically presents as an infantile toxic encephalopathy with a prominent acute metabolic anion gap acidosis. MUT deficiency is caused by mutations in the gene encoding methylmalonyl-CoA mutase (*MUT* on 6p12.3).

Confirmatory Testing: Analysis of ACP will reveal elevations in propionylcarnitine (C3) and the ratio of propionylcarnitine to acetylcarnitine (C3/C2). Measurement of plasma methylmalonic acid levels will show significant elevations (typically 10 to 1000 μM). Analysis of UOA and UAG will demonstrate the presence of 3-methylmalonic acid, methylcitric acid, and tiglylglycine. Biochemical confirmation can be obtained by demonstration of enzyme deficiency in SFC. Sequencing of *MUT* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Propionic Acidemia (Propionyl-CoA Carboxylase Deficiency)

Primary Screening Analyte: C3

Secondary Screening Analyte(s): C3/C2 ratio

Other Metabolic Markers: Propionic acid, 3-OH propionic acid, methylcitric acid, and tiglylglycine

Clinical Summary: Propionic acidemia is a disorder of amino acid metabolism caused by the deficiency of propionyl-CoA carboxylase affecting the catabolism of isoleucine, valine, methionine, and threonine. It is an autosomal recessive disorder with an estimated incidence of 1:75,000 to 1:300,000.[33,34] Propionic acidemia classically presents as an infantile toxic encephalopathy with a prominent acute metabolic anion gap acidosis, mild hyperammonemia, and neutropenia. Propionic acidemia is caused by mutations in either the gene encoding the α-subunit (*PCCA* on 13q32.3) or β-subunit (*PCCB* on 3q22.3) of propionyl-CoA carboxylase.

Confirmatory Testing: Analysis of ACP will reveal elevations in propionylcarnitine (C3) and the ratio of propionylcarnitine to acetylcarnitine (C3/C2). Measurement of PAA will typically show elevations of glycine and alanine. Analysis of UOA and UAG will demonstrate the presence of propionic acid, 3-hydroxy-propionic acid, methylcitric acid, and tiglylglycine. Biochemical confirmation can be obtained by demonstration of enzyme deficiency in SFC or leukocytes. Sequencing of *PCCA* or *PCCB* and identifying known or suspected pathogenic mutations in the homozygous or compound heterozygous state will provide molecular confirmation.

Disorders of Cobalamin Metabolism (CblA, B, C, D, F)

Primary Screening Analyte: C3

Secondary Screening Analyte(s): C3/C2 ratio

Other Metabolic Markers: Methylmalonic acid, homocysteine (normal with Cbl A, B; ↑ with Cbl C, D, F)

Clinical Summary: Cobalamin (Vitamin B_{12}) is a cobalt-containing water-soluble vitamin that is converted into two distinct bioactive forms intracellularly. Adenosyl cobalamin (AdoCbl) is a required cofactor for the mitochondrial methylmalonyl-CoA mutase, and methylcobalamin (MeCbl) is a required cofactor for the cytoplasmic methionine synthase. Inherited disorders affecting the intracellular metabolism of cobalamin, which result in deficiency of AdoCbl, will present with MMA. Biochemical complementation studies of fibroblasts from patients with defects of intracellular metabolism of cobalamin defined seven genetically distinct complementation groups, CblA-G.[35] Of these, CblA and CblB present as isolated MMA similar to MUT deficiency, while CblC, CblD, and CblF (which are involved in intracellular processing of cobalamin prior to conversion to either AdoCbl or MeCbl) present with combined MMA and hyperhomocysteinemia that can include megaloblastic anemia and neurological abnormalities. These disorders are rare autosomal recessive conditions for which true incidences are currently unknown. However, in our experience, CblC is by far the most common of these disorders. CblA is caused by mutations in the MMAA gene (*MMAA* on 4q31.21); CblB is caused by mutations in the MMAB gene (*MMAB* on 12q24.11); CblC is caused by