Lysosomal Storage Disorders: A Practical Guide

Edited by
Atul Mehta, Professor of Haematology, University College London, Royal Free Hospital, London, UK
Bryan Winchester, Emeritus Professor of Biochemistry, UCL Institute of Child Health at Great Ormond Street Hospital, University College London, London, UK

The last two decades have seen a huge expansion in research in the area of lysosomal storage disorders, which has substantially extended our understanding of both the scientific and the clinical basis of these diseases. Lysosomal Storage Disorders: A Practical Guide is the fruit of an ambitious project aiming to review both the scientific and the clinical aspects of lysosomal storage disorders, resulting in this accessible volume, which gives an up-to-date overview of the subject.

There is substantial scientific interest in these diseases: new advances in small molecule therapy are likely to be useful in the near future, and trials are already underway. Lysosomal storage disorders offer a unique platform for teaching modern clinical science, from basic genetics through to clinical applications.

The first part of the book reviews and classifies our current understanding of the physiology and pathophysiology of lysosomal storage disorders. The second part of the book reviews individual diseases, and gives perspectives from patients and experts looking towards future therapeutic directions.

Lysosomal Storage Disorders: A Practical Guide is the ideal guide for a wide audience including scientists, clinicians, health care workers and administrators, those working in the pharmaceutical industry, patients and their organisations.

TITLES OF RELATED INTEREST

Haematology at a Glance · Mehta · ISBN 97814051779706
Atlas of Endocrine and Metabolic Disease · Pozzilli · ISBN 9780470656273
Lysosomal Storage Disorders

A Practical Guide

EDITED BY

Atul Mehta
Clinical Director, Lysosomal Storage Disorders Unit
Professor of Haematology, University College London
Royal Free Hospital
London, UK

Bryan Winchester
Emeritus Professor of Biochemistry
UCL Institute of Child Health at Great Ormond Street Hospital
University College London
London, UK

WILEY-BLACKWELL
A John Wiley & Sons, Ltd., Publication
Contents

List of Contributors, v
Preface, viii
Foreword, x

Part 1  General Aspects of Lysosomal Storage Diseases, 1

1 The Lysosomal System: Physiology and Pathology, 3
   Matthew C. Micsenyi and Steven U. Walkley

2 Clinical Aspects and Clinical Diagnosis, 13
   J. Edmond Wraith and Michael Beck

3 Laboratory Diagnosis of Lysosomal Storage Diseases, 20
   Bryan Winchester

4 Genetics of Lysosomal Storage Disorders and Counselling, 29
   John J. Hopwood

5 Classification of Lysosomal Storage Diseases, 37
   Bryan Winchester

Part 2  The Individual Diseases, 47

6 Gaucher Disease, 49
   Deborah Elstein and Ari Zimran

7 Fabry Disease, 58
   Atul Mehta and Uma Ramaswami

8 The Gangliosidoses, 63
   Joe T.R. Clarke

9 Metachromatic Leukodystrophy and Globoid Cell Leukodystrophy, 70
   Volkmar Gieselmann, David A. Wenger and Ingeborg Krägeloh-Mann

10 Types A and B Niemann–Pick Disease, 80
    Melissa P. Wasserstein, Robert J. Desnick, and Edward H. Schuchman

11 Niemann–Pick Disease Type C, 87
    Marie T. Vanier and Marc C. Patterson
12 The Mucopolysaccharidoses, 94
   Roberto Giugliani

13 Pompe Disease, 101
   Arnold J.J. Reuser and Ans T. van der Ploeg

14 Glycoproteinoses, 107
   Dag Malm, Hilde Monica F. Riise Stensland and Øivind Nilssen

15 Defect in Protective Protein/Cathepsin A: Galactosialidosis, 115
   Alessandra d’Azzo and Erik J. Bonten

16 Multiple Enzyme Deficiencies, 121
   16.1 Defects in Transport: Mucolipidosis II alpha/beta, Mucolipidosis III alpha/beta and Mucolipidosis III gamma, 121
   Annick Raas-Rothschild, Sandra Pohl and Thomas Braulke
   16.2 Multiple Sulfatase Deficiency, 127
   Graciana Diez-Roux and Andrea Ballabio

17 Lysosomal Membrane Defects, 131
   Michael Schwake and Paul Saftig

18 Neuronal Ceroid Lipofuscinoses, 137
   Jonathan D. Cooper and Ruth E. Williams

19 Other Lysosomal Disorders, 142
   Bryan Winchester and Timothy M. Cox

Part 3 Therapy and Patient Issues, 151

20 Current Treatments, 153
   Timothy M. Cox

21 Central Nervous System Aspects, Neurodegeneration and the Blood–Brain Barrier, 166
   David J. Begley and Maurizio Scarpa

22 Emerging Treatments and Future Outcomes, 174
   T. Andrew Burrow and Gregory A. Grabowski

23 Newborn, High Risk and Carrier Screening for Lysosomal Storage Disorders, 181
   Gabor E. Linthorst and Carla E.M. Hollak

24 The Patient Perspective on Rare Diseases, 186
   Alastair Kent, Christine Lavery, and Jeremy Manuel

Index, 193
Contributors

**Andrea Ballabio, MD**
Director, TIGEM (Telethon Institute of Genetics and Medicine)
Naples, Italy;  
Professor, Department of Molecular and Human Genetics  
Baylor College of Medicine  
Houston, TX, USA;  
Jan and Dan Duncan Neurological Research Institute  
Texas Children's Hospital  
Houston, TX, USA;  
Medical Genetics  
Department of Pediatrics  
Federico II University  
Naples, Italy

**Michael Beck**
Children's Hospital  
University of Mainz  
Mainz, Germany

**David J. Begley, BSc, PhD**
Senior Lecturer in Physiology  
Kings College London  
London, UK

**Erik J. Bonten, PhD**
Department of Genetics  
St. Jude Children's Research Hospital  
Memphis, TN, USA

**Thomas Braulke, PhD**
Department of Biochemistry  
Children's Hospital  
University Medical Center Hamburg-Eppendorf  
Hamburg, Germany

**T. Andrew Burrow, MD**
Assistant Professor  
The Division of Human Genetics  
University of Cincinnati Department of Pediatrics and  
Cincinnati Children's Hospital Medical Center  
Cincinnati, OH, USA

**Joe T.R. Clarke, MD, PhD**
Professor Emeritus (Pediatrics)  
University of Toronto  
Toronto, ON, Canada  
Professor densignement clinique  
Centre Hospitalier Universitaire  
Sherbrooke, QC, Canada

**Jonathan D. Cooper, BSc(Hons), PhD**
Professor of Experimental Neuropathology  
Pediatric Storage Disorders Laboratory, Neuroscience  
Centre for the Cellular Basis of Behaviour  
King's Health Partners Centre for Neurodegeneration Research  
James Black Centre  
Institute of Psychiatry  
King's College London  
London, UK

**Timothy M. Cox, MD, FMedSci**
Professor of Medicine  
University of Cambridge  
Addenbrooke's Hospital  
Cambridge, UK

**Alessandra d’Azzo, PhD**
Member and Endowed Chair  
Department of Genetics  
St. Jude Children's Research Hospital  
Memphis, TN, USA
Contributors

Robert J. Desnick, PhD, MD
Dean for Genetic and Genomic Medicine
Professor and Chairman Emeritus
Department of Genetics and Genomic Sciences
Mount Sinai School of Medicine
New York, NY, USA

Graciana Diez-Roux
Telethon Institute of Genetics and Medicine
Naples, Italy

Deborah Elstein, PhD
Clinical Research Coordinator
Gaucher Clinic
Shaare Zedek Medical Center
Jerusalem, Israel

Volkmar Gieselmann, MD
Professor of Biochemistry
Institut fuer Biochemie und Molekularbiologie
Rheinische-Friedrich-Wilhelms Universitaet
Bonn, Germany

Roberto Giugliani, MD, PhD
Professor, Department of Genetics,
Federal University of Rio Grande do Sul
Director, WHO Collaborating Centre
Medical Genetics Service, HCPA
Porto Alegre, RS, Brazil

Carla E.M. Hollak, MD, PhD
Internist, Professor of Inherited Metabolic Diseases in Adults
Department of Endocrinology and Metabolism
Academic Medical Center
Amsterdam, OH, USA

John J. Hopwood, AM, FAA, PhD
Lysosomal Diseases Research Unit
SA Pathology at Women's and Children's Hospital
North Adelaide, SA, Australia

Alastair Kent
Director
Genetic Alliance UK
London, UK

Ingeborg Krägeloh-Mann, MD
Professor of Pediatrics
Director, Pediatric Neurology and Developmental Medicine
University Children's Hospital
Tübingen, Germany

Christine Lavery, MBE
Chief Executive
Society for Mucopolysaccharide Diseases
Amersham, Buckinghamshire, UK

Gabor E. Linthorst, MD, PhD
Internist-Endocrinologist
Department of Internal Medicine, Endocrinology and Metabolism
Academic Medical Center
Amsterdam, The Netherlands

Dag Malm, MD, PhD
The Tromsø Centre of Internal Medicine (TIS)
Tromsø, Norway

Jeremy Manuel, OBE
Chairman
European Gaucher Alliance
Dursley, Gloucestershire, UK

Atul Mehta
Clinical Director
Lysosomal Storage Disorders Unit
Department of Haematology, University College London
Royal Free Hospital
London, UK

Matthew C. Micsenyi
The Dominick P. Purpura Department of Neuroscience
Albert Einstein College of Medicine
Bronx, NY, USA

Øivind Nilssen
Professor
Department of Clinical Medicine-Medical Genetics
University of Tromsø; Department of Medical Genetics
University Hospital of North-Norway
Tromsø, Norway

Marc C. Patterson, MD, FRACP
Chair, Division of Child and Adolescent Neurology
Professor of Neurology, Pediatrics and Medical Genetics
Mayo Clinic
Rochester, MN, USA
Contributors

Sandra Pohl, PhD
Department of Biochemistry
Children’s Hospital
University Medical Center Hamburg-Eppendorf
Hamburg, Germany

Annick Raas-Rothschild, MD
Associate Professor of Genetics
Department of Human Genetics and Metabolic Diseases
Hadassah Hebrew University Medical Center
Ein Kerem
Jerusalem, Israel

Uma Ramaswami, MBBS, MSc, MD, FRCPCH
Consultant Metabolic Paediatrician
The Willink Biochemical Genetics Unit
Genetic Medicine
St Mary’s Hospital
Manchester, UK

Arnold J.J. Reuser
Department of Clinical Genetics
Center for Lysosomal and Metabolic Diseases
Erasmus MC University Medical Center
Rotterdam, The Netherlands

Paul Saftig, PhD
Biochemical Institute
Christian-Albrechts-University Kiel
Kiel, Germany

Maurizio Scarpa
Department of Paediatrics
University of Padova
Padova, Italy

Edward H. Schuchman, PhD
Genetic Disease Foundation – Francis Crick Professor
Vice Chairman for Research
Department of Genetics and Genomic Sciences
Mount Sinai School of Medicine
New York, NY, USA

Michael Schwake, PhD
Biochemical Institute
Christian-Albrechts-University Kiel
Kiel, Germany

Hilde Monica F. Riise Stensland, DrSc
Senior Scientist
Department of Medical Genetics
University Hospital of North-Norway
Tromsø, Norway

Ans T. van der Ploeg
Department of Pediatrics
Center for Lysosomal and Metabolic Diseases
Erasmus MC University Medical Center
Rotterdam, The Netherlands

Marie T. Vanier, MD, PhD
Director of Research (Emeritus)
Institut National de la Santé et de la Recherche Médicale
and Hospices Civils de Lyon
Lyon, France

Steven U. Walkley, DVM, PhD
Director, Rose F. Kennedy Intellectual and Developmental Disabilities Research Center
Professor of Neuroscience, Pathology and Neurology
Albert Einstein College of Medicine
Bronx, NY, USA

Melissa P. Wasserstein
Department of Genetics and Genomic Sciences
Mount Sinai School of Medicine
New York, NY, USA

David A. Wenger, PhD
Professor of Neurology
Thomas Jefferson University, Jefferson Medical College
Philadelphia, PA, USA

Ruth E. Williams
Department of Paediatric Neurology
Evelina Children’s Hospital
Guy’s and St Thomas’ NHS Foundation Trust
London, UK

Bryan Winchester
Biochemistry Research Group
UCL Institute of Child Health at Great Ormond Street Hospital
University College London
London, UK

J. Edmond Wraith, MBChB, FRCPCH
Professor of Paediatric Metabolic Medicine
Manchester Academic Health Sciences Centre;
Willink Biochemical Genetics Unit
Royal Manchester Children’s Hospital
Manchester, UK

Ari Zimran, MD
Gaucher Clinic
Shaare Zedek Medical Center
The Hebrew University – Hadassah Medical School
Jerusalem, Israel
The concept of a lysosomal storage disorder is now almost 50 years old – an appropriate time, we feel, for a new review of the subject.

The term lysosome was coined by Christian de Duve [1], the discoverer of this organelle, to reflect its role as the major intracellular site for the enzymatic ‘lysis’ of macromolecules so that they may be recycled. The concept of a lysosomal storage disorder was first proposed by H G Hers [2], following the discovery that one of the glycogen storage diseases (Pompe disease, acid maltase deficiency) was due to deficiency of a lysosomal enzyme. The concept of ‘cross-correction’, formulated by Elizabeth Neufeld [3] and her group after the discovery that co-cultured fibroblasts derived from two patients with different lysosomal storage disorders mutually corrected each other, led to the notion of ‘enzyme replacement therapy’ (ERT). Roscoe Brady not only discovered the enzymatic basis for Gaucher disease and Fabry disease but also pioneered ERT for humans [4,5].

The last two decades, however, have seen a huge expansion in research in this area which has substantially extended our understanding of both the scientific and the clinical basis of lysosomal storage disorders [6]. Thus, at a scientific level it is now very well recognised that lysosomes are part of an endosomal/lysosomal network which plays a critical role in a whole range of cellular processes including the recycling of membrane and other organelles, the turnover of molecules and ingested matter through endocytosis and phagocytosis, and an emerging role in apoptosis and autophagy. At a clinical level successful treatments have been employed which reduce substrate accumulation or promote substrate degradation but it is increasingly recognised that the protean multi-system manifestations of these conditions result from pathologic processes over and above simple lysosomal storage and damage.

This book is the fruit of an ambitious project which aims to review both the scientific and the clinical aspects of lysosomal storage disorders. We perceive a need for an accessible volume giving an up to date overview of the subject. Even when effective treatments are available, there remains an urgent need to highlight awareness of the diseases so that early and appropriate treatment may be sought [7]. Furthermore, in a rapidly changing world, there is a real need to improve access to expensive treatments. The first section of the book reviews current understanding of the physiology and pathophysiology of lysosomal storage disorders and we again to attempt to classify the conditions. The second part of the book reviews individual diseases, and gives perspectives from patients and experts looking towards future therapeutic directions. The book is aimed at a wide audience including scientists, clinicians, health care workers and administrators, those working in the pharmaceutical industry, patients and their organisations.

We are highly indebted to Christine Lavery, the Founder and Chief Executive of the Society for Mucopolysaccharidosis Diseases (MPS Society, UK). Christine has been an integral part of the project from the very beginning, a partner during its production and a driver towards its completion. The extremely high regard in which she is held internationally has allowed us to assemble a glittering array of distinguished contemporary scientists and clinicians working in this area.
Furthermore, all contributors and the editors have donated their royalties to the MPS society, which is dedicated to promote research into these diseases and to the support of patients and families who suffer from them. We are also grateful to Shire HGT which has made the project possible through an unrestricted educational grant given to the MPS Society. The Editors and contributors take full responsibility for the contents of the book and confirm that Shire HGT, the MPS Society and Wiley-Blackwell have not had any role in influencing the content of the work.

We would also like to thank Elisabeth Dodds, Production Manager, Nick Godwin and Rob Blundell at Wiley-Blackwell who have helped us at every stage of the project. Our distinguished contributors have made time in their busy schedules to prepare and revise their contributions and we thank them for their patience, timeliness, clarity and charity in delivering their chapters to us.

We would each like to acknowledge some of our many, academic mentors. For Atul this has to be Lucio Luzzatto, a clinician and scientist who guided his early academic career, emphasising the need for meticulous and reflective observation and record. Atul would also like to thank Victor Hoffbrand, who has provided invaluable encouragement during his career as a clinician, academic – and as a writer. Bryan would like to thank Don Robinson for introducing him to lysosomal storage diseases and giving him his first job, and Bob Jolly, who taught him the importance of linking pathology and biochemistry through the study of animal models. Finally, we would both like to thank our respective wives and families for their continuing forbearance and support.

Atul Mehta
Bryan Winchester

References

Foreword

Soon after the discovery of metabolic abnormality in Gaucher disease, a recommendation was made to examine the potential benefit of enzyme replacement therapy for patients with hereditary enzyme deficiency disorders [1]. A search for a suitable source of the requisite enzymes was initiated. Because of the possibility of sensitizing recipients to a foreign protein, one wished to avoid the administration of an enzyme obtained from a non-human source. Eventually it occurred to me that human placental tissue might be an appropriate starting material and an effort to isolate glucocerebrosidase, the enzyme that is deficient in Gaucher patients, was begun.

Because of its high hydrophobicity, and lack of experience in the handling of such a protein, much difficulty was encountered in obtaining useful quantities of the requisite enzyme. Eventually a small amount of sufficiently purified glucocerebrosidase was obtained and administered to a patient with Type 3 Gaucher disease and a second with Type 1 Gaucher disease [2]. There was a 26% reduction of glucocerebroside in the liver of both patients, as well as a striking reduction in the quantity of glucocerebroside associated with circulating erythrocytes in the recipients. Another long delay was encountered before consistent clinical benefit of enzyme replacement therapy was demonstrated in a cohort of patients with Gaucher disease [3].

This book is a timely review of this rapidly developing field. The Editors are to be congratulated on securing contributions from so many distinguished scientists and clinicians. The benefits, as well as the disappointments, of enzyme replacement therapy (ERT) in various sphingolipid and mucopolysaccharide storage disorders are discussed in individual chapters devoted to these topics. Despite the remarkable successes of ERT with regard to the systemic manifestations of metabolic storage disorders, one is still confronted with the disappointing inability to achieve comparable benefit with regard to the central nervous system manifestations of these conditions. This area is under active investigation and ultimately success in this endeavor is anticipated. Several additional approaches are under consideration. One is the use of small molecules, such as molecular chaperones, to enhance the stability and delivery of mutated enzymes to lysosomes where they can function. Another is to determine the effect of histone deacetylase inhibitors on the quantity and function of mutated enzymes. Eventually an ultimate goal is the development of effective gene therapy to provide permanent cures for patients with lysosomal storage disorders.

Finally, I would like to endorse the work of the MPS Society, which is devoted to research to improve the lives of sufferers and their families. The editors and authors have donated their royalties to this valuable charitable cause and I very much hope this book will succeed in raising awareness of these diseases.

Roscoe O. Brady, MD

References

PART 1

General Aspects of Lysosomal Storage Diseases
CHAPTER 1

The Lysosomal System: Physiology and Pathology

Matthew C. Micsenyi and Steven U. Walkley

The Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA

Introduction

The lysosome and its constituent parts – what has been referred to as the greater lysosomal system [1] – constitute a major metabolic regulatory network in eukaryotic cells. This system includes secretory streams transporting newly synthesized enzymes and other proteins to lysosomes, endosomal and retromolecular streams contributing to signal transduction and related processing, autophagic streams delivering intracellular material for lysosomal degradation, and salvage streams facilitating egress of lysosomal degradation products to other sites in the cell for reutilization. Operating in close parallel are additional proteolytic mechanisms such as the ubiquitin–proteasome system (UPS), which assists in efficient protein turnover. The central coordinator of this remarkable intracellular network is ultimately the lysosome itself, an acidic membrane-bound organelle that functions to degrade and reprocess a vast array of cellular material. Hydrolytic enzymes localized to the lysosomal lumen are optimally active at an acidic pH and have the capacity to degrade most macromolecules including proteins, carbohydrates, lipids, RNA and DNA. Following the breakdown of this material, the resultant amino acids, sugars, simple glycolipids, cholesterol and nucleotides are salvaged by transport through the lysosomal membrane with the aid of specific transporter proteins for delivery to other cell organelles and membranes for subsequent use in biosynthetic processes. Although traditionally depicted as a terminal compartment, this role in recycling molecular precursors brings the importance of the lysosome full circle. Taken as a whole, the greater lysosomal system therefore functions at the very hub of cellular metabolic homeostasis. With the recent discovery of an overarching gene regulatory network referred to as CLEAR (Coordinated Lysosomal Expression and Regulation) and its master gene transcription factor EB (TFEB), many components of the greater lysosomal system have been shown to be linked at the transcriptional level [2]. Indeed, these studies further establish the lysosomal system as a highly efficient and coordinated network. As such, proper lysosomal function is essential since failure of this system leads, inexorably, to catastrophic consequences for cells, organs, and individuals, with nearly 60 different types of lysosomal diseases documented to date (see Classification in Chapter 5).

The greater lysosomal system

Our understanding of the lysosome and its role in cells has evolved significantly since its discovery by Christian de Duve more than 50 years ago. This organelle and the constituent streams or pathways to which it is linked comprise a processing and recycling centre essential to all cells. While each component is typically defined separately, it is important to conceptualize the various parts functioning as a highly orchestrated cellular mechanism (Figure 1.1).

Endocytosis

The endolysosomal pathway consists of the major delivery streams and molecular machinery necessary for the internalization of cell surface and extracellular material linking
The Greater Lysosomal System

**Figure 1.1** Schematic highlighting the major pathways and mechanisms contributing to the greater lysosomal system including: the secretory pathway involved in targeting of acid hydrolases from the Golgi complex to late endosomes/lysosomes; the endocytic pathway where extracellular components are internalized; the autophagic pathway – macroautophagy (MA), chaperone-mediated autophagy (CMA), and microautophagy (McA) – where intracellular proteins and organelles are targeted for degradation; and the ubiquitin-proteasome system (UPS) which is allied with the autophagic/lysosomal system in maintaining proteolytic quality control. Salvage streams are depicted as solid red lines where simple molecular products are transported out of lysosomes and trafficked to organelles throughout the cell to be reutilized. (APH, autophagosome; EE, early endosome; LE, late endosome; RE, recycling endosome; MPR, mannose 6-phosphate receptor; NPC1, Niemann–Pick type C1 protein).

cells with their external environment. The full scope of the complexity of the endocytic system continues to evolve with the characterization of diverse forms of endocytosis and the elucidation of key molecular components associated with these pathways. Endocytic processes are often grouped by the morphological characteristics of the invagination of the membrane. Clathrin-mediated endocytosis (CME) is defined by clathrin-coated pits localized to the plasmalemma that internalize receptor/cargo complexes into vesicles that are sorted and targeted to various intracellular locations. In the CNS, neurons rely on CME for the cycling of neurotransmitter receptors regulating signaling and activity-dependent neuroplasticity. Clathrin-independent endocytosis has also been described and most often occurs at flask-like invaginations along the plasmalemma called caveolae. Caveolae are long-lived plasma membrane microdomains composed of caveolins, cholesterol, sphingolipids including glycosphingolipids (GSLs) and sphingomyelin, GPI-anchored proteins and various receptor proteins. Such specialized membrane structures which are ultimately processed by the endolysosomal system are known to play a critical role as platforms for cell signalling and as regulators of lipid components within the plasmalemma.

The canonical endocytic pathway progresses along an increasing lumen-acidic gradient from early endosomes retrogradely trafficked from the plasma membrane, to multivesicular bodies or late endosomes, and finally to perinuclear-localized lysosomes. Deviating from this pathway, early endosomes can be recruited back to the plasmalemma or to other organelles as sorting/recycling endosomes. These divergent streams allow for the recycling and reinsertion of cell surface receptors, delivery of signaling ligands throughout the cell, and internalization...
of membrane components to be reorganized. Such carefully orchestrated processing and its related signal transduction events may be interrupted in diseases of the lysosomal system in which endocytosed components including cholesterol and GSLs accumulate. While this accumulation is typically associated with late endosomes and lysosomes, recent evidence has emerged suggesting additional involvement of early endosomal compartments [3], raising the likelihood that the consequences of lysosomal disease extend well beyond the lysosome itself.

**Autophagy**

In addition to endocytic pathways, autophagic streams feed into the lysosome and are involved in targeting intracellular material including effete organelles, long-lived proteins and pathogens for degradation [4]. Autophagy, which is often activated following starvation stress, is divided into three distinct subtypes – microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy – defined by the delivery method of substrate to lysosomes. Microautophagy involves the direct engulfment of cytosolic material by the lysosome either by direct invagination of the lysosomal membrane, or projected arm-like extensions that sequester material into intralysosomal vesicles. CMA is selective for soluble monomeric proteins containing the peptide signalling motif Lys-Phe-Glu-Arg-Gln (KFERQ). This motif allows monomeric proteins containing the peptide signalling motifs to leave the lysosome and enter the cytosol means that it is unavailable for conversion into enzyme cofactors methylcobalamin and adenosylcobalamin, which are critical for multiple metabolic processes. Another example of salvage compromise involves the reutilization of lysosomal degradation products directly in synthetic pathways in the Golgi and elsewhere in the cell. In Niemann–Pick type C disease (NPC), lack of the NPC1 protein and the resulting compromise in egress of unesterified cholesterol to other sites in the cell is known to cause compensatory increases in cholesterol synthesis [6] and a similar phenomenon involving glycosphingolipids (GSLs) may occur following sequestration of GM2 and GM3 gangliosides [3]. Following synthesis in the Golgi and transport to the plasmalemma, GM1 and other complex gangliosides are eventually endocytosed and degraded in lysosomes. Salvage of simple gangliosides (e.g., GM2 and GM3) from lysosomes and their transport back to the Golgi allows for efficient production of complex gangliosides without the need for complete de novo synthesis from ceramide. Even in other lysosomal disease caused by defects in degradation rather than egress – such as Sandhoff disease in which GM2 accumulates following absence of β-hexosaminidase – neurons may similarly be deprived of this precursor for complex ganglioside synthesis. Reutilization of simple molecular components derived from lysosomal degradation would be energetically favourable over full de novo synthesis. What storage may lead to in terms of cell energy consumption and altered regulatory processing is only now beginning to be explored in depth. Thus, while lysosomal diseases have been historically viewed as states of overabundance of non-degraded material, it is only logical to assume that they are also likely deficiency diseases in which critical components for multiple metabolic pathways are in reduced supply.

**Ubiquitin–Proteasome System (UPS)**

In addition to pathways directly feeding into and out of the lysosome, the lysosomal system includes mechanisms functionally allied in maintaining proteolytic quality
control, namely the UPS. As the primary degradative pathway for most soluble short-lived proteins, the UPS plays a pivotal role in cellular regulatory processes, including the endoplasmic reticulum associated degradative system (ERAD). The ERAD system is responsible for turning over aberrantly misfolded proteins immediately following their translation thereby serving as a quality control check-point. Additionally, the UPS has been found to coordinate proteolysis with the autophagic/lysosomal system in certain instances. For example, UPS inhibition promotes the upregulation of macroautophagy in an apparent effort to redirect and sustain efficient protein degradation through the lysosome. Furthermore, the UPS and autophagic/lysosomal systems rely on several of the same key molecules – most notably ubiquitin and p62 – selectively targeting substrates for degradation. This complementation is limited, however, as the UPS only has the ability to degrade monomeric proteins that have been properly unfolded and fed into the proteasome catalytic core, while macroautophagy is capable of bulk degradation of organelles and oligomerized proteins. In lysosomal disease states in which the degradative capacity of the lysosome is compromised it is conceivable that the UPS may be employed to compensate for some of the proteolytic load, although the molecular machinery and sequence of events involved in this cross-talk is largely unknown. Finally, it is unclear whether there is a threshold at which the UPS may too become overwhelmed, perhaps contributing to ER stress and thereby causing complete proteolytic failure in lysosomal disease states.

Lysosomal diseases

After more than a half century of clinical recognition and classification, lysosomal storage diseases were conceptualized as disorders resulting from deficiencies in single lysosomal hydrolases followed by the subsequent accumulation of a specific substrate for that enzyme [7]. Today, lysosomal diseases are known to include nearly 60 monogenic disorders with a combined frequency of approximately 1:7000 live births. They are known to be caused by deficiencies not only in lysosomal hydrolases, but also in other lysosomal and non-lysosomal proteins including enzymes, soluble non-enzymatic proteins, and membrane-associated proteins critical for proper function of the greater lysosomal system [8]. Categorizing lysosomal disorders is not completely straightforward as several diseases exhibit significant overlap of pathological features, storage material, and so forth. However, grouping lysosomal diseases based on the traditional biochemical nature of the primary storage material is often preferred; these include the lipidoses, mucopolysaccharidoses (MPSs), glycosogenoses, neuronal ceroid lipofuscinoses (NCLs), mucolipidoses (MLs), glycoproteinoses and others (see Classification in Chapter 5).

In general, most lysosomal diseases afflict children. Age of onset and clinical course can vary significantly, but nearly all lysosomal diseases have a delayed non-congenital onset, and progressive course ultimately leading to premature death. Even as our grasp of the genetic, molecular and biochemical bases of these disorders has advanced in recent years there are still many gaps in our understanding as to pathogenic mechanisms and the reversibility of disease-induced cell damage. Some of the most important questions in this regard involve the brain.

CNS Involvement (See also Chapter 21) Adding to lysosomal disease complexity is the broad systemic involvement of multiple tissues and organs. Clinical presentation often involves bone, muscle, liver, kidney and spleen, while nearly two-thirds of these disorders also exhibit extensive neurological impairment (see Chapter 2). Intellectual disability, dementia, seizures, motor system deficits, visual impairment and hearing loss are common manifestations associated with several lysosomal diseases. The progressive clinical and pathological course presented in these disorders highlights the indispensable role of the lysosome, especially in postmitotic neurons which are predominately affected. Interestingly, this vulnerability becomes manifest in a highly specified manner with different neuronal subtypes and brain regions exhibiting distinct pathophysiological changes.

One of the more perplexing phenomena associated with lysosomal disease is neurodegeneration. While many of these disorders exhibit some degree of patterned neuronal loss, the question remains why some neurons appear more vulnerable to this fate than others and what effect neurodegeneration has on disease course. A common theme implicated in motor system impairment is Purkinje cell (PC) death. PC death in mice with NPC begins early and progresses in an anterior to posterior lobe pattern within the cerebellum. This cell loss is severe and nearly total, with the conspicuous exception of lobule X (flocculonodular lobe in humans) where PCs are well preserved. Remarkably, almost identical patterns of PC loss occur across a wide spectrum of lysosomal diseases, including mucolipidosis type IV and the gangliosidoses. It should also be noted that in the case of NPC disease that both PCs and cerebrocortical neurons exhibit extensive pathological
storage of cholesterol and ganglioside, however unlike PC degeneration, cortical neuron death is often not as conspicuous in the early stages of the disease.

CNS inflammation is an additional feature of lysosomal diseases that in many cases has been shown to be spatially and temporally correlated with neuronal dysfunction and neurodegeneration. Of particular interest is how this initial protective response to disease may become deleterious with chronic induction, ultimately leading to secondary damage and exacerbation of pathogenic processes. In the normal brain, microglia play an essential house-keeping role intimately coupled to neuronal function. As the resident macrophage of the CNS, microglia are also critical for synapse maintenance, axon and spine pruning, clearing extracellular debris and apoptotic cells, glutamate and trophic factor regulation, and probing their surroundings for homeostatic deviations in the extracellular environment. Conversely, during disease/injury activated microglia proliferate, and, along with infiltrating macrophages, can generate cytotoxic components including reactive oxygen species, nitric oxide and pro-inflammatory cytokines. Microglia and macrophages exhibit altered morphological states in many lysosomal diseases, while activated astrocytes are also a prominent feature contributing to the pathological landscape in the CNS. Some NCLs, gangliosidoses, MPSs, MLs and neuronopathic Gaucher disease exhibit neuroinflammatory features. A study in Sandhoff disease mice showed that microglial activation precedes neuronal cell death, and that bone marrow transplantation ameliorates the expansion of microglia and neurodegeneration [9]. Interestingly, however, this improvement did not coincide with any significant decrease in GM2 ganglioside storage, suggesting that microglial activation is a significant component of neuronal death independent of storage. As such, the use of anti-inflammatory drugs to treat lysosomal diseases may be a relevant therapeutic strategy for providing benefit in certain instances. Future efforts to decipher the protective roles of microglial activation and inflammation from pathogenic stimulating events, and to determine the temporal window for using anti-inflammatory drugs in the treatment of lysosomal disease states, will be critical.

Intracellular storage

Ever since the concept of lysosomal disease was developed by H.G. Hers, intracellular storage material has been a defining characteristic of these disorders. As the complexity of this pathological feature has evolved well beyond the original single enzyme/single substrate theory, research has focused on understanding how storage contributes to pathogenic cascades. The relationship between primary and secondary storage cascades is a fundamental cause of neuronal dysfunction remains an important question. Studies in NCL disease have shown no direct correlation between the accumulation of saposins A and D and subunit c of mitochondrial ATP synthase (SCMAS) and neuron loss (Figure 1.2). In fact, storage pathology in NCL and other lysosomal diseases is typically prevalent long before the onset of any behavioural phenotype in animal models [8]. Similarly, neurons in many lysosomal diseases present significant storage pathology early, and yet survive for extended periods of time (decades) suggesting that accumulation is
not immediately cytotoxic. Yet this does not mean that neurons remain functionally normal. Indeed, as described below, given the presence of metabolic compromise and of axonal, dendritic and synaptic abnormalities, it is highly likely that affected neurons are not optimally functioning even from early time points in the storage process. Eventually the presence of such variously malfunctioning neurons in given neural networks would be anticipated to reach a tipping-point at a systems level, with clinical disease emanating as a result, even in the absence of frank neurodegeneration. Such clinical deficits would be solidified, and possibly worsened, with the eventual death of neurons that participate in these neural circuits. It is essential to understand these issues in the face of emerging therapies that may rescue the storage phenotype long after it has been established, but before neuron death. Interestingly, substrate reduction therapy either aimed at inhibiting the biosynthesis of GSLs in gangliosides [11], or at enhancing the egress of cholesterol and GSLs in NPC disease, has proven effective in delaying clinical onset and increasing life-span [12]. These studies clearly indicate that reducing storage is beneficial in lysosomal disorders but reveal little about the precise link between storage and brain dysfunction.

In contrast to a primary role in disease pathogenesis, storage may have a more indirect and broader influence on hindering metabolic homeostasis in cells. Impairment in lysosomal salvage probably represents a progressive and deleterious metabolic burden forcing cells to expend energy synthesizing simple molecules to replace those trapped within residual lysosomes. Owing to its tight regulation of peripheral energy stores via the hypothalamus, as well as to additional local support from astrocytes, the brain in general, and neurons in particular, are considered resistant to starvation events. Lysosomal diseases, however, may represent a unique pathophysiological state within the CNS characterized by chronic energy depletion. This could perhaps explain why neurons which need to sustain high levels of activity are particularly susceptible in these disorders. It is also interesting to consider how this scenario might be further compounded in lysosomal disorders that have been speculated to involve suboptimal mitochondrial function (e.g. NCL disorders and MLIV), and whether this could be a cause, or a result, of chronic energy strain. Once again, clues as to how storage correlates with CNS dysfunction and why specific neuronal subtypes are more susceptible to pathological changes in lysosomal diseases may lie in the different metabolic requirements of individual neuronal populations. These so-called metabolic signatures may determine a cell’s vulnerability to downstream deleterious events like starvation stress, oxidative stress, ER stress and even the capacity to tolerate protein aggregates and storage accumulation.

In addition to interfering with metabolic homeostasis, lysosomal storage may inhibit the destined function of the stored material within the cell. For instance, glycosaminoglycans (GAGs) are the predominant storage material in MPS diseases. Normally, mature proteoglycans are decorated with GAG side chains and localized to the outer leaflet of the plasma membrane to function in intercellular signalling cascades. Specifically, proteoglycans play a significant role in CNS development during axon guidance.
and synapse formation through their interaction with growth factors and other extracellular components. Although MPS disorders, like other lysosomal diseases, are not believed to be associated with abnormal brain development per se, it is conceivable that the buildup of GAGs in MPS disease may over time hinder appropriate signalling events – eventually reaching a tipping-point for neuron and neural circuit dysfunction as described above.

**Axonal spheroids**

Neuroaxonal dystrophy, also known as axonal spheroid formation, occurs in a wide spectrum of lysosomal diseases, from the gangliosidoses to the glycoproteinoses [8]. Notably, this feature appears to occur predominantly in γ-aminobutyric acid (GABA)ergic neurons, particularly PCs in the cerebellum (Figure 1.3). Morphologically, spheroids are characterized as focal swellings along axons where mitochondria, multivesicular and dense bodies, tubulovesicular profiles and possibly autophagic vacuoles accumulate. Interestingly, this material is ultrastructurally similar across different lysosomal diseases, yet distinct from the storage found in neuronal cell bodies. Given the large surface area of neurons and their highly polarized nature, functional anterograde and retrograde trafficking is essential. Spheroids appear to reflect a compromise in such transport and may as a result hinder critical growth factor support for the development, maintenance, and survival of target neurons. They are also large enough to impact normal action potential propagation and thereby contribute to neuronal dysfunction. Indeed, the incidence of spheroids appears to correlate with the onset and progression of CNS impairment in animal models, suggesting a similar, significant role in human clinical neurological disease [8].

**Calcium signalling**

Calcium plays a critical role as a second messenger involved in a wide range of cellular functions, and altered