

# The Mousetrap: What We Can Learn When the Mouse Model Does Not Mimic the Human Disease

Sarah H. Elsea and Rebecca E. Lucas

## Abstract

In recent years, mouse models for human metabolic diseases have become commonplace because the information gained from in vivo study of biochemical pathways is invaluable, and many metabolic diseases are relatively easy to recreate in mice through gene knockout technology in embryonic stem cells. In certain cases, however, the knockout mice may reproduce only some of the human disease phenotype, may be more severely affected than human cases, or may have no clinical phenotype at all. Under these circumstances, the disease pathology can become more complex, causing the researcher to evaluate basic differences in mouse and human biology as well as questions of genetic background, alternate pathways, and possible gene interactions. This review is a brief analysis of gene knockout models for Lesch-Nyhan syndrome, Lowe syndrome, X-linked adrenoleukodystrophy, Fabry disease, galactosemia, glycogen storage disease type II, metachromatic leukodystrophy, and Tay-Sachs disease, which produce a biochemical model of disease but often do not reproduce clinical symptoms. These mice may be useful for studying the biochemical and physiological pathways in which certain metabolites function toward embryonic and fetal development, as well as specific functions in various organs, and they may provide an inexpensive and useful model system for development of new therapeutic techniques.

**Key Words:** gene knockout; metabolic disease; mouse model

## Introduction

The use of genetically engineered mice as models has become commonplace in the study of many human metabolic diseases as well as diseases with a more complex origin. Many classic “one gene, one enzyme” metabolic diseases are relatively easy to recreate in mice through gene-targeting technology in embryonic stem (ES<sup>1</sup>)

Sarah H. Elsea, Ph.D., F.A.C.M.G., is Assistant Professor and Director of the DNA Diagnostic Program, Departments of Zoology and Pediatrics and Human Development, Michigan State University, East Lansing, Michigan. Rebecca E. Lucas is a Ph.D. Candidate in the Genetics Graduate Program at Michigan State University.

<sup>1</sup>Abbreviations used in this article (Note: Gene names in italic type denote genes, with all capital letters for human and initial capital and subsequent

cells. In many cases, the phenotype of the knockout mice replicates most of the important features of the human disease and can be extremely useful for analyzing the disease pathophysiology as well as the biochemical pathways that are disrupted. However, in certain cases, the knockout mice may reproduce only some of the human disease phenotype, may be more severely affected than human cases, or may have no clinical phenotype at all. Under these circumstances, the disease pathology can become more complex, causing the researcher to evaluate basic differences in mouse and human biology as well as questions of genetic background, alternate pathways, and possible gene interactions. This review is a noncomprehensive analysis of several mouse models for metabolic diseases that do not replicate the features of the human disease. Often, many of the storage metabolites and biochemical hallmarks of disease are detected, but the mice do not exhibit significant clinical disease. Table 1 is a summary of the diseases covered in this review.

To gain a greater understanding of the metabolic pathways that are disturbed in the knockout mice, several groups have created alternate gene knockouts or double gene knockouts of genes in the same or overlapping/interacting metabolic pathway. Table 2 is a summary of these secondary mouse models. This technique was especially useful in the study of a few diseases and will be discussed briefly.

Even though these mutant mice may not replicate the human disease, they are still quite useful for studying the biochemical and physiological pathways in which certain metabolites function toward embryonic and fetal development as well as specific functions in various organs. In

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lower case letters for mouse; gene names not in italic type denote the protein product of the gene): *ABCD1/Abcd1*, ATP-binding cassette D1 (gene for X-ALD); ALD, adrenoleukodystrophy;  $\alpha$ -gal A,  $\alpha$ -galactosidase A; *APRT/Aprt*, adenine phosphoribosyltransferase; *ARSA*, human arylsulfatase A gene; *As2*, murine arylsulfatase A gene; CF, cystic fibrosis; *Cftr*, cystic fibrosis transmembrane conductance regulator; ES, embryonic stem; *GAA/Gaa*,  $\alpha$ -glucosidase gene; gal-1-p, galactose-1-phosphate; GALE, uridine diphosphate galactose 4'-epimerase; GALK, galactokinase; GALT, galactose-1-phosphate uridylyltransferase; *GLA/Gla*,  $\alpha$ -galactosidase A gene; *GM2A/Gm2a*, G<sub>M2</sub> activator; GSDII, glycogen storage disease type II; HexA,  $\beta$ -hexosaminidase A isoenzyme; *HEXA/HexA*,  $\beta$ -hexosaminidase A gene; *HexB*,  $\beta$ -hexosaminidase B isoenzyme; *HEXB/HexB*,  $\beta$ -hexosaminidase B gene; *HPRT1/Hprt*, hypoxanthine-guanine phosphoribosyltransferase; *INPP5B/Inpp5b*, inositol polyphosphate-5-phosphatase; MLD, metachromatic leukodystrophy; OMIM, Online Mendelian Inheritance in Man; ORCL, oculocerebrorenal syndrome of Lowe; *OCRL1/Ocrl1*, human Lowe syndrome gene; TS, Tay-Sachs disease; VLCFA, very long-chain fatty acids; X-ALD, X-linked adrenoleukodystrophy.

**Table 1 Mouse models of the human metabolic diseases discussed in this review that do not have a similar phenotype to humans with the corresponding disease**

Metabolic disease	Human gene <sup>a</sup>	Mouse gene <sup>a</sup>	Summary of the phenotype observed in the knockout mice (see text for complete description)	References (see text)
Lesch-Nyhan syndrome	<i>HPRT1</i>	<i>Hprt</i>	No clinical phenotype; purine content in mouse brain is normal; however, de novo purine synthesis is accelerated	Kuehn et al. 1987; Finger et al. 1988; Hooper et al. 1987; Jinnah et al. 1993, 1999
Lowe syndrome	<i>OCRL1</i>	<i>Ocr11</i>	No clinical phenotype; no tissue differences observed between knockout mice and controls	Janne et al. 1998
X-linked adrenoleukodystrophy	<i>ABCD1</i>	<i>Abcd1</i>	No clinical phenotype; abnormal storage of VLCFAs <sup>a</sup> and reduction of $\beta$ -oxidation observed	Forss-Petter et al. 1997; Kobasyashi et al. 1997; Lu et al. 1997
$\alpha$ -Galactosidase A deficiency/Fabry disease	<i>GLA</i>	<i>Gla</i>	No clinical phenotype; extensive lipid accumulation in liver and kidney	Oshima et al. 1997
Galactosemia	<i>GALT</i>	<i>Galt</i>	No clinical phenotype; mice have high levels of gal-1-p, <sup>a</sup> galactitol, and galactose	Leslie et al. 1996; Ning et al. 2000, 2001
Glycogen storage disease type II/Pompe disease	<i>GAA</i>	<i>Gaa</i>	Mild phenotype and later disease onset observed on 129 x C57BL/6 x FVB murine genetic background; similar phenotype to human GSD II patients on 129 x C57BL/6 genetic background	Bijvoet et al. 1998; Raben et al. 1998, 2000
Metachromatic leukodystrophy	<i>ARSA</i>	<i>As2</i>	Milder phenotype than human MLD <sup>a</sup> patients and no dysmyelination; storage of sulfatide throughout white matter	Hess et al. 1996
Tay-Sachs disease	<i>HEXA</i>	<i>HexA</i>	No clinical phenotype; significant accumulation of G <sub>M2</sub> ganglioside	Sango et al. 1995; Yamanaka et al. 1994

<sup>a</sup>*ABCD1/Abcd1*, ATP-binding cassette D1 (gene for X-ALD); *ARSA*, human arylsulfatase A gene; *As2*, murine arylsulfatase A gene; *GAA/Gaa*,  $\alpha$ -glucosidase gene; gal-1-p, galactose-1-phosphate; *GALT*, galactose-1-phosphate uridylyltransferase; *GLA/Gla*,  $\alpha$ -galactosidase A gene; *HEXA/HexA*,  $\beta$ -hexosaminidase A gene; *HPRT1/Hprt*, hypoxanthine-guanine phosphoribosyltransferase; MLD, metachromatic leukodystrophy; *OCRL1/Ocr11*, human Lowe syndrome gene; VLCFA, very long-chain fatty acids.

addition, they may provide an inexpensive and useful model system for development of new therapeutic techniques.

## X-Linked Disorders

### Lesch-Nyhan Syndrome

Lesch-Nyhan syndrome (Online Mendelian Inheritance in Man [OMIM]<sup>1,2</sup> #300322) is an X-linked recessive disorder

<sup>2</sup>Online Mendelian Inheritance in Man (OMIM) is an electronic compendium of bibliographic material and summaries on inherited disorders and genes, which is maintained by geneticists and molecular biologists at the National Library of Medicine, National Institutes of Health (<http://www.ncbi.nlm.nih.gov/omim>).

that results from the deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT<sup>1</sup>) in the purine salvage pathway (Figure 1). The degree of neurological involvement is dependent on the amount of residual HPRT enzyme activity present, but most persons with classical Lesch-Nyhan syndrome have less than 1.5% HPRT activity (Jinnah and Friedmann 2001). Classical HPRT deficiency results in overproduction of uric acid, debilitating neurological disability (choreic and athetoid movements, dysarthric speech, hyperreflexia, and hypertonia), varying degrees of cognitive disability, and behavioral abnormalities that often include impulsive and self-injurious behaviors (Jinnah and Friedmann 2001). In addition, megaloblastic anemia, growth retardation, and unexplained fevers are often reported in these individuals (Jinnah and Friedmann 2001). Diminished dopamine levels in the basal ganglia

**Table 2 Genetically engineered mice developed to elucidate pathways or mechanisms in the mouse that may differ from the human**

Mouse model	Human gene/s	Mouse gene/s	Summary of the phenotype observed in the knockout mice (see text for complete description)	References (see text)
<i>Hprt</i> <sup>a</sup> / <i>Aprt</i> <sup>a</sup> double knockout	<i>HPRT1/APRT</i>	<i>Hprt/Aprt</i>	Phenotype similar to humans with APRT deficiency (adenine excretion and kidney disease)	Engle et al. 1996
<i>Hprt</i> /urate oxidase double knockout	Urate oxidase/uridase is not present in humans	<i>Hprt/Uox</i>	No neurobehavioral defects	Wu et al. 1994
<i>Ocr1</i> <sup>a</sup> / <i>Inpp5b</i> <sup>a</sup> double knockout INPP5B	<i>ORCL1/INPP5B</i>	<i>Ocr1/Inpp5b</i>	Embryonic lethal	Janne et al. 1998
	<i>INPP5B</i>	<i>Inpp5b</i>	No clinical phenotype; later onset testicular degeneration	Janne et al. 1998
Galactokinase	<i>GALK1</i>	<i>Glk1</i>	No clinical phenotype; galactosemic cataracts developed when aldose reductase was overexpressed	Ai et al. 2000; Lee et al. 1995
Sandhoff disease	<i>HEXB</i>	<i>Hexb</i>	Ganglioside storage in nearly all neurons; severe neurological phenotype	Phaneuf et al. 1996; Sango et al. 1995
G <sub>M2</sub> activator protein	<i>GM2A</i>	<i>Gm2a</i>	Restricted pattern of ganglioside storage; balance and coordination abnormalities	Liu et al. 1997
<i>HEXA</i> <sup>a</sup> / <i>HEXB</i> <sup>a</sup> double knockout	<i>HEXA/HEXB</i>	<i>Hexa/Hexb</i>	Severe dysmorphology and mucopolysaccharidosis	Sango et al. 1996

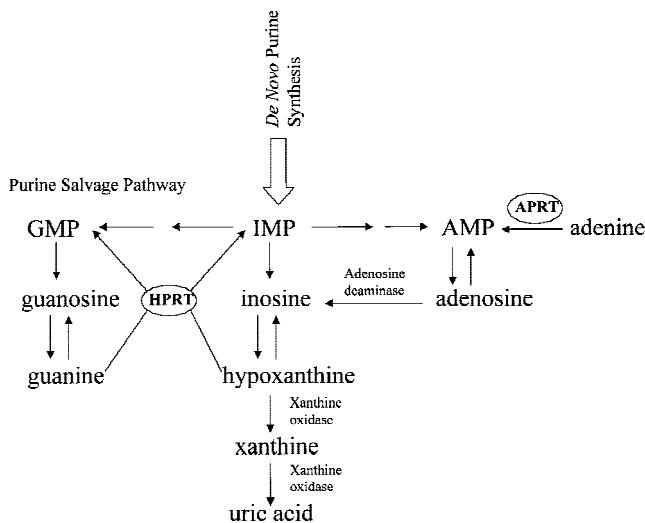
<sup>a</sup>*APRT*, adenine phosphoribosyltransferase; *HEXA*, β-hexosaminidase a; *HEXB*, β-hexosaminidase B; *HPRT*, hypoxanthine-guanine phosphoribosyltransferase; *INPP5B*, inositol polyphosphate-5-phosphatase; *OCRL1*, human Lowe syndrome gene.

(60-90% depletion) and cerebrospinal fluid have also been reported throughout patients' lives, suggesting that dopamine deficiency may account for some neurological symptoms of the disorder (Jinnah and Friedmann 2001). The mechanism by which features of Lesch-Nyhan syndrome result from impaired purine metabolism is still not well understood.

In an attempt to study the underlying cause of the complex phenotype observed in persons with Lesch-Nyhan syndrome, at least two mouse models have been created that exhibit *Hprt* deficiency (Table 1) (Hooper et al. 1987; Kuehn et al. 1987). The model of Kuehn and coworkers (1987) used retroviral insertion to mutagenize mouse ES cells, ultimately creating an *Hprt*-deficient mouse. The model that Hooper and colleagues (1987) developed was derived from a spontaneously mutated ES cell line. In both of these models, male mice have a total lack of HPRT

activity; however, these mice function and behave normally and have no apparent phenotype (Finger et al. 1988; Hooper et al. 1987; Kuehn et al. 1987). *Hprt*-deficient mice do show reduced levels of basal ganglia dopamine (~20-60% depending on the strain, subregion of basal ganglia studied, and age of the mice); however, other studies have shown that dopamine depletion must be at least 70% to manifest phenotype effects (Breese et al. 1985, 1986; Finger et al. 1988; Jinnah et al. 1999). In fact, a dopamine depletion of 70 to 90% in rats (induced with dopamine receptor antagonists) resulted in self-mutilation behaviors, but lower levels of depletion did not result in these effects (Breese et al. 1985, 1986).

Other studies indicate that tyrosine hydroxylase and aromatic amino acid decarboxylase, both enzymes involved in the synthesis of dopamine, are reduced (~20-40%) in *Hprt*-deficient mice, and the magnitude of the loss was dependent



**Figure 1** Purine salvage pathway. The roles of hypoxanthine-guanine phosphoribosyltransferase (HPRT) and adenine phosphoribosyltransferase (APRT) in the purine salvage pathway are depicted. Lesch-Nyhan syndrome arises from a deficiency of HPRT.

on the genetic background of the mice (Jinnah et al. 1999). Furthermore, studies of dopaminergic neurons from *Hprt* mutant mice demonstrate a decreased rate of dendrite outgrowth (Boer et al. 2001). Because dopamine depletion is much greater in humans with Lesch-Nyhan syndrome than in the mouse models of *Hprt* deficiency, it may be that the dopamine pathway is either not as severely affected or is regulated differently than in humans, or perhaps an alternative pathway exists for dopamine synthesis.

*Hprt*-deficient mouse models are valuable for exploring the purine synthesis pathways with regard to the interactions with the dopaminergic pathways and the resulting effects on the basal ganglia. Can the mouse compensate for lack of HPRT enzyme activity through an as yet unidentified pathway of purine salvage? Some studies suggest that the mouse may depend less on HPRT than the human to maintain purine levels (Finger et al. 1988; Jinnah et al. 1993). Jinnah and colleagues (1993) have shown that purine content in the *Hprt*-deficient mouse brain is within the normal range; however, de novo purine synthesis is accelerated four- to five-fold in the brains of *Hprt* mutant mice. A similar increase in de novo purine synthesis (~10-fold) has been documented in several studies of human fibroblasts and lymphoblasts as well as in intravenous studies in affected humans (Hershfield and Seegmiller 1977; Michener 1967; Nuki et al. 1973; Seegmiller et al. 1967; Wood et al. 1973). However, although the assumption has been that this observed acceleration in purine synthesis does occur in the human brain, it has never been demonstrated. Perhaps the increase in purine synthesis in the mouse brain protects these *Hprt*-deficient mice from the devastating effects seen in humans, but such protection is only speculation.

The combination of increased purine synthesis and de-

creased or absent purine recycling is responsible for the excess uric acid produced in the Lesch-Nyhan syndrome. *Hprt*-deficient mice do not have hyperuricemia due to the presence of uricase, which is not found in humans. Excess uric acid has not been implicated in the neurobehavioral features of Lesch-Nyhan syndrome, nonetheless, a double knockout of HPRT and uricase has no neurobehavioral deficits (Table 2) (Wu et al. 1994).

In a further attempt to stress the purine synthesis pathway, Wu and Melton (1993) reported that *Hprt* mice given adenine phosphoribosyltransferase (*Aprt*<sup>1</sup>) inhibitors (caffeine and 9-ethyladenine) exhibited self-injurious behaviors, although other investigators have not been able to replicate this model (Edamura and Sasai 1998; Engle et al. 1996; Jinnah and Friedmann 2001). *Hprt/Aprt*-deficient mice (Table 2) were created to test the hypothesis that total absence of a purine salvage pathway would produce the aberrant behaviors observed in humans with Lesch-Nyhan syndrome (Engle et al. 1996). However, the doubly deficient mice exhibited only the typical biochemical abnormalities observed in both mice and humans with *Aprt* deficiency (adenine and 2,8-dihydroxyadenine excretion and kidney disease), although to a somewhat lesser extent than that observed in the *Aprt*-deficient mice (Engle et al. 1996). HPRT deficiency appears to be protective for the negative effects of *Aprt* deficiency (Engle et al. 1996). Furthermore, the original studies of Wu and Melton utilized caffeine and 9-ethyladenine as *Aprt* inhibitors. Subsequent studies have shown that 9-ethyladenine is not a sufficiently effective inhibitor of *Aprt* (Edamura and Sasai 1998) and that caffeine itself can cause self-injurious behavior in wild-type mice (Engle et al. 1996; Wu and Melton 1993). Thus, the studies of Wu and Melton are not appropriate for the discussion of a mouse model of *Hprt* deficiency and Lesch-Nyhan syndrome. To date, an appropriate model of the human disease has yet to be generated.

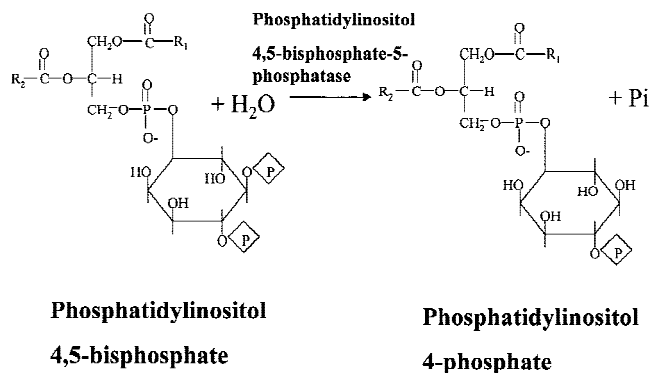
Even though the *Hprt*-deficient mice do not exhibit similar neurological features, they do have a similar biochemical phenotype compared with humans with Lesch-Nyhan syndrome. These mice may be exploited pharmacologically to develop a more complete mouse model of Lesch-Nyhan syndrome and eventually to develop other pharmacological therapies for the treatment of the human disorder. Development of more effective strategies for treatment requires a more thorough understanding of the dopamine and purine pathways and interactions. The fact that the mice are healthy and otherwise unaffected provides an excellent tool for evaluation of potential therapies from the biochemical perspective. In addition, these models provide the necessary reagents for the identification of modifiers and other players in the purine synthesis pathways and the interactions with the dopaminergic pathways. If basal ganglia dopaminergic neuronal loss is key to the development of the neurobehavioral features of Lesch-Nyhan syndrome, then perhaps we can identify the differences between mice and humans in the regulation of these interacting pathways and identify potential modifiers in the

threshold effect seen with dopaminergic loss and neurobehavioral dysfunction.

## Low Syndrome

The oculocerebrorenal syndrome of Lowe (OCRL<sup>1</sup>; OMIM \*309000) is an X-linked human genetic disorder characterized by mental retardation, renal Fanconi syndrome, and congenital cataracts (Nussbaum and Suchy 2001). The gene responsible for Lowe syndrome is *OCRL1*, which maps to Xq26.1 and encodes phosphatidylinositol-4,5-bisphosphate-5-phosphatase (Figure 2). The defect is in inositol metabolism. The enzyme removes one molecule of phosphate from phosphatidylinositol 4,5-bisphosphate (or PtdIns[4,5]P<sub>2</sub> or PIP<sub>2</sub>), converting it into phosphatidylinositol 4-phosphate (PtdIns[4]P or PIP) (Figure 2). This protein functions in the *trans*-Golgi complex and is expressed in all tissues examined, with a similar pattern of gene expression observed in both humans and mice (Janne et al. 1998; Nussbaum and Suchy 2001). The role this enzyme and the related pathways play in development and in the features of the disease are not well understood.

In 1998, Janne and coworkers attempted to create a Lowe syndrome mouse model by knocking out the murine *Ocr11* utilizing gene-targeting strategy to disrupt the gene at the 3'-end (Table 1). They created mice that exhibited no *Ocr11* enzyme activity (Janne et al. 1998); however, these mice had no apparent phenotype. All tissues and organs examined were normal, and no differences were observed between *Ocr11*-deficient mice and their normal littermates, even after 1 yr of age (Janne et al. 1998). Numerous biochemical studies were performed, again, with normal outcomes. Behavioral studies did not reveal statistically significant differences between knockout mice and controls (Janne et al. 1998).



**Figure 2** *OCRL1* activity. The gene for Lowe syndrome, *OCRL1*, encodes a phosphatidylinositol 4,5-bisphosphate-5-phosphatase, which is active in the *trans*-Golgi complex. This phosphatase removes one molecule of phosphate from phosphatidylinositol 4,5-bisphosphate, converting it to phosphatidylinositol 4-phosphate. Mouse knockouts for *OCRL1* and a similar enzyme, inositol polyphosphate-5-phosphatase (*INPP5*), are discussed in the text.

To address the possibility of biochemical redundancy, the same group created a knockout for inositol polyphosphate-5-phosphatase (*Inpp5b*<sup>1</sup>) (Table 2), which is very similar to the *Ocr11* protein and shares similar enzyme activity (Janne et al. 1998). Expression analysis indicated that murine *Inpp5b* is more widely expressed in mice than is *INPP5b* in humans, suggesting that the murine enzyme could compensate for the *Ocr11* deficiency (Janne et al. 1998). A mouse was created with a disruption of *Inpp5b*, and similar to the *Ocr11* deficient mice, no phenotype was observed (Janne et al. 1998). However, after sexual maturity, testicular degeneration leading to sterility did occur in the *Inpp5b*-deficient mice. The *Inpp5b* mice were bred to the *Ocr11* mice to create double knockouts (Table 2), but the double mutation appeared to cause embryonic lethality (Janne et al. 1998). Although more than 40 embryos were examined, no doubly mutant embryos were identified and no live-born double mutant mice were obtained (Janne et al. 1998).

It is clear that in mice, but not in humans, *Inpp5b* can compensate for the deficiency of *Ocr11*, and this crossover activity may provide an alternative avenue for therapy for this disorder. By enhancing, increasing, or altering the expression of *INPP5b*, some of the features of Lowe syndrome might be lessened. Because Lowe syndrome is clearly a development disorder and prevention of the disease would need to occur prenatally, only disease severity might be altered. The mouse models deficient for these two enzymes in inositol metabolism will be important for our understanding of these metabolic pathways and the potential disruption to signal transduction pathways that may occur in the absence of one or both of these enzymes. This understanding is only beginning to develop as additional interacting pathways are explored.

## X-Linked Adrenoleukodystrophy

In the human form of X-linked adrenoleukodystrophy (X-ALD<sup>1</sup>; OMIM \*300100), affected males accumulate unusually high levels of unbranched very long-chain fatty acids (VLCFAs<sup>1</sup>) in their tissues (particularly the brain and adrenal cortex) as a result of defective VLCFA activation and degradation that normally occurs in the peroxisome (Berger et al. 1997; Moser et al. 2001). With the classic cerebral form of the X-ALD, onset typically occurs within 3 to 10 yr of age, with death occurring within 3 yr of onset of symptoms. A significant number of male X-ALD patients may also experience adrenocortical insufficiency. Female heterozygotes may also show some phenotypic effects, including elevated VLCFAs and various neurological symptoms including hyperreflexia, impaired vibration sense, paraparesis, Addison disease, and rapidly progressing dementing illness (Moser et al. 1991). The occurrence and severity of all of these features increase with age (Moser et al. 1991).

The gene for X-ALD (*ABCD1*<sup>1</sup>), which is a member of the ATP-binding cassette transporter family, has been

mapped to Xq28 (Moser et al. 2001). The ABCD1 protein is localized to the peroxisomal membrane and is a member of a family of transporter proteins involved in import of fatty acids or fatty acyl-CoAs into the peroxisome. This peroxisomal membrane protein is likely involved in the peroxisomal transport or catabolism of VLCFAs. All known peroxisomal ABC transporters are half transporters and require a partner half transporter molecule to form a functional homodimeric or heterodimeric transporter. The partner transporter for ABCD1 has not yet been identified.

It is still unclear how this transporter protein normally interacts with the enzymes that process the VLCFAs (called very long-chain acyl-CoA synthetases), or the true role of ABCD1 in the defective formation of these VLCFA coenzyme-A derivatives in X-ALD (Moser et al. 2001). Furthermore, the relation between elevated levels of VLCFAs and the loss of myelin on nerve fibers is not known. Analysis of the more than 340 *ABCD1* mutations in human X-ALD patients (see [www.x-ald.nl](http://www.x-ald.nl)) does not demonstrate a clear genotype-phenotype correlation, and variable clinical presentations of X-ALD can exist within families that carry the same mutation (Berger et al. 1997; Moser et al. 2001), suggesting the presence of modifiers.

The mouse X-ALD gene (designated *Abcd1*) consists of 10 exons and maps to band B of the mouse X chromosome (Kennedy et al. 1996). Three groups subsequently reported the production of gene-targeted mice that disrupt the *Abcd1* gene (Table 1) (Forss-Petter et al. 1997; Kobayashi et al. 1997; Lu et al. 1997). By reverse transcriptase-polymerase chain reaction analysis, Lu and colleagues (1997) demonstrated that no adrenoleukodystrophy protein mRNA was present in gene-targeted mice. Surprisingly, all groups report the significant accumulation of VLCFAs in the tissues of the mutant mice, especially in the brain and adrenal gland; however, no clinical or neurological phenotype has been observed. Forss-Petter et al. (1997) used the rotarod test to determine that there were no significant differences between the motor development of the X-ALD and control mice.

Biochemically, the X-ALD mice proved to be a useful model for studying  $\beta$ -oxidation. Lu and coworkers (1997) measured VLCFA  $\beta$ -oxidation in the fibroblasts of knockout mice and found a 60% reduction compared with normal mice; in males with X-ALD, the reduction is typically 60 to 80%. Forss-Petter and colleagues (1997) reported a 43% reduction in  $\beta$ -oxidation, compared with a 57% reduction in human X-ALD fibroblasts. Levels of VLCFAs were elevated in all tissues of X-ALD mice (Forss-Petter et al. 1997; Kobayashi et al. 1997; Lu et al. 1997), although the levels varied from tissue to tissue; and contrary to findings with humans with X-ALD, no detectable elevation of the C24:0 or C26:0 fatty acids were discovered in plasma (Lu et al. 1997). This result suggests that differences in the processing or metabolism of VLCFAs, perhaps specific to certain tissues, may exist between mice and human. In addition, these data could suggest that the increased quantity of VLCFAs present may not be the cause of the abnormalities observed in humans. Perhaps it is not the presence of

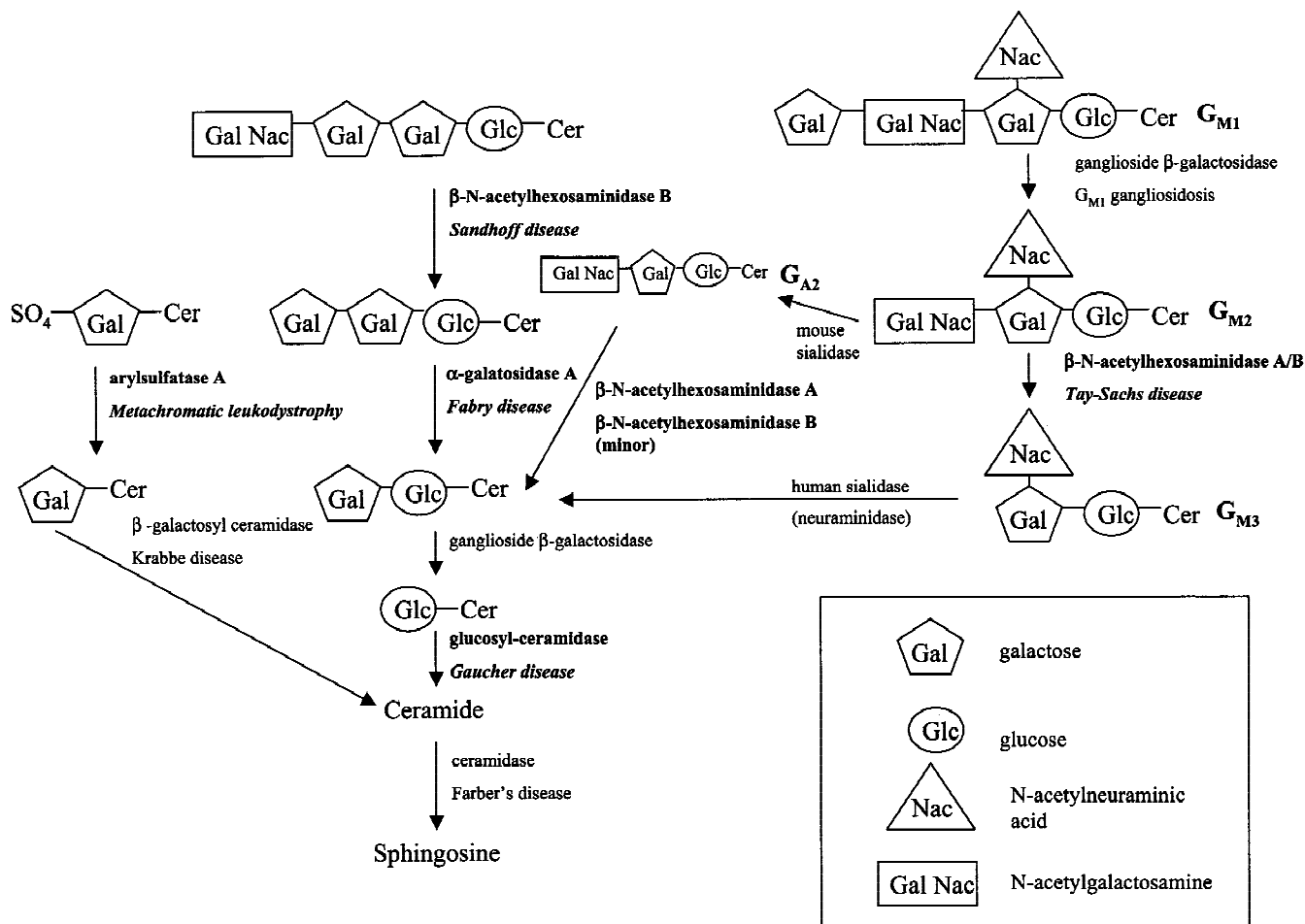
these VLCFAs in these tissues that is the problem; it instead may be the absence of either the VLCFAs or their by-products in other tissues that may be the most important.

Histologically, Lu and coworkers (1997) observed clear lipid clefts in the adrenocortical cells of X-ALD mice, although no abnormal findings were noted in the cerebral cortex, cerebellum, or brainstem. Perhaps most interestingly, they reported the absence of dysmyelination in the central nervous system of X-ALD mice, and Kobayashi and colleagues (1997) reported similar findings. Lack of dysmyelination most likely contributes to the absence of a clinical phenotype, although the reasons for this difference are still poorly understood (Forss-Petter et al. 1997; Kobayashi et al. 1997; Lu et al. 1997). It is clear that the role of ABCD1 in normal processing and transport of VLCFAs is crucial to our understanding of this disease.

### $\alpha$ -Galactosidase A Deficiency/Fabry Disease

$\alpha$ -Galactosidase A deficiency, or Fabry disease (OMIM \*301500), is an X-linked metabolic disorder that results from deficiency of the lysosomal hydrolase  $\alpha$ -galactosidase A ( $\alpha$ -gal A<sup>1</sup>), which leads to the accumulation of glycosphingolipids with a terminal  $\alpha$ -linked galactosyl moiety (Figure 3) in the tissues of affected males (Desnick et al. 2001). Significant storage occurs in the lysosomes of perithelial, endothelial, and smooth-muscle cells of blood vessels as well as the ganglion and most other tissues, including the heart and kidney (Desnick et al. 2001; Ohshima et al. 1997). Hemizygous males have no detectable  $\alpha$ -gal A activity and typically present with angiokeratoma, painful paresthesias, corneal dystrophy, renal impairment, gastrointestinal pain and diarrhea, as well as cardiac disease, leading to early mortality (Desnick et al. 2001).

Human *αgalA* (hereafter *GLA*<sup>1</sup>) cDNA was cloned (Bishop et al. 1988) and the genomic structure of the gene was determined (Bishop et al. 1988) and mapped to Xq22 (Desnick et al. 2001). In 1995, the highly similar genomic structure of the murine *Gla* was reported (Ohshima et al. 1995), and in 1997, a mouse model for Fabry disease was produced by disruption of the mouse *Gla* by homologous recombination in ES cells (Table 1) (Ohshima et al. 1997). All of the models demonstrate a lack of  $\alpha$ -gal A enzyme activity in tissues and fibroblasts, although the mice appeared clinically normal through 10 wk of age (Ohshima et al. 1997). Biochemically and pathophysiologically, the mutant mice resemble Fabry disease patients. Extensive lipid accumulation was demonstrated in the liver and kidney, and lamellar inclusions in the lysosomes of renal cells were observed by electron microscopy (Ohshima et al. 1997). A specific fluorescent label that binds to  $\alpha$ -D-galactosyl residues revealed intense fluorescence in the kidneys of *Gla* knockout mice compared with controls (Ohshima et al. 1997). The authors were able to correct the enzyme deficiency and clear accumulated  $\alpha$ -galactosyl storage through the transduction of human *Gla* cDNA retrovirus construct (Ohshima et al. 1997).



**Figure 3** Stepwise lysosomal degradation of glycosphingolipids. The lysosomal degradation pathway of glycosphingolipids is depicted with appropriate enzymes. Lysosomal storage diseases (associated with enzyme deficiencies at certain steps in the pathway) discussed in the text are italicized.

It remains to be determined exactly which metabolite causes toxicity in humans with Fabry disease. It is possible that human physiology reacts differently to significant lipid accumulation. It is also possible that these mice were evaluated too young or that subtle signs of disease, such as peripheral neuropathy, may be difficult to detect in the mutant mice. However, the *Gla*-deficient mice were a valuable pre-clinical model for enzyme replacement therapy and remain a promising model for gene therapy research.

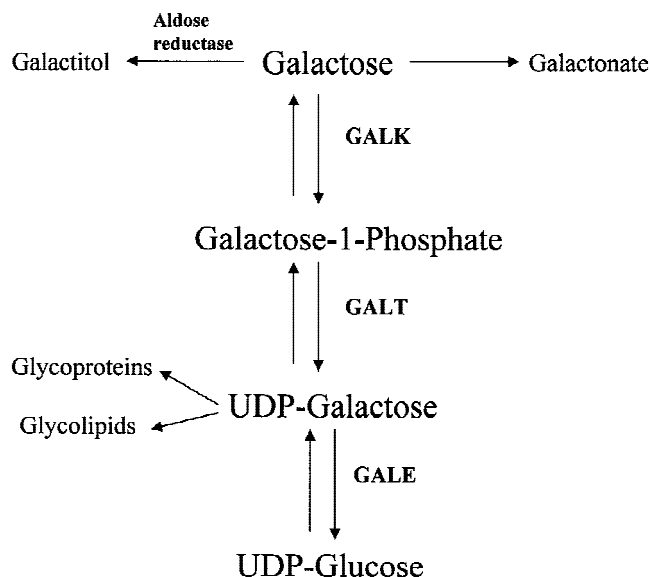
## Autosomal Disorders

### Galactosemia

Galactosemia (OMIM \*230400) typically results from enzymatic deficiency of any one of three enzymes involved in the metabolism of galactose (Figure 4), including galactokinase (GALK<sup>1</sup>), galactose-1-phosphate uridylyltransferase (GALT<sup>1</sup>), and uridine diphosphate galactose 4'-epimerase

(GALE<sup>1</sup>) (Holten et al. 2001). GALK deficiency (OMIM #230200) is limited to the formation of cataracts (associated with the presence of galactitol), although red cell GALE deficiency (OMIM \*230350) appears to be typically asymptomatic (generalized GALE deficiency has been reported only twice in the literature, is extremely rare, and results in a severe lethal phenotype very similar to untreated classical galactosemia) (Holten et al. 2001). Classical galactosemia typically results from GALT deficiency and presents in the first few days or weeks of life as failure to thrive with poor feeding, vomiting, diarrhea, jaundice, weight loss, lethargy, and often hypotonia. Congenital cataracts, liver dysfunction, renal tubular acidosis, and septicemia are often identified on clinical examination (Holten et al. 2001). Newborn screening for galactosemia is performed in many US states and in several European countries to identify individuals at risk for disease so that treatment can begin as early as possible to avoid the acute complications.

Treatment for galactosemia includes a diet with restricted galactose intake. However, there is some evidence



**Figure 4** Galactose metabolism. The activities of the galactokinase (GALK), galactose-1-phosphate uridylyltransferase (GALT), uridine diphosphate galactose 4'-epimerase (GALE), and aldose reductase enzymes are shown in a simplified diagram of galactose metabolism. GALT deficiency is the primary cause of galactosemia. Uridine diphosphogalactose (UDP-gal) can be converted through the activity of other enzymes to glycolipids and glycoproteins.

for endogenous production of galactose, so it appears that diet alone cannot control galactose levels in the individual (Holten et al. 2001). Lack of treatment for galactosemia can result in death of the newborn or severe developmental delay and neurological disease in those who survive. Early and consistent treatment, however, has been shown to result in variable outcomes, from apparently normal to severely affected persons (Holten et al. 2001). A significant percentage of galactosemics will develop neurological disease later in life, including ataxia, tremor, and poor coordination. Difficulty with articulation has been reported in more than 90% of persons with galactosemia, and virtually all females with galactosemia will have primary ovarian failure (Holten et al. 2001).

To study the pathogenesis of *GALT* deficiency and to understand the galactosemic phenotype further, mice deficient in Galt activity were created by gene-targeting methods (Table 1) that removed exons 6-8 of the murine *Galt* gene (Leslie et al. 1996). These mice have very high levels of galactose-1-phosphate (gal-1-p), galactitol, and galactose and are completely deficient in hepatic Galt activity. Biochemically, these mice are very similar to galactosemic humans due to *GALT* deficiency; however, these mice do not show any of the toxic signs associated with *GALT* deficiency in humans. Growth, reproduction, and overall viability are similar to wild-type mice. In recent studies, these mice showed no adverse effects when placed on a high galactose diet or when given injections of galactose (Ning et al. 2001).

One theory for the differences observed between humans and mice is that galactose transport across the blood brain barrier may differ between humans and mice, with decreased transport occurring in mice compared with humans (Ning et al. 2001). A second theory that may explain the absence of the toxic effects is that mice are purported to have a very low level of aldose reductase activity, which is required to form galactitol, one of the two compounds thought to cause the early toxicity observed in humans with galactosemia (the other compound being gal-1-p) (Leslie et al. 1996; Ning et al. 2000). These studies suggest that absence of Galt is necessary but not sufficient to cause disease and indicate that the pathophysiology of Galt deficiency and galactose metabolism are not yet well understood.

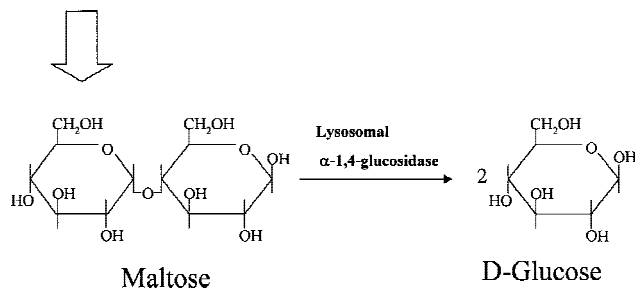
To investigate galactose metabolism further, mice deficient in galactokinase (*Glk1*) (Table 2) were also generated by gene targeting and found to have high levels of galactose and galactitol; however, these mice did not develop cataracts (even on a high galactose diet), as has been observed in the human condition (Ai et al. 2000). Further studies indicated that galactosemic cataracts could be induced but only when crossed to mice on a genetic background that overexpressed aldose reductase (Ai et al. 2000; Lee et al. 1995). Mice overexpressing aldose reductase develop cataracts when fed a high galactose diet (Lee et al. 1995). Perhaps there is an alternative galactose metabolic pathway, such as that involving aldose reductase, that is utilized or regulated differently between mouse and human, which could possibly be exploited to provide better therapy and better long-term outcomes in humans. These studies suggest that aldose reductase inhibitors have potential as therapeutic reagents for the treatment of galactosemia.

## Glycogen Storage Disease Type II

Glycogen storage disease type II (GSDII<sup>1</sup>; OMIM \*232300), also known as Pompe disease, is an autosomal recessive disorder that results from a deficiency of the lysosomal enzyme  $\alpha$ -1,4-glucosidase (encoded by *GAA*<sup>1</sup>; Figure 5), which leads to abnormal lysosomal accumulation of glycogen in the tissues of affected individuals (Hirshhorn and Reuser 2001). Confirmation of the diagnosis is made through the demonstration of absence or significant reduction of  $\alpha$ -glucosidase activity, although the clinical presentation of the disease can be variable. The most severe type of GSDII is the infantile-onset type, which is characterized by hypotonia, cardiomegaly, hepatomegaly, and fatal cardiorespiratory failure by approximately 2 yr of age (Hirshhorn and Reuser 2001). The adult onset form of GSDII is usually characterized by progressive myopathy, primarily in skeletal muscle and the diaphragm. Muscle weakness is usually found in other intermediate forms of GSDII, which may or may not have cardiac disruption (Hirshhorn and Reuser 2001).

To understand the pathological development of GSDII better, mouse models were created in 1998 by two separate

## Glycogen hydrolysis



**Figure 5** Acid  $\alpha$ -glucosidase activity. GSDII or Pompe disease is a disease of glycogen metabolism that results from a deficiency of lysosomal acid  $\alpha$ -glucosidase. This enzyme hydrolyzes  $\alpha$ -1,4- and  $\alpha$ -1,6-glycosidic linkages at an acid pH level. The natural substrate for acid  $\alpha$ -glucosidase is glycogen, although it is also active toward many other substrates, including maltose, as depicted in the figure.

groups (Bijvoet et al. 1998b; Raben et al. 1998) (Table 1). Raben and colleagues (1998) reported the disruption of the murine  $\alpha$ -glucosidase gene (*Gaa*<sup>1</sup>) through insertion of the neomycin phosphotransferase gene in exon 6 (*6<sup>neo</sup>/6<sup>neo</sup>*), as well as a second model with a deletion of exon 6 ( $\Delta 6/\Delta 6$ ). Homozygous knockout mice for both mutations did not produce normal mRNA or GAA protein and had very low levels of  $\alpha$ -glucosidase enzyme activity. Although the two models appeared to be biochemically similar, the phenotypes were somewhat different. The *6<sup>neo</sup>/6<sup>neo</sup>* mice accumulated glycogen in the skeletal muscle and diaphragm and developed progressive muscle weakness as early as 3.5 wk of age, which degenerated to muscle wasting by 8 to 9 mo (Raben et al. 1998). These mice were less mobile and were unable to grip onto a wire screen as long as heterozygous mice. Surprisingly, the  $\Delta 6/\Delta 6$  mutant mice also accumulated glycogen in skeletal muscle and heart, yet these mice did not have impaired movement or wire hanging ability (Raben et al. 1998). The  $\Delta 6/\Delta 6$  mice did not develop locomotor abnormalities until ~6.5 mo of age (Raben et al. 1998). The authors discuss the possibility that the genetic background of the mice might play a role in the phenotypic differences seen in the two mouse models.

Similarly, the human phenotype of GSDII can also be variable, and patients that bear the same mutation may not have the same clinical presentation, presumably due to differences in genetic background (Raben et al. 1998). This group further investigated the role of the genetic background by breeding additional murine *Gaa* mutant models and determined that  $\alpha$ -glucosidase deficiency on 129  $\times$  C57BL/6 background (the background of the *6<sup>neo</sup>/6<sup>neo</sup>* mice) resulted in progressive muscle wasting and cardiomyopathy common to human GSDII (Raben et al. 2000). In contrast,  $\alpha$ -1,4-glucosidase deficiency on 129  $\times$  C57BL/6  $\times$  FVB background (the background of the  $\Delta 6/\Delta 6$  mice) produced a milder phenotype and later age of onset for disease (Raben et al. 2000).

At the same time Raben and coworkers (1998) reported their initial findings, Bijvoet and colleagues (1998b) reported a disruption of murine *Gaa* exon 13 through a *neo* insertion; homozygous mutant mice did not produce *Gaa* mRNA or protein. The authors reported that these mutant mice appeared clinically normal at birth and remained virtually normal up to 9 mo of age, although several of the mice developed cardiomyopathy and an enlarged heart. Locomotor evaluations were not performed, so a true comparison with the knockout models described above is not possible. Glycogen storage was present in the lysosomes of the liver, heart, and skeletal muscle cells, similar to that demonstrated in the previous models (Bijvoet et al. 1998b). By 9 to 10 mo, *Gaa* (–/–) mice developed gait and postural abnormalities, and some of the affected mice lost weight, although several maintained normal weight. Fat cells were severely affected by glycogen storage (Bijvoet et al. 1998b). These authors also discussed the importance of genetic background and modulation of clinical phenotype (Bijvoet et al. 1998b).

The  $\alpha$ -glucosidase knockout mouse model mimics the infantile form of GSDII with regard to the genetic, biochemical, and pathological criteria; however, this model resembles the adult onset form with regard to clinical course, age of onset, and age of death. It is possible that these model systems will play an important role in determining the modifier genes that may influence the severity of disease in GSDII mice and perhaps also in humans with GSDII. In addition, these models provide a means to test potential therapies for treatment of GSDII, including enzyme replacement and gene therapy. Enzyme replacement has great potential and has, so far, met with at least some success in human and animal models (Bijvoet et al. 1998a, 1999; Kikuchi et al. 1998; Van den Hout et al. 2000). Initial studies performed in a variety of animal model systems showed that the human recombinant protein administered into the knockout mice (or other animal models) resulted in significant correction of the enzyme deficiency in virtually all tissues (with the exception of brain) and a decrease in lysosomal glycogen storage (Bijvoet et al. 1998a, 1999).

## Metachromatic Leukodystrophy

The autosomal recessive disorder metachromatic leukodystrophy (MLD<sup>1</sup>; OMIM \*250100) is a lysosomal storage disease in which the glycolipid cerebroside-3-sulfate (sulfatide) accumulates in the lysosomes of affected tissues due to a deficiency of the enzyme arylsulfatase A (Figure 3) (Gieselmann et al. 1994; von Figura et al. 2001). Because sulfatide is a major component of the myelin sheath, sphingolipids mainly accrue in the oligodendrocytes throughout the central and peripheral nervous system. Visceral organs also demonstrate characteristic metachromatic staining due to the presence of abnormal amounts of sulfated glycolipids, although organ function may not be impaired (von Figura et al. 2001). The clinical manifestation of MLD can

be variable, although patients typically display widespread demyelination and progressive degenerative neurological symptoms. The infantile form is usually fatal in early childhood (von Figura et al. 2001). The juvenile and adult forms of MLD are usually first recognized by gait abnormalities, followed by mental regression, blindness, speech difficulties, neuropathy, and seizures (von Figura et al. 2001).

The human arylsulfatase A gene (designated *ARSA*<sup>1</sup>) maps to 22q13.33, although the murine ortholog (*As2*) maps to mouse chromosome 15. In 1996, Hess and colleagues produced *As2*(*-/-*) mice through gene targeting (Table 1) and subsequently demonstrated by Northern analysis that *As2* mRNA was absent in *As2*(*-/-*) mice. *As2* activity was measured by a specific sulfatide loading assay and demonstrated that *As2*(*-/-*) mice did not convert sulfatide to galactosyl ceramide and ceramide, although (+/+) mice were able to turn over 72% of the sulfatide, and (+/-) mice converted 61% (Hess et al. 1996). Through behavioral, auditory, and neuromotor assessment of *As2* knockout and control mice, the authors determined that the (*-/-*) mice did not have a general motor defect, although the distance of hind limb paw prints was significantly smaller in (*-/-*) mice. The ability of knockout mice to stay on a rotarod was also significantly impaired (Hess et al. 1996). The Morris water maze was used to determine the spatial learning acuity of the mice, although the swimming ability of the mutant mice was deficient and confounded further analysis. The authors also noted from auditory brainstem-evoked potentials that the *As2*(*-/-*) were deaf, and within 2 yr, the mice developed a head tremor (Hess et al. 1996).

Despite the abnormal findings observed in the *As2* mutant mice, the phenotype of these mice is mild compared with human MLD patients. The devastating neurological effects seen in human patients with MLD are not present in the *As2* deficient mice, although various histopathological findings, such as astrogliosis and neuronal storage, are similar (Hess et al. 1996). Storage of sulfatide in the mouse brain was noted throughout the white matter in a pattern similar to that seen in human patients, but the widespread demyelination that occurs in humans was not present in the mutant mice. Myelin sheaths appeared normal but did have a significantly smaller cross-sectional area in the corpus collosum and optic nerve (Hess et al. 1996). In 8- to 11-month-old (*-/-*) mice, the neuronal damage in the inner ear was very severe; neurons and myelinated nerve fibers were reduced, presumably causing deafness (Hess et al. 1996). A recent study demonstrated that the distribution of sulfatide storage in the visceral organs of *As2*-deficient mice is similar to human MLD patients (Schott et al. 2001). Insights into the human form of MLD from these *As2* mutant mice may come from studying the role of neuronal and microglial pathology and astroglial disruption in the development of the disease and the possible relation of these factors to demyelination. However, there may be critical differences in substrate accumulation and distribution or an additionally disrupted pathway in humans that contributes to eventual demyelination.

## Tay-Sachs and Sandhoff Diseases (G<sub>M2</sub> Gangliosidoses)

Tay-Sachs disease (TS<sup>1</sup>) (OMIM \*272800) and Sandhoff disease (OMIM \*268800) are autosomal recessive lysosomal storage disorders that involve the stepwise hydrolysis of the ganglioside G<sub>M2</sub> through the action of the  $\beta$ -hexosaminidase enzyme (Hex<sup>1</sup>) (Figure 3), of which there are two isoenzymes, Hex A (heterodimer structure  $\alpha\beta$ ) and Hex B (homodimer structure  $\beta\beta$ ) (Gravel et al. 2001). A cofactor, the G<sub>M2</sub> activator, is also required for Hex A to hydrolyze the G<sub>M2</sub> and activator complex. TS results from mutations in *HEXA*, which encodes the  $\alpha$  subunit of the Hex A protein (Gravel et al. 2001). Sandhoff disease is caused by mutations in *HEXB*, which encodes the  $\beta$  subunit of Hex A and Hex B. Hex A or Hex B enzyme deficiency leads to gangliosidosis in both TS and Sandhoff disease and accumulated G<sub>M2</sub> storage material in neurons throughout the nervous system, causing profound neurological degeneration (Gravel et al. 2001). Mutations in the *GM2A* activator gene also can lead to disease, and although Sandhoff disease often has visceral organ involvement not typically seen in TS, the pathological changes in TS and Sandhoff disease as well as in activator deficiency are similar and are characterized by membranous cytoplasmic bodies. The phenotype of TS and Sandhoff disease can be variable; however, the most devastating and common is the early-onset, rapidly progressive neurological phenotype, which usually leads to early death. The typical clinical presentation is mental and motor deterioration leading to a completely unresponsive state (Gravel et al. 2001; Yamanaka et al. 1994). Milder, later-onset and chronic forms of TS and Sandhoff disease are also rarely seen and usually correlate with the amount of residual Hex A or Hex B activity (Gravel et al. 2001).

To understand the pathophysiology of TS and Sandhoff disease better, *Hexa* and *Hexb* knockout mice, respectively, were created. Surprisingly, the clinical presentation of the TS mice did not recapitulate that of the human disease, and the phenotypes of the TS and Sandhoff disease mice were very different. The *Hexa* (*-/-*) mice were created by gene targeting (Table 1) in murine ES cells, and the authors demonstrated through Hex A enzyme assay using an artificial substrate that the mutant mice retained <1% of normal Hex A activity (Yamanaka et al. 1994). They subsequently reported that the brains of the *Hexa* (*-/-*) mice progressively accumulated G<sub>M2</sub> ganglioside, and that the pathological features of TS were present in the neurons of the knockout mice, including the membranous cytoplasmic bodies (Yamanaka et al. 1994). However, unlike the human form of TS, in which the neuronal storage affects nearly all regions of the brain, the mutant mice displayed only localized damage (Yamanaka et al. 1994). Despite neuropathology similar to humans with TS, the *Hexa* (*-/-*) mice surprisingly had no clinical phenotype and exhibited normal balance, motor, and behavioral patterns (Yamanaka et al. 1994).

In 1995, Sango and colleagues reported that they also had created *Hexa* and *Hexb* knockouts through targeted dis-

ruption of the genes in ES cells. The *Hexa* ( $-/-$ ) mice were found to be clinically normal, and the pathological findings were similar to Yamanaka et al. (Sango et al. 1995). In contrast, the *Hexb* ( $-/-$ ) mice (Table 2) displayed progressive deterioration in motor function and gait abnormalities on a rotarod by 12 to 16 wk of age. By 5 mo of age, these mice were completely incapacitated, necessitating euthanasia (Sango et al. 1995). As seen in Sandhoff disease patients, extensive ganglioside storage was observed in nearly all neurons of the central nervous system in the *Hexb* mutant mice (Sango et al. 1995). In general, the *Hexb* mice more closely mirrored the human later-onset Sandhoff disease cases inasmuch as these patients often present with motor neuron dysfunction, ataxia, and muscle wasting although the mutant mice have more extensive cortical pathology (Sango et al. 1995). The authors also performed thin-layer chromatography in an effort to resolve the types of gangliosides that were present in the tissues of the knockout mice. In *Hexb* ( $-/-$ ) mice,  $G_{M2}$  and  $G_{A2}$  were greatly elevated compared with control animals. In *Hexa* ( $-/-$ ) mice, only  $G_{M2}$  was present, but at approximately one fourth the amount found in the *Hexb* ( $-/-$ ) mice (Sango et al. 1995). Further analysis of the ganglioside degradation pathway in mice led the authors to conclude that in mice, there are two independent pathways for the degradation of  $G_{M2}$ , whereas in humans,  $G_{M2}$  is primarily converted by Hex A to  $G_{M3}$  (Sango et al. 1995). Murine sialidase is able to convert  $G_{M2}$  to  $G_{A2}$  (for further degradation) (Figure 3); and in *Hexa* mutant mice, this pathway is still available and may alleviate excessive ganglioside storage (Sango et al. 1995). It is suggested that Hex B might participate in the breakdown of  $G_{A2}$  inasmuch as this pathway is blocked in the *Hexb* mutant mice, leading to greater ganglioside storage (Sango et al. 1995). A further study published on the phenotype of TS and Sandhoff disease mice reported similar conclusions (Phaneuf et al. 1996).

Knockout mice for the  $G_{M2}$  activator protein (Liu et al. 1997) and double knockout mice for *Hexa* and *Hexb* (Table 2) have also been made (Sango et al. 1996). The *Gm2a* ( $-/-$ ) mice had a restricted pattern of neuronal ganglioside storage similar to that of the TS mice but also demonstrated significant accumulation of ganglioside in the cerebellum (Liu et al. 1997). These mice also had balance and coordination abnormalities (Liu et al. 1997). The authors were able to demonstrate that the activator protein is essential for  $G_{M2}$  degradation by Hex A (Liu et al. 1997). The *Hexa* and *Hexb* double knockout mice (Table 2) have a more severe phenotype than those with the individual gene deficiencies, including pronounced physical dysmorphism and mucopolysaccharidosis (Sango et al. 1996). The authors conclude that the mucopolysaccharidosis is most likely due to the secondary activity of hexosaminidase in the degradation of glycosaminoglycans (Sango et al. 1996).

The TS and Sandhoff disease mouse models have contributed greatly. They have elucidated the pathway for ganglioside degradation in mice as well as in humans, and they

have emphasized the role of hexosaminidases in multiple pathways.

## Discussion

### Alternate Avenues into the Mousetrap

This review of metabolic models is not intended to be comprehensive. Many other interesting mouse models have been created that also do not mirror all the features of the human disease. One example is cystic fibrosis (CF<sup>1</sup>), in which numerous mouse models have been created that produce complete cystic fibrosis transmembrane conductance regulator (*Cftr*<sup>1</sup>) null alleles (Colledge et al. 1992; Hasty et al. 1995; O'Neal et al. 1993; Ratcliff et al. 1993; Rozmahel et al. 1996; Snouwaert et al. 1992). Although these various models were created using similar technology, and each of the models has abnormalities in cAMP-mediated chloride ion transport, none of the models completely replicate the human disease. Pulmonary complications are the major cause of morbidity and mortality in humans with CF; however, these complications were not noted to occur spontaneously in any CF mouse model (Dickenson et al. 1995). Pancreatic pathology was also not observed in any of the transgenic models. Nevertheless, some CFTR knockouts exhibited variable, minor abnormalities, including some pancreatic duct obstructions, acinar atrophy, and mild dilation of pancreatic ducts (Grubb and Boucher 1999). However, compared with humans, the level of *Cftr* gene expression is very low in the mouse pancreas (Manson et al. 1997). Other CF mouse models have also been created that contain specific targeted mutations, primarily  $\Delta F508$  (Colledge et al. 1995; van Doorninck et al. 1995; Zeiher et al. 1995). A more detailed review of the pathogenesis of various mouse models for cystic fibrosis is available in the literature (Grubb and Boucher 1999).

It is also intriguing that some mouse models demonstrate a more severe phenotype than most human patients. One example is Gaucher disease (Figure 3), in which the glucocerebrosidase knockout mouse most resembles the severe type II infantile onset Gaucher subtype rather than the more variable type I or type III Gaucher (Suzuki and Proia 1998; Tybulewicz et al. 1992). Methionine synthase deficiency, which causes homocysteinemia, homocysteinuria, and hypomethioninemia in humans, confers embryonic lethality in homozygous knockout mice (Swanson et al. 2001). Erythropoietic protoporphyria, in which a mouse model was identified following mutagenesis with ethylnitrosourea, displays photosensitivity similar to humans with this condition yet exhibits a more severe anemia and hepatic dysfunction than observed in human cases (de Verneuil et al. 1995). These more severe phenotypes often reflect the fact that many induced mouse mutant alleles are null, whereas human mutations are often point mutations. Nevertheless, these models provide insight into the variability often seen across the human spectrum of disease and force

another look into the interacting biochemical pathways and the effects of genetic background, type(s) of mutations, and genetic modifiers on the pathogenesis of the disease.

## Getting out of the Mousetrap

In attempting to examine some of the interesting physiological questions that arise in the study of mouse models for human genetic disease that do not replicate the human phenotype, it becomes obvious that we do not fully understand the pathophysiology of many metabolic diseases and that these models may provide clues to alternate pathways, possible interacting proteins, modifier genes, and synergistic effects that contribute to produce disease. Determining the toxic effects of certain metabolites on the developing embryo and infant can be difficult, as in the examples of the Fabry disease and X-ALD mice, in which a significant accumulation of storage products were noted yet there was no significant phenotypic effect. The lack of a clinical phenotype in the X-ALD mice may be related to the lack of dysmyelination, which may also be the case for the MLD mice. It will be a challenge to understand fully the relation between the obvious biochemical disruption and secondary effects, thus further complicating the one gene, one enzyme concept of inherited metabolic disorders.

As demonstrated in the Fabry disease knockout mice, animal models with biochemical abnormalities but no or few clinical features can also be used to develop new treatments for disease—whether with drugs, gene-replacement (gene-transfer), or enhancing/exploiting any possible alternative pathways present in the mouse that also may be present (but underutilized) in humans. In addition, they contribute to our understanding of biology with respect to the similarities and differences between species and various organ systems, and they provide models for testing therapeutics. Furthermore, animal models can save time and money in the development or identification of treatments for disease and can also be useful for identification of variants and other factors in disease (i.e., environmental modifiers of severity). These models provide unique opportunities to stress the biochemical pathways to elicit responses that may further delineate the metabolic pathways in both humans and mice and identify various interacting pathways. Genetic background analysis and breeding onto different strains may uncover other modifier genes that may facilitate understanding and treatment of disease.

In the mouse models addressed in this review, simply recreating a metabolic disruption did not lead to a clinical phenotype. Often, additional genetic constructs or environmental stresses were required to discern a very complicated biochemical pathway. Furthermore, many of the mouse models were not assessed in a complete manner with regard to phenotypic analysis, particularly with respect to neurological phenotypes. Although many of these animals expressed the biochemical phenotype, the clinical phenotype was either not apparent or was not thoroughly evaluated.

This issue may be due in part to the lack of expertise or lack of available behavioral testing facilities where the mouse models were created and in part to the fact that we cannot always correlate human behavior with mouse behavior. The real challenge that lies ahead is how to analyze these mutant mouse models effectively, especially with regard to the neurological phenotype. Although there are many excellent mouse behavioral assays, we may need to think outside the box to identify or develop new techniques for analyzing behavior that encompass not only locomotor tasks but also learning and memory, with and without requiring locomotor activity, so that we can effectively separate the locomotor defects from the behavioral and other neurological abnormalities. Mice can be very useful models not only in reproducing a human condition but also in challenging investigators' true understanding of the pathogenesis of an otherwise "simple" single-gene disease.

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