J. Biochem. 124, 8-19 (1998)

Induced Mouse Models of Abnormal Sphingolipid Metabolism[1]

Kunihiko Suzuki,*,† Marie T. Vanier,‡ and Kinuko Suzuki*,‡,2

**Neuroscience Center, †Departments of Neurology and Psychiatry, and ‡Department of Pathology & Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA; and ‡INSERM-CNRS U189, Lyon-Sud School of Medicine and Fondation Gillet-Mérieux, Lyon-Sud Hospital, F-69921 Oullins Cedex, France*

Received for publication, March 16, 1998

Only a limited number of genetic mouse models of abnormal sphingolipid metabolism are known to occur spontaneously. However, recent progress in the combined homologous recombination and embryonic stem cell technology allows inactivation of any genes of choice once they are cloned. Not only is it possible to generate mutant mouse lines that are equivalent to known human genetic disorders but genetic conditions unknown or highly unlikely to occur in humans, such as simultaneous inactivation of more than one gene, can also be created. Most of the human disorders due to genetic defects in sphingolipid catabolism have been duplicated in the mouse. With increasing activity in cloning of the enzymes involved in sphingolipid biosynthesis, genetic mouse models of abnormal sphingolipid biosynthesis are beginning to appear. These models have already provided invaluable insight into the metabolism and physiological functions of sphingolipids and are expected to be utilized extensively for evaluation of the pathogenesis and of treatment approaches of these genetic disorders.

Key words: brain development and function, gene targeting, mouse model, sphingolipid, sphingolipidosis.

Sphingolipids comprise a group of complex lipids that are characteristic integral amphiphilic constituents of the plasma membrane in vertebrates (*1-3*). Their composition varies in different cell types and in different developmental stages. The pattern can be further altered by viral transformation and oncogenesis (*4, 5*). The biochemical pathways of biosynthesis and degradation of sphingolipids have been largely clarified but the cell biological mechanism by which they are transported from sites of synthesis to other membranes and from the membranes to the lysosome, the site of their degradation, is the current subject of intensive investigation. It is generally assumed (*6, 7*), as is the case in glycoproteins, that routing of glycosphingolipids is coupled with a vesicular membrane flow, from the endoplasmic reticulum (ER) through the cisternae of the Golgi complex and finally to the plasma membrane (*8*). There is also a general agreement that a portion of the plasma membrane destined for degradation is endocytosed and carried by endosomes to the lysosome, where the constituent glycosphingolipids are degraded. However, several glycosphingolipid-binding or transfer proteins have been described in the literature and their involvement in the intracellular trafficking of glycosphingolipids cannot yet be excluded (*9, 10*). Inherited deficiencies of protein components essential for degradation of glycosphingolipids cause serious metabolic disorders.

Despite the large body of circumstantial evidence and speculations, the importance of sphingolipids in the physiological functions of biological membranes remains to be more clearly defined. To this goal, genetic animal models, particularly in small laboratory animals, in which sphingolipid metabolism is abnormal in specifically defined manner would be highly useful. Genetic lysosomal diseases have been known to occur spontaneously in many mammalian species (*11*). However, most of them are among larger, domestic or farm animals. Larger animals offer their own advantages but for many experiments, models in smaller laboratory animals, such as mice or rats, would be desirable. The twitcher mutant (galactosylceramidase deficiency globoid cell leukodystrophy, Krabbe disease) is the only known spontaneous mouse model of well-delineated genetic sphingolipidoses. It has been well characterized and used extensively for studies of Krabbe disease (*12*).

The state of the available small animal models changed dramatically in recent years with the advent of the combined homologous recombination and embryonic stem cell technology, which now allows induction of mutant mouse lines of any genes as long as the genes are cloned and the basic information about their genomic structures available (*13*). This development coincided with the cloning activity of the genes responsible for sphingolipid synthesis and degradation. Almost all of the lysosomal sphingolipidoses known in humans have already been duplicated in the

[1] Work from the authors' laboratories was supported in part by research grants, RO1-NS24289, RO1-NS28997 and a Mental Retardation Research Center Core Grant, P30-HD03110, from the USPHS to Ku. S, RO1-NS24453 to Ki. S. and a research grant from the ELA to M.T.V.

[2] To whom correspondence should be addressed. Neuroscience Center, CB#7250, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7250, USA, Tel: +1-919-966-2405, Fax: +1-919-966-1322, E-Mail: kuni@css.unc.edu

mouse. In addition, induced mouse mutants involving sphingolipid synthesis are beginning to provide insight into biochemistry and physiological functions of sphingolipids.

Induced mouse mutants of abnormal sphingolipid metabolism

As indicated above, the only known spontaneous mouse model for human sphingolipidoses is the twitcher mutant. However, genetically equivalent models of nearly all of the known human sphingolipidoses have been artificially induced in the mouse using the homologous recombination and embryonic stem cell technology. Not only duplicating existing human diseases, mouse lines with genetic conditions that are not known or highly unlikely to exist in humans, such as double mutations of related genes, have also been generated. Furthermore, mouse mutants that are abnormal in sphingolipid biosynthesis are beginning to be generated. Such genetic conditions are not known in humans. Such an exploitation of the mouse genetics allows analyses of the physiological functions of tissue sphingolipids that could not have been imagined just a short while ago.

Mutants of abnormal sphingolipid degradation

All genetic sphingolipidoses known in humans are inherited as autosomal recessive trait except for Fabry disease which is an X-linked disorder.

Gaucher disease (glucosylceramidase deficiency). Gaucher disease in humans is caused by deficient activity of glucosylceramidase (glucocerebrosidase), resulting in massive accumulation of glucosylceramide in the reticuloendothelial system. These storage cells ("Gaucher cells"), and hepatosplenomegaly are the most conspicuous features in human patients. According to the traditional classification, type I is a non-neuronopathic form affecting mostly adults, type II is a severe, primarily infantile neuronopathic form, and type III is phenotypically intermediate. In addition, a rapidly progressive fulminating phenotype in neonates, a subtype of type II Gaucher disease, was recognized recently.

Tybulewicz and co-workers (*14*) generated a mouse model through gene targeting. The mouse glucosylceramidase gene was disrupted by the neomycine-resistance (Neo) gene that replaced the entire intron 9 and the flanking segments of exons 9 and 10, which encode a part of the active site of the enzyme. The glucosylceramidase activity in homozygous mutant mice was less than 4% of control. Increased amounts of glucosylceramide were demonstrated in the liver, lung, brain, and bone marrow. The affected mice, however, were akinetic with irregular respiration and poor feeding and died within 24 h of birth with rapidly progressive cyanosis. The typical Gaucher cells were not found in any of the tissues that showed increased glucosylceramide. The stored lipid, which had the same morphological appearance as the inclusions found in cells of Gaucher patients, was demonstrated by the electron microscope in the macrophages in the liver, spleen, and bone marrow. However, the extent of glucosylceramide accumulation in these organs did not appear to be sufficient to explain the death within one day of birth. In the CNS, no Gaucher cells were observed although tubular Gaucher-type inclusions were present in microglia, neurons of the red nucleus and of vestibular nucleus, and in sensory and motor neurons (*15*). These mice did not show hepatosplenomegaly. The most significant pathology was in the skin, which showed abnormally prominent rugation and hyperkeratosis. These features were nearly identical to those seen in patients with the severe neonatal subtype of type II Gaucher disease ("collodion" babies, or "Gaucher infants having congenital ichthyosis" or "cellophane like" skin) (*16*). Hydrolysis of glucosylceramide and consequent accumulation of ceramide normally occurs during the maturational process in the skin structure to establish a permeability barrier. The absence of glucosylceramidase may reduce normal production of ceramide and thus affect functional skin integrity. Thus, the glucosylceramidase deficiency appears directly responsible for the abnormal skin of this subtype of Gaucher infants and the mutant mice. Histologically, the thickened overlying keratin layer was found in the skin of both the homozygous mutant mice and neonatal Gaucher patients. Furthermore, these mice demonstrated markedly elevated transepidermal water loss as well as altered permeability barrier function as shown by an electron-dense tracer study (*17*). Such changes were not detected in the skin of type 1 or III Gaucher patients (*18*). Thus, the complete inactivation of the glucosylceramidase gene results in a mouse model that phenotypically resembles the rare and the most severe neonatal subtype of type II Gaucher disease (*16*).

The mouse models of point mutations have been generated very recently to duplicate the molecular lesions in human Gaucher disease types 2 and 3 (*19*). These authors used the single insertion mutagenesis technology to introduce point mutations into the glucosylceramidase gene, L444P (type 3), and a combined L444P and A456P (type 2, RecNicI mutation). The mouse with the double mutation, L444P and A456P, gave little enzyme activity and an accumulation of glucosylceramide in the liver and brain. The mouse with only L444P gave higher residual glucosylceramidase activity and no accumulation of the substrate in the liver or brain. However, in contrast to the human patients with these genotypes of Gaucher disease, both mice with the point mutations died within 48 h of birth apparently due to an abnormal epidermal permeability barrier as in the mice with complete gene inactivation. This conspicuous difference between the mouse and human diseases may well be due to the fact that mice are born at a much earlier stage of development than human neonates.

GM2-gangliosidosis

GM2-gangliosidoses are caused by deficient activity of β-hexosaminidase or GM2-activator protein. Three distinct lysosomal proteins, the β-hexosaminidase α and β-subunits and the GM2-activator protein are required in normal degradation of GM2-ganglioside *in vivo*. The two subunits of β-hexosaminidase form three forms of dimers, hexosaminidase A ($\alpha\beta$), hexosaminidase B ($\beta\beta$) and hexosaminidase S ($\alpha\alpha$). Thus, abnormalities in these three genes cause three genetically distinct forms of GM2-gangliosidoses; Tay-Sachs disease (β-hexosaminidase A and S deficiency), Sandhoff disease (β-hexosaminidase A and B deficiency), and AB variant (GM2-activator deficiency). In all forms, a massive accumulation of GM2-ganglioside occurs in neuronal lysosomes. These glycolipids are stored as multilamellar structures known as membranous cytoplasmic bodies (MCBs) (*20, 21*). In Sandhoff disease, the

additional absence of β-hexosaminidase B results in accumulation of other substrates, such as GA2 (asialo-GM2-ganglioside), globoside, oligosaccharides, and glycosaminoglycans, in the visceral organs. Since GM2-activator protein is required for *in vivo* degradation of GM2-gangioside by β-hexosaminidase A, its genetic abnormalities result in a phenotype similar to Tay-Sachs disease. The infantile forms of all GM2-gangliosidoses are rapidly progressive, resulting in death in early childhood. The late onset forms that usually retain low but detectable level of enzyme activity, progress more slowly with a wide spectrum of clinical phenotypes (*22, 23*).

Through targeted disruption of the *Hexa, Hexb*, and *Gm2a* genes (corresponding to human *HEXA, HEXB*, and *GM2A* genes) by homologous recombination, the three genetic forms of human GM2-gangliosidoses have been duplicated in the mouse (*24-29*).

Tay-Sachs disease (β-hexosaminidase α subunit deficiency). Mouse models of β-hexosaminidase α subunit deficiency have been generated independently in three laboratories. Affected mice with the *Hexa* null mutation are profoundly deficient with β-hexosaminidase A activity with an accumulation of GM2-ganglioside in the brain. Storage neurons contain MCBs. No histological abnormalities were noted in the liver, spleen and other visceral organs, although modest lysosomal storage was reported in the hepatocytes by one group (*27*). In these respects, *Hexa*−/− mice are similar biochemically and pathologically to Tay-Sachs disease in humans. However, there are many features in the mouse model that are distinctly different from the human disease. First of all, these *Hexa*−/− mice are clinically normal, fertile and have a normal life span. The accumulation of GM2-ganglioside is progressive but its degree is much milder than that seen in the brain of human patients with Tay-Sachs disease, reaching only 15% of total brain ganglioside at 6 months (*24*). The localization of storage neurons is limited to certain regions in the brain such as cerebral cortex, amygdala, piriform cortex, hippocampus, hypothalamus, in contrast to the infantile human disease in which GM2-ganglioside accumulation is widespread in all neurons. Notably absent is storage in the Purkinje and granular cells in the cerebellar folia and in neurons in the spinal cord. The storage materials stain positively with antibody against GM2-ganglioside (*26*). Identical distribution of the storage neurons and GM2-ganglioside immunoreactivity were reported in the *Hexa*−/− mice generated by Phaneuf *et al.* (*29*). However, in the mice described by Cohen-Tannoudji and co-workers, no storage was noted in the hippocampus at 150 days (*27*). The mild accumulation of GM2-ganglioside, the limited neuronal storage and the apparently normal clinical phenotype can be explained by a difference in the GM2-ganglioside degradation pathway between humans and mouse. In humans GM2-ganglioside cannot be degraded to GA2 by sialidase, while the mouse sialidase is active toward GM2-ganglioside. Since GA2 can be further degraded by β-hexosaminidase B, which is unaffected in the *Hexa*−/− mice, there is an effective bypass for degradation of GM2-ganglioside in the mice (see next section for further clarification).

Sandhoff disease (β-hexosaminidase β subunit deficiency). The *Hexb*−/− mice were generated by disruption of exon 13 of the *Hexb* gene with the neomycin-resistant gene (*25*). Liver extracts from *Hexb*−/− mice were deficient in both β-hexosaminidase A and B activity. The *Hexb*−/− mice appear normal at birth. However, progressive worsening of motor coordination and balance can be detected by the rotorod test starting around 12 weeks of age. Generalized locomotion in a Digiscan open field and passive avoidance learning are not significantly different from wild type controls at this age. The first overt signs of severe motor dysfunction begin also at around 3 months. Gait abnormalities with spastic movements start from hind limbs and progressing to forelimbs. By 4 to 4.5 months, muscles over the hind limbs become atrophic, and the mice are no longer able to effectively take food or water. Both male and female *Hexb*−/− mice are fertile, however. Thus, unlike Tay-Sachs and Sandhoff diseases in humans, where the clinical course is nearly identical, the corresponding mouse models, *Hexa*−/− and *Hexb*−/− show quite different clinical phenotypes. Extensive neuronal storage is present throughout the cerebrum, cerebellum, brainstem, spinal cord, trigeminal, and dorsal root ganglia, retina, and myenteric plexus. These storage materials are best demonstrated in frozen sections with the periodic acid Schiff (PAS) stain. Storage materials in small RCA-1-positive cells (macrophage/microglia), but not those in neurons, stain positive with Alcian blue and colloidal iron consistent with their being glycosaminoglycans. Similarly PAS and Alcian blue/colloidal iron positive cells are noted in the hepatic sinusoids. These histological features are identical to those in human Sandhoff disease. The cerebral white matter is diffusely hypomyelinated compared with the *Hexa*−/− or wild type mice. Clinical as well as pathologic phenotypes of *Hexb*−/− generated by Phaneuf and co-workers (*29*) are essentially identical to those generated by Sango and co-workers (*25*). In the brain of *Hexb*−/− mice, a massive and progressive accumulation of both GM2-ganglioside and GA2 is readily demonstrated from early stages of development. Thus, *Hexb*−/− mice are very similar to human Sandhoff disease in its clinical, pathological, and biochemical phenotype. As already indicated, the presence of the alternate pathway for GM2-ganglioside degradation in mice can explain the differences. In humans, GM2-ganglioside is degraded almost exclusively by β-hexosaminidase A and GM2 activator protein to GM3. Mice have a bypass through which GM2-ganglioside is converted to GA2 by the action of sialidase. In both human Tay-Sachs patients and *Hexa*−/− mice, the conversion of GM2 to GM3 is blocked by the absence of β-hexosaminidase A. However, in mice the alternate degradative pathway, the conversion of GM2 to GA2 by sialidase, is available. In the *Hexa*−/− mice, GA2 can then be degraded by the genetically intact β-hexosaminidase B, explaining their limited GM2-ganglioside storage and the lack of neurological symptoms. In the *Hexb*−/− mice, however, both GM2 and GA2 cannot be further degraded leading to extensive neuronal storage of both GA2 and GM2-ganglioside and a severe clinical phenotype similar to infantile GM2-gangliosidosis in humans. An analogous bypass pathway through sialidase also exists in the mouse for degradation of GM1-ganglioside (see below).

GM2-gangliosidosis AB variant (GM2 activator protein deficiency). The *Gm2a* gene was inactivated by a deletion of a portion of exon 3 and all of the coding region of exon 4 (*28*). *Gm2a*−/− mice grow normally and are

fertile. Although displaying a normal life span, they exhibit subtle neurological dysfunction. When tested beginning at 13 and ending at 33 weeks, overall rotorod performance was significantly impaired. Passive-avoidance task as a test for learning and memory suggested possible memory deficit. Similar to human patients with GM2-activator deficiency, the *Gm2a−/−* mice had normal levels of β-hexosaminidase activity in liver extracts when tested with artificial substrates. Analysis at 4 months demonstrated an accumulation of GM2-ganglioside in the brain of *Gm2a−/−* mice at a level comparable to the *Hexa−/−* mice but less than the *Hexb−/−* mice. In *Hexb−/−* mice, both GM2-ganglioside and GA2 accumulate in the brain, while only GM2-ganglioside accumulate in the brain of *Hexa−/−* mice at about 1/4 level of that in the *Hexb−/−* mice. In *Gm2a−/−* mice, in addition to GM2-ganglioside, a slight accumulation of GA2 is detectable. The distribution of storage neurons are generally very similar to that of *Hexa−/−* with the exception of the cerebellum where storage materials are present in the Purkinje and granular cells and also in some cells in the molecular layer. Very little storage is present in these cells in the cerebellum of *Hexa−/−* mice even at age 18 months. The significant storage in the cerebellum in *Gm2a−/−*, but not in *Hexa−/−* mice, can explain the impaired motor coordination in the former. The minimal GA2 storage in the *Gm2a−/−* mice indicates that the hexosaminidase-mediated degradation of GA2 can proceed at least to some extent, if not optimally, in the absence of the activator protein.

β-Hexosaminidase α and β subunit double deficiency. Mice in which both *Hexa* and *Hexb* genes are inactivated have been produced by interbreeding double heterozygotes (*Hexa+/−* *Hexb+/−*) or by mating *Hexa−/−* and *Hexb+/−* mice (*30*). The "double knockout" (*Hexa−/−Hexb−/−*) mice are totally deficient in all forms of lysosomal β-hexosaminidase including β-hexosaminidase S present in the *Hexb−/−* mice. Total deficiency of lysosomal β-hexosaminidase is not known in humans. Unexpectedly, these mice show clinical, pathological, and biochemical features of a severe mucopolysaccharidosis. Affected mice are indistinguishable from their littermates at birth but they are clearly smaller and dysmorphic by 4-5 weeks. Their heads are shorter and the snouts broader. Their feet are thick and broad with flexion contracture of the digits. Corneal opacity is present. Occasional seizure-like activities and unresponsiveness to sudden loud noise suggestive of deafness are observed. A radiographic study showed kyphosis, an abnormally shaped rib cage and short and thickened long bones. Their life span was only 1 to 4 months. There is a massive accumulation of GM2-ganglioside and GA2 to a degree comparable to the level of *Hexb−/−* mouse brain. They excrete into urine large amounts of glucosaminoglycans consisting of *N*-acetylgalactosamine and iduronic acid consistent with dermatan sulfate. However, the activity of enzymes involved in the classical mucopolysaccharidoses, such as α-*N*-acetylglucosaminidase, α-L-iduronidase, β-glucuronidase, and β-galactosidase were higher than wild type control mice.

Pathology is similar to other mucopolysaccharidoses (*31*). In the central nervous system, extensive neuronal storage, similar to that seen in *Hexb−/−* mice is present throughout. In addition, PAS, Alcian blue and colloidal iron-positive materials, consistent with glycosaminoglycans are stored in macrophages, chondrocytes, splenic sinusoidal cells, and in some neurons. Similar storage macrophages are also present in the bone marrow, skin, adipose, and connective tissues, the myocardium, heart valves, lung, liver, spleen, and kidney. These cells stain strongly positive with RCA-1. The white matter is diffusely hypomyelinated with increased numbers of GFAP-positive astrocytes and RCA-1-positive microglia/macrophages. At the terminal stage, nerve fiber tracts in the white matter contain many axonal spheroids and scattered myelin figures suggestive of myelin degeneration. In the PNS, neuronal storage is seen in the trigeminal and dorsal root ganglia, and in neurons in the myenteric plexus and visceral autonomic ganglia. Macrophages similar to those found in the brain are also very conspicuous in the peripheral nerves, although nerve fibers appear well preserved. These morphological features together with biochemical data indicate that β-hexosaminidase is a crucial enzyme in some steps of degradation of some glycosaminoglycans. Although a few colloidal iron-containing cells are found in the brain of *Hexb−/−* mice, lack of significant glycosaminoglycan storage in the mice with single gene defect in human patients with Tay-Sachs and Sandhoff disease suggest possible functional redundancy in the β-hexosaminidase system. These completely hexosaminidase-deficient mice are not a model of a known human disease but are a valuable tool for studying the role of the β-hexosaminidase in the degradation of glycosaminoglycans and in understanding the pathological consequences of excessive accumulation of glycosaminoglycans.

Metachromatic leukodystrophy (arylsulfatase A deficiency). Metachromatic leukodystrophy (MLD) is caused by deficiency of arylsulfatase A (ASA) (*32*). Deficiency of ASA results in an accumulation of the substrate, sulfatide, in various organs including the brain. Sulfatide is a major myelin lipid and perturbed sulfatide metabolism causes diffuse demyelination of the CNS white matter and peripheral nerves. Late infantile, juvenile, and adult types are known clinically. The late infantile MLD is the most common type with rapidly progressive neurological symptoms such as psychomotor deterioration with blindness, seizures, quadriparesis, and peripheral neuropathy, usually manifesting in the second year of life. Patients with this type usually die within a few years. Juvenile and adult types are more slowly progressive and neurological symptoms appear between age 4 and 12 years or between mid-teens and the seventh decade, respectively. Clinical manifestations of the late-onset forms are widely variable. Behavioral disturbance, dementia or progressive peripheral neuropathy are often the major presenting signs. More than 30 disease-causing mutations have been identified in human patients (*33*). The pathology of MLD in the nervous system is primarily a diffuse demyelination in association with deposits of abnormal storage materials in the brain, kidney, gallbladder, liver, pancreas, and various other visceral organs. The storage materials stain brown with acidic cresyl violet in frozen sections (metachromasia). In the brain, metachromatic materials accumulate primarily in oligodendrocytes, although neurons in certain regions also contain metachromatic materials (*34*).

The mouse model of MLD was generated by targeted disruption of the arylsulfatase A gene (*35*). ASA mRNA is

undetectable in the *ASA−/−* mice. No turnover of sulfatide can be detected in cultured fibroblasts in a sulfatide loading test. Thus, biochemically the *ASA−/−* mice resemble the severe late infantile form of human MLD. However, *ASA−/−* mice show a much milder clinical phenotype. Affected mice develop normally for several months without overt clinical signs. At 12-14 months of age, statistically significant but subtle abnormalities can be detected in the neuromuscular function and behavior when they are evaluated by the walking pattern, rotorod test, and the Morris water maze. The most notable neurological sign in *ASA−/−* mice is total absence of auditory brainstem evoked potentials. In the second year of life, *ASA−/−* mice develop low frequency tremors of the head. However, the storage is not as widespread as that seen in human patients and there is no evidence of storage in hepatocytes, adrenal glands, and skeletal muscle. In the brain of 11 month old *ASA−/−* mice, storage of metachromatic material (sulfatide) was noted in the white matter (corpus callosum, hippocampal fimbria, internal capsule, and optic nerve) but demyelination is not apparent. Sulfatide storage is also observed histologically in the kidney, gall bladder, and bile duct at 6-11 months. Ultrastructurally, lamellar deposits, herringbone, and tuffstone like materials, similar to those found in human MLD, can be demonstrated in astrocytes, oligodendrocytes, microglia, and Schwann cells. The myelin sheath has a normal ultrastructure. However, there is a statistically significant reduction of the cross-sectional area of myelinated axons in the corpus callosum and optic nerve. Astrogliosis is present at 1 year and activation of microglia become conspicuous with increasing age. Dramatic pathology is seen in the inner ear of *ASA−/−* mice. The number of acoustic ganglion cells and myelinated nerve fibers are greatly reduced. The Schwann cells in the acoustic and vestibular ganglia show marked sulfatide storage. Storage is also evident in the Schwann cells in the peripheral nerve but demyelination is not apparent. Thus, basic pattern of pathological process in *ASA−/−* mice is similar to human MLD but the process is slower and milder.

Fabry disease (α-galactosidase A deficiency). Fabry disease is the only X-linked disorder among human sphingolipidoses and is caused by a deficiency of α-galactosidase A (α-Gal A) (*36*). Neutral glycosphingolipids with a terminal α-linked galactose—predominantly globotriaosylceramide, accumulate in the endothelial, perithelial, and smooth muscle cells of blood vessels and many other cell types in the liver, heart, spleen, kidney, and also in the plasma of the patients. Classical clinical manifestations include pain and paresthesia in the extremities, angiokeratoma of the skin and mucous membranes, and hypohidrosis during childhood or adolescence. With increasing age, severe renal failure caused by progressive glycosphingolipid accumulation in blood vessels results in hypertension and uremia. The hemizygous male patients usually die of renal, cardiac or cerebrovascular complications. A late-onset milder form with predominant cardiac involvement ("cardiac variant") is known. Heterozygous females may exhibit an attenuated form of the disease. The characteristic pathology is widespread deposits of birefringent crystalline glycosphingolipids. The lipid deposits have also been reported in neurons in certain region in the brain and spinal cord (*37, 38*).

The mouse model of Fabry disease has recently been generated by targeted gene disruption (*39, 40*). α-Gal A activity is undetectable in liver homogenates from *α-Gal A−/0* mice. Hemizygous mutant mice are clinically normal at 10-14 weeks of age. No obvious histological lesions are detected in the kidney, liver, heart, spleen, lung, and brain with hematoxylin and eosin stain. However, lipid inclusions consisting of concentric lamellar structures can be demonstrated by electron microscopy in the renal tubular epithelium. They appear similar to those seen in human patients. With fluorescent-labeled *Griffonia (Bandeiraea) simplicifolia* lectin which selectively binds to α-D-galactosyl residues, significant accumulation of compounds containing α-D-galactosyl residues was demonstrated in the kidney of 10-week-old mutant mice. Skin fibroblasts from *α-Gal −/0* mouse embryos also displayed significant accumulation of α-Gal A substrates. Significant accumulation of globotriaosylceramide is present in the liver and kidney. Thus, the mouse model of Fabry disease closely resembles the human disease. A new model of Fabry disease with a specific point mutation has also been recently developed (*41*).

Niemann-Pick type A and B (acid sphingomyelinase deficiency). Niemann-Pick disease (NPD) is caused by deficient activity of the lysosomal acid sphingomyelinase (*42, 43*). Two clinical types, A and B, are known. Type A patients have severe neurovisceral storage and usually die by three years of age. Type B patients have similarly severe hepatosplenomegaly but little or no neurological involvement and often survive until adulthood. The underlying mechanism for such a phenotypic difference is not well understood. The acid sphingomyelinase gene locus is on chromosome 11 and many mutations that cause type A or type B disease have been identified. Sphingomyelin and cholesterol accumulate within the cells of the reticuloendothelial system causing the massive hepatosplenomegaly. In addition, diffuse neuronal storage is a conspicuous neuropathological finding in the type A patients. Consistent with the pathological finding, abnormal accumulation of sphingomyelin in the brain appears to occur only in patients with the type A disease (*44*).

Recently two laboratories generated murine models of NPD by disrupting exon 2 or exon 3 of the acid sphingomyelinase gene (*45, 46*). While the locations of the gene disruption were in adjacent exons, the two laboratories reported significantly different findings in their respective mutant mice.

The acid sphingomyelinase null mutant (*asmase−/−*) mice generated by Otterbach and Stoffel (*46*) were normal until 8-10 weeks when fine tremors of the entire body and severe intention tremors developed. Their gait became increasingly ataxic and tottering with zigzag movements characteristic of cerebellar dysfunction. Around 60 days, hepatosplenomegaly became apparent and the affected mice died around 4 months of age after a period of severe dyspnea. Acid sphingomyelinase mRNA was absent and no acid sphingomyelinase activity was measurable in the liver, spleen, and brain. Neutral sphingomyelinase activity in the brain, however, was similar to that of wild type mice. Accumulation of sphingomyelin was present in the liver and spleen and to a lesser extent in the brain. The size of the liver and spleen of the affected mice increased about 1.5 and 2 times in weight, respectively, in the terminal stage.

Massive accumulation of lipid material was histologically evident in the Kupffer cells and in macrophages in the bone marrow, spleen, and lung. The brain had normal size and weight. Neurons in the central nervous system were frequently swollen with pale vacuolation. The most striking feature in the brain was loss of Purkinje cells in the cerebellum which occurred within 60-90 days after birth. The cerebellar molecular cell layer became very narrow in the final stage of the disease at 210 and 225 days (*47*). Thus, this murine model is phenotypically closely similar to Niemann-Pick disease type A in humans.

The acid sphingomyelinase knockout mice generated by Horinouchi and co-workers (*45*) showed ataxia, mild tremor, and feeding difficulty around 8 weeks but survived longer and were fertile. Death occurred at between 6 and 8 months of age, much later than the affected mice described by Otterbach and Stoffel. Unlike the mice of Otterbach and Stoffel which showed no significant brain atrophy, the brain of the mice generated by Horinouchi *et al.* were atrophic and less than half the normal weight and size. A remarkable loss of Purkinje cells and general atrophy of the cerebellum and midbrain were similar in both models. Hepatosplenomegaly, that was a striking feature in the mice generated by Otterback and Stoffel, was not evident in these mice, although lipid-laden foam cells (NPD cells) were found in most major organs, particularly in bone marrow and spleen. Multilamellar inclusions were demonstrated in the neurons in both mutant mice by electron microscopy. The reason for such phenotypic differences between these two acid sphingomyelinase deficient mouse lines is not clear.

GM1-gangliosidosis (acid β-galactosidase deficiency). GM1-gangliosidosis is a progressive neurological disease caused by a genetic deficiency of lysosomal acid β-galactosidase (*23, 48*). Neurological manifestations are similar to those of GM2-gangliosidosis. The clinical course is most rapidly progressive in the infantile form and is usually associated with visceromegaly and mucopolysaccharidosis-like dysmorphic features. Patients with the infantile form of GM1-gangliosidosis usually succumb to the disease within a few years. In the later-onset types, the clinical course is more prolonged and visceral involvement is less common. The adult type is the most chronic form and often manifests itself as a movement disorder. Radiologic evidence of bony abnormalities is frequently present in the adult form. Morquio B disease is allelic to GM1-gangliosidosis and is also caused by genetic abnormalities in the acid β-galactosidase gene. Unlike the cerebral forms of the disease mentioned, Morquio B disease does not affect the nervous system directly. The main clinical symptoms are related to severe bony deformities. Patients with clinical forms intermediate between the primarily neurological GM1-gangliosidosis and the primarily skeletal Morquio B disease have also been described. The infantile type shows diffuse and extensive neuronal storage of GM1-ganglioside and, to a lesser extent, its asialo derivative, GA1. Oligosaccharides derived from keratan sulfate and glycoproteins are the primary storage materials in the visceral organs (*49*) and are excreted into the urine. In the chronic adult type, neuronal storage tends to be more localized in the basal ganglia and cerebellum (*50*). The human acid β-galactosidase cDNA and the gene have been cloned and many disease-causing mutations have been identified (*48*).

Recently mouse models lacking the functional β-galactosidase gene (*β-gal−/−*) have been generated by homologous recombination and embryonic stem cell technology in two laboratories (*51-53*). The *β-gal* gene was inactivated by disruption of exon 6 (*51*) or exon 15 (*52*). The *β-gal−/−* mice are fertile and appear phenotypically normal until about 4-5 months of age when generalized tremor, ataxia, and abnormal gait become apparent. No β-gal mRNA is detectable by Northern blot analysis of kidney, brain, and liver. β-Galactosidase activity in the kidney, brain, liver, and spleen as measured with a fluorescent substrate is markedly decreased (ranging from 1% in the spleen to 4% in the brain) compared with wild type littermates. Degradation of the natural substrate, GM1-ganglioside, is not detectable at all. By thin layer chromatography, markedly increased GM1-ganglioside and GA1 are noted in the brains of *β-gal−/−* mice. In the brain of human patients with infantile GM1-gangliosidosis, a similar degree of GM1-ganglioside accumulation occurs but accumulation of GA1 is only slight. Thus, marked accumulation of GA1 in this mouse model suggests that, analogous to GM2-ganglioside degradation (see above), the murine sialidase is more active toward GM1-ganglioside than the corresponding human enzyme, a difference between human and mouse. In *β-gal−/−* mice, GM1-ganglioside increases dramatically from 3 weeks to 3.5 months. Histologically, neuronal storage is already widespread as early as 3 weeks. By 5 weeks, PAS-positive storage materials are present in almost all neurons in the cerebrum, cerebellum, brainstem, spinal cord, and the spinal dorsal root ganglia. Diffuse neuronal storage in these mice is very similar to the pattern of neuronal storage noted in the human infantile GM1-gangliosidosis. Ultrastructural features of neuronal storage materials are also similar to those of human infantile GM1-gangliosidosis. Unlike in the human disease, *β-gal−/−* mice show no hepatosplenomegaly and no morpholgical evidence of storage materials in the liver even at 3.5 months. Biochemical analysis show only a minimal storage of oligosaccharides in the liver and a low level of abnormal urinary oligosaccharides. The *β-gal−/−* mice reported by Matsuda and co-workers (*52*) were also apparently healthy for the first 4 months. Subsequently, horizontal movement became slower and rearing or vertical climbing became less frequent. Definite gait disturbance was noted by 6-8 months. Spastic diplegia progressed and the mice died of extreme emaciation at 7-10 months because of difficulty feeding. They described ballooning of neurons in various areas of the central nervous system and a 20-30-fold increase of GM1-ganglioside in the brain of these mice. β-Galactosidase activity in *β-gal−/−* fibroblasts was reported to be 0-1% of control value. Thus, both of these mouse models are similar and appear to be excellent models of the infantile form of human GM1-gangliosidosis as far as the CNS involvement is concerned. Minimal involvement of visceral organs in the mouse models suggest differences in the metabolic pathway in these organs between humans and mice.

Total SAP deficiency (sphingolipid activator protein deficiency). Sphingolipid activator proteins (SAP, saposins) are small non-enzymatic lysosomal glycoproteins which function as essential cofactors for physiological degradation of some sphingolipids with relatively short hydrophilic head groups (*54*). Two genes encode all estab-

lished sphingolipid activator proteins. One is the GM2-activator protein localized on chromosome 5 that stimulates degradation of GM2-ganglioside and asialo GM2-ganglioside (GA2) (see GM2-gangliosidosis section above) (*55*). The other gene is on chromosome 10 coding for the SAP precursor, which is processed to four homologous activator proteins, *sap*-A, *sap*-B, *sap*-C, and *sap*-D. Genetic deficiencies of *sap*-B (sulfatide activator) (*56*) and *sap*-C (glucosylceramide activator) (*57*) result in human disorders mimicking metachromatic leukodystrophy and Gaucher disease, respectively. In 1989, Harzer and co-workers reported two sibs in a consanguineous family, affected by a rapidly fatal disorder (*58*). None of the *saps* were detected in the tissue from these sibs (*59*) and they were later found to be homoallelic for a mutation in the initiation codon of the SAP precursor gene (*60*). The clinical phenotype of the first patient in the family resembled the severe infantile type of Gaucher disease (type II). Shortly after birth, the patient showed hyperkinetic or cloniform motor abnormalities and generalized clonic seizures. The patient had fasciculation of tongue and periauricular muscle, spontaneous Babinski signs and an exaggerated Moro reflex. Massive hepatosplenomegaly was present and storage macrophages resembling Gaucher cells were observed in a bone marrow smear at the age of 5 weeks. The enzyme studies with leukocytes and cultured fibroblasts showed reduced activities of galactosylceramidase and glucosylceramidase. The ultrastructure of liver, nerve, and skin biopsies revealed the presence of vesicular inclusions and membranous bodies suggestive of a lysosomal storage disease. An *in situ* test with cultured fibroblasts demonstrated a defect in ceramide catabolism similar to Farber disease. Neuroimaging indicated an atrophic brain with hydrocephalus. Postmortem examination of the brain was not performed, however. The second patient, an aborted female sib of the first patient, showed similar defect in the ceramide catabolism and had contracted hand joints consistent with a Farber-like disease. The SAP precursor and mature *saps* were completely absent in this fetus who had generalized accumulation of multiple sphingolipids (ceramide, glucosylceramide, galactosylceramide, sulfatide, lactosylceramide, digalactosylceramide, GM1-ganglioside) with exception of sphingomyelin in the brain and systemic organs.

The mouse SAP gene was disrupted by insertion of the neomycin-resistant gene within exon 3 to generate a *SAP*$-/-$ mouse line (*61*). SAP mRNA was completely absent in the tissue of *SAP*$-/-$ mice. Of the first 126 offspring from heterozygous mating, 36 (26%) wild type, 68 (55%) heterozygotes, and 16 (13%) homozygotes were obtained. Five of the 9 mice that died within a day or two of birth were *SAP*$-/-$ mice. These results suggest that a disproportionate percentage of *SAP*$-/-$ mice die *in utero* or within a day or two after birth. Those *SAP*$-/-$ mice that survive the neonatal period are apparently healthy until about 18-20 days, although they were slightly smaller in size than their normal littermates. Tremulousness of the head and mild weakness/ataxia are the initial symptoms. These symptoms progress rapidly and *SAP*$-/-$ mice usually die around 35 days in an emaciated general condition. The pathology of *SAP*$-/-$ mice is that of a combined neuronal storage and a leukodystrophy. The brain and visceral organs are of normal size except for the kidneys which are significantly smaller than controls. There is an extensive neuronal storage throughout the cerebrum, cerebellum, brainstem, spinal cord, and retinal ganglion cells. The storage materials stain brilliantly with PAS on frozen sections. Many axonal spheroids and macrophages/microglia containing storage materials are also conspicuous. The white matter is hypomyelinated but myelin ovoids suggestive of myelin degeneration are also present in the corpus callosum, internal capsule, spinal white matter, spinal roots, trigeminal, and sciatic nerves. No metachromatic or sudanophilic materials are found in frozen sections of the cerebrum. In the liver, clusters of histiocytes with abundant eosinophilic cytoplasm are present in the hepatic sinusoids. Similar cells are also seen in the spleen and lymphnodes. No significant abnormalities are detected in the kidney despite the smaller size. The inclusions in neurons are pleomorphic; some consist of concentric or short lamellar structures combined with an electron dense granular structure and others are electron dense granular structure surrounded by a single membrane. Small clusters of electron dense granular material or small concentric lamellar structures are scattered within the cytoplasm of some neurons. These inclusions resemble those in cultured skin fibroblasts from the patients with total SAP deficiency. The inclusions within the cells in the hepatic sinusoids also consist of membranous and vesicular structures and are more complex than neuronal inclusions (*62*). Biochemically the most conspicuous specific abnormality is a major accumulation of lactosylceramide, which appears to account for the strong PAS staining on frozen sections. The total ganglioside sialic acid is increased significantly with a large relative increase in the monosialogangliosides, GM1, GM2, and GM3. However, on the wet weight or protein basis, many of the disialo- and trisialo-gangliosides are also significantly increased, a condition known so far only in a genetic disorder occurring in the emu {Bermudez, Johnson, *et al.* 1995 ID: 228} {Freischütz, Tokuda, *et al.* 1997 ID: 850}. Similar to the brain, lactosylceramide is conspicuously increased in the liver and kidney. Additionally, ceramide, glucosylceramide, globotriaosylceramide and globoside are all significantly increased in the liver. Galactosylceramide and sulfatide appear to be increased in the kidney. These galactolipids are reduced in the brain, most probably secondary to the significant myelin loss. Thus, accumulation of varieties of sphingolipids noted in patient with SAP deficiency is also present in the *SAP*$-/-$ mice. As noted in human patients, activity of glucosylceramidase and galactosylceramidase are also decreased in *SAP*$-/-$ mice. The results of the serine-loading tests on cultured fibroblasts were remarkably similar to the results obtained with the fibroblasts from the human patients. Therefore, *SAP*$-/-$ mice closely mimic biochemical changes of the known human patients with total SAP deficiency. Neuropathology cannot be compared, however, since postmortem investigation was not carried out on the original patient with SAP deficiency.

Mutants of abnormal sphingolipid synthesis

Genetically-determined blocks in sphingolipid biosynthesis are known to occur in humans without detrimental consequences, *e.g.* in the blood group P/p system. Individuals with small *p* blood type lack lactosylceramide galactosyltransferase and thus are unable to synthesize globotriao-

sylceramide. However, no spontaneous genetic diseases, either in humans or other mammalian species, of abnormal sphingolipid biosynthesis are known. Induced mouse mutants of sphingolipid biosynthesis are still limited because cloning and characterization of the enzymes involved in sphingolipid synthesis have been more recent than those of the degradative lysosomal enzymes. However, this situation is rapidly changing and we can expect the number of mouse models in which specific steps of sphingolipid biosynthesis are genetically impaired will increased rapidly. As of now, only two such examples are known. However, the insight afforded by these two models clearly points to the importance of these models.

Galactocerebroside synthase (CGT) deficient mouse

Galactosylceramide (galactocerebroside) and its sulfate ester, sulfatide, are uniquely concentrated in the myelin sheath. Although also present in other tissues, most notably in the kidney, these galactolipids are quantitatively limited in the nervous system, particularly in the myelin-forming cells, the oligodendrocytes in the CNS and the Schwann cells in the PNS. Expression of galactosylceramide is considered a reliable marker for differentiated oligodendrocytes, and some investigators suggested that its expression in fact might be essential to trigger the myelination process (*65*). UDP-galactose:ceramide galactosyltransferase (galactocerebroside synthase, CGT) catalyzes the last step of galactosylceramide synthesis. Its expression is tightly regulated developmentally during the active period of myelination (*66*). Earlier enzymological studies indicated that the enzyme transfers galactose to ceramide as well as to sphingosine (*67*). A question remained for many years, however, whether a single enzyme galactosylates both ceramides containing either normal fatty acid (NFA) or α-hydroxy-fatty acid (HFA) or whether enzymes specific for the respective acceptors exist. Furthermore, CGT is likely to catalyze synthesis of the precursor for seminolipid, which is supposed to be essential for normal spermatogenesis. The gene coding for the enzyme was cloned and characterized independently in three laboratories (*68-70*). The gene is mapped to 4q26 in humans (*71*) and to band E3 F1 in the mouse (*72*). Mouse lines in which the gene was inactivated have been generated in two of these laboratories (*73, 74*). Several important questions had been raised in generating this mouse model; (a) do the oligodendrocytes develop and differentiate normally in the absence of galactosylceramide?, (b) does the process of myelination proceed normally, *i.e.* does it follow the normal developmental clock?, (c) are other myelin-specific genes expressed without galactosylceramide expression? (d) is any membrane resembling normal myelin formed without galactosylceramide and sulfatide?, (e) if such a membrane is generated, does it function like normal myelin?, (f) are both NFA- and HFA-galactosylceramide synthesized by the same enzyme?, (g) what happens to the unused HFA-ceramide which is normally almost exclusively in galactosylceramide and sulfatide?, (h) what is the consequence to spermatogenesis if seminolipid biosynthesis is blocked?

In the CGT-deficient mouse generated by Coetzee *et al.* (*73*), exon 2 was disrupted by the neomycin-resistant gene. Exon 2 is the major exon among the 6 exons of the gene and contains most of the protein coding sequence. Homozygous affected mice appear to develop normally until about 12-14 days, when they start exhibiting jerking of the head and ataxic locomotion. Complex and progressive neurological signs reminiscent of many "myelin mutants" develop. About a half of affected mice die by 35 days but remainder die more slowly and some survive up to 100 days. After 60 days, paralysis becomes prominent. Affected females are fertile but unable to raise pups. Homozygous affected mice are totally devoid of CGT mRNA, while heterozygotes express, as expected, an approximately half normal amount of CGT mRNA. However, the genes encoding myelin-specific proteins, myelin basic protein, proteolipid protein, and myelin-associated glycoprotein, are expressed at or near normal levels in the brain of affected mice, an indication that coordinate expression of myelin-specific constituents does occur without galactosylceramide expression.

The normal expression of the myelin proteins was the first in the series of surprises in that myelination starts and proceeds with the normal developmental timing in the brain and peripheral nerves of the CGT-deficient mice and that the generated myelin appears normal on both light and electron microscopic levels. Morphometric analyses showed that myelin thickness is slightly reduced in the dorsal column and reduced by 30% in the ventral column of the spinal cord but was normal in the optic and sciatic nerves. More recent electron microscopic studies showed that there are subtle abnormalities in the CNS myelin, most notably in the structure of the node of Ranvier (*75*). When myelin was isolated by the standard procedure from $25 \pm$ day old whole brains, the yield was approximately 50% of normal. However, despite the apparently normal morphology but consistent with the severe clinical phenotype, compound action potentials measured with isolated spinal cord indicated drastic abnormalities. Biochemical analysis confirmed the complete absence of galactosylceramide and sulfatide in the brain. In their place, a smaller quantity of HFA-glucosylceramide was present, perhaps 10-15% of all galactolipids in the normal brain combined. No NFA-glucosylceramide could be detected. In addition, HFA-containing sphingomyelin could be detected in the whole brain at approximately 30% of total sphingomyelin. Surprisingly, there were no abnormalities in the lacto-series gangliosides in the brain. The lipid composition of the isolated myelin fraction looked essentially identical to that of the whole brain. The only quantitative exception was that HFA-sphingomyelin was the predominant molecular species in sphingomyelin in the isolated myelin fraction. It was also notable that, at 16- and 24-days, heterozygous carriers showed clearly retarded accumulation of both galactosylceramide and sulfatide, particularly those with NFA-ceramide.

When affected mice survived beyond 40 days, increasing intramyelinic vacuolization was noted regionally, most prominently in the ventral column of the spinal cord. The myelin sheath was split at the intraperiod line indicating that the fluid was extracellular. This pathology appears consistent with the progressively severe paralysis that develops in animals that survive longer than 40-45 days.

The initial findings in the CGT-deficient mice generated by Bosio *et al.* (*74*) were essentially identical with those of Coetzee *et al.* More detailed analytical data have been published recently by Bosio *et al.* (*76*). An intriguing finding, which was also among their initial observation, was

that, in the peripheral nerves, there was a component that migrated on thin-layer chromatography to the area expected for HFA-glucosylsulfatide. Such a component was not seen in the central nervous system. While more definitive identification must still be made, presence of HFA-glucosylsulfatide only in the peripheral nerves, if confirmed, will pose some intriguing questions regarding biosynthesis of these sphingolipids. As expected, they found no monogalactosyldiglyceride in the CGT knockout mice. The reduced synthesis of galactosylceramide in the CNS of heterozygous mice was less than expected from the single dose of the gene, most likely due to a compensatory metabolic mechanism. These authors described the reduction of galactosylceramide in the heterozygous mice to be primarily in HFA-galactosylceramide. However, the accompanying thin-layer chromatogram indicates that the reduction is primarily in NFA-galactosylceramide.

This mutant has provided answers to some of the important questions; (a) the oligodendrocytes can develop and differentiate according to the normal biological clock in the absence of galactosylceramide synthesis, (b) the process of myelination occurs and other myelin-specific constituents can be expressed without galactosylceramide biosynthesis, (c) without the usual galactolipids, morphologically normal myelin can be formed but it is functionally abnormal, (d) the total lack of both HFA- and NFA-galactosylceramides proves that a single enzyme makes both of these molecular species, (e) HFA-ceramide which is normally used exclusively for synthesis of galactosylceramide and sulfatide is diverted to glucosylceramide and sphingomyelin and the former partially substitutes for the missing galactosylceramide, (f) nevertheless, HFA-glucosylceramide is not converted to lactosylceramide, since the higher gangliosides are normal, most likely explanation being that the galactosyltransferase that synthesizes lactosylceramide may not recognize HFA-glucosylceramide as an acceptor, (g) at least in the central nervous system, HFA-glucosylceramide is not sulfated to the corresponding sulfatide but the situation may not be the same in the peripheral nervous system. A number of questions are still to be answered. If HFA-glucosylsulfatide is present in the peripheral nerves, does this mean there are more than one sulfatide-forming sulfotransferase? Any compounds that are synthesized by CGT should not be present in these mice, including monogalactosyldiglyceride (77) and probably also the precursor for seminolipid, 1-alkyl,2-acyl-galactosylglycerol. The consequent lack of seminolipid may interfere with normal spermatogenesis in the testis. Any number of lipids that must be synthesized though these primary CGT products should not be present (Table I). A major weakness of this model is that it is nearly impossible to dissect out the consequences of lack of galactosylceramide from that of sulfatide. For example, is the physiological dysfunction of myelin due to lack of all galactolipids or due to loss of sulfatide which contributes a major portion of negative charge to normal myelin? Cloning of the sulfatide synthase has been reported recently (*78*). It is hoped that availability of this clone would help clarify these major issues that cannot be resolved by the CGT-deficient model.

TABLE I. **Primary and secondary products of CGT.** These compounds are found to be or are likely to be absent in the CGT-deficient mice.

Primary product	Secondary product
Galactosylceramide	Sulfatide
	Digalactosylceramide and its sulfate ester
	GM4-ganglioside (sialylgalactosylceramide)
	Acyl-ester of galactosylceramide
Galactosylsphingosine (psychosine)	
Monogalactosyldiglyceride	Monogalactosyldiglyceride sulfate
	Digalactosyldiglyceride
1-Alkyl,2-acyl, galactosylglycerol[a]	Seminolipid

[a]No data available but very probably also a primary CGT product.

GM2/GD2-ganglioside synthase deficient mouse

The β1,4 *N*-acetylgalactosaminyl transferase (β1,4 galNAc-T) that synthesizes both GM2- and GD2-gangliosides from GM3 and GD3, respectively, was first cloned in 1992 (*79*). Essentially no higher gangliosides beyond GM3 and GD3 can be generated in the absence of this enzyme. In view of the multiple postulated physiological functions of gangliosides, dire consequences had been predicted in the mouse which is totally defective in the activity of this enzyme. However, when the knockout mouse was generated by disrupting exon 4, the phenotype was surprisingly mild (*80*).

In the homozygous affected mice, the β1,4 galNAc-T mRNA was undetectable and so was the enzymatic activity. Consequently, no higher gangliosides were present in the liver and brain. Compensatory increases of the precursors, GM3 and GD3, were observed. These authors in fact estimated that the total brain gangliosides might not be significantly lower than in the wild-type animals because of the increase in the GM3 and GD3 levels. There were no detectable abnormalities in the brain development. Extensive histological examination of various areas of the cerebrum, cerebellum, and brain stem indicated that the cellular proliferation and migration were normal and that myelination and synapse formation also appeared normal even on the ultrastructural level. Affected mice developed normally without an overt clinical phenotype. A series of behavioral tests were similarly normal, including the inclined plane test, swimming ability, flinch hearing, and the Morris water maze tests. Nerve conduction as assessed by evoked potentials at the cerebral cortical and lumbar levels as the results of stimulation at the Achilles tendon level gave a statistically significant reduction by approximately 30% in the homozygous affected mice only when measured at the cortical level, although there was a tendency toward reduced velocity even at the lumbar level. These initial observations on the mice that lack all higher gangliosides normally present abundantly in the brain, such as GM2, GM1, GD1a, GD1b, GT1b, GQ1b, were contrary to the generally held expectations. A very large number of potentially important physiological functions have been proposed for these gangliosides in neuronal differentiation, neuronal arborization, myelination, regeneration, protection from external insults, cell-to-cell interactions, *etc.* Although a moderate reduction in the conduction velocity was observed, these higher gangliosides are not as essential as they had been thought. Potentially significant in interpretation of these findings is the apparent compensatory increases in the simpler gangliosides, GM3 and GD3. The

metabolic machinery to degrade these gangliosides is intact in the affected mice and thus their increase in fact may indicate active compensatory increase. If that is the case, it would represent a clear demonstration of the much overused concept of redundancy. These authors carefully pointed out that a complete shutdown of all ganglioside biosynthesis, including GM3 and GD3 would be necessary to answer this question. GM3-ganglioside synthase has very recently been cloned and the answer to this extremely important question would hopefully be forthcoming shortly. Outside the nervous system, a surprisingly drastic pathology in the testis of affected mice has been presented in meetings. From the known ganglioside composition of the testis, it is difficult to explain such extensive histological abnormalities and its pathogenetic mechanism needs to be clarified in the future.

REFERENCES

1. Svennerholm, L. (1984) Biological significance of gangliosides in *Cellular and Pathological Aspects of Glycoconjugate Metabolism* (Dreyfus, H., Massarelli, R., Freysz, L., and Rebel, G., eds.) pp. 21-44, INSERM, Paris
2. Ledeen, R.W. and Yu, R.K. (1982) Gangliosides: Structure, isolation, and analysis. *Methods Enzymol.* **83**, 139-191
3. van Echten, G. and Sandhoff, K. (1989) Modulation of ganglioside biosynthesis in primary cultured neurons. *J. Neurochem.* **52**, 207-214
4. Hakomori, S.I. (1984) Glycosphingolipids as differentiation-dependent, tumor-associated markers and as regulators of cell proliferation. *Trends Biochem. Sci.* **9**, 453-458
5. Markwell, M.A.K., Svennerholm, L., and Paulson, J.C. (1981) Specific gangliosides function as host cell receptors for Sendai virus. *Proc. Natl. Acad. Sci. USA* **78**, 5406-5410
6. Fishman, P.H. and Brady, R.O. (1976) Biosynthesis and function of gangliosides. *Science* **194**, 906-915
7. Morré, D.J., Kartenbeck, J., and Franke, W.W. (1979) Membrane flow and interconversions among endomembranes. *Biochim. Biophys. Acta* **559**, 71-152
8. Schwarzmann, G. and Sandhoff, K. (1990) Metabolism and intracellular transport of glycosphingolipids. *Biochemistry* **29**, 10865-10871
9. Thompson, L.K., Horowitz, P.M., Bentley, K.L., Thomas, D.D., Alderete, J.F., and Klebe, R.J. (1986) Localization of the ganglioside-binding site of fibronectin. *J. Biol. Chem.* **261**, 5209-5214
10. Tiemeyer, M., Yasuda, Y., and Schnaar, R.L. (1989) Ganglioside-specific binding protein on rat brain membranes. *J. Biol. Chem.* **264**, 1671-1681
11. Suzuki, K. (1984) "Authentic animal models" for biochemical studies of human genetic disease in *Developmental Disabilities* (Arima, M., Suzuki, Y., and Yabuuchi, H., eds.) pp. 129-138, Tokyo University Press, Tokyo
12. Suzuki, K. and Suzuki, K. (1995) The twitcher mouse: A model for Krabbe disease and for experimental therapies. *Brain Pathol.* **5**, 249-258
13. Capecchi, M.R. (1994) Targeted gene replacement. *Sci. Am.* **270**, 52-58
14. Tybulewicz, V.L.J., Tremblay, M.L., LaMarca, M.E., Willemsen, R., Stubblefield, B.K., Winfield, S., Zablocka, B., Sidransky, E., Martin, B.M., Huang, S.P., Mintzer, K.A., Westphal, H., Mulligan, R.C., and Ginns, E.I. (1992) Animal model of Gaucher's disease from targeted disruption of the mouse glucocerebrosidase gene. *Nature* **357**, 407-410
15. Willemsen, R., Tybulewicz, E., Sidransky, E., Eliason, W.K., Martin, B.M., LaMarca, M.E., Reuser, A.J.J., Tremblay, M., Westphal, H., Mulligan, R.C., and Ginns, E.I. (1995) A biochemical and ultrastructural evaluation of the type 2 Gaucher mouse. *Mol. Chem. Neuropathol.* **24**, 179-192
16. Sidransky, E., Sherer, D.M., and Ginns, E.I. (1992) Gaucher disease in the neonate: A distinct Gaucher phenotype is analogous to a mouse model created by targeted disruption of the glucocerebrosidase gene. *Pediatr. Res.* **32**, 494-498
17. Holleran, W.M., Ginns, E.I., Menon, G.K., Grundmann, J.-U., Fartasch, M., McKinney, C.E., Elias, P.M., and Sidransky, E. (1994) Consequences of β-glucocerebrosidase deficiency in epidermis. Ultrastructure and permeability barrier alterations in Gaucher disease. *J. Clin. Invest.* **93**, 1756-1764
18. Sidransky, E., Fartasch, M., Lee, R.E., Metlay, L.A., Abella, S., Zimran, A., Gao, W., Elias, P.M., Ginns, E.I., and Holleran, W. M. (1996) Epidermal abnormalities may distinguish type 2 from type 1 and type 3 of Gaucher disease. *Pediatr. Res.* **39**, 134-141
19. Liu, Y., Suzuki, K., Reed, J., Grinberg, A., Westphal, H., Hoffmann, A., Döring, T., Sandhoff, K., and Proia, R.L. (1998) Mice with type 2 and 3 Gaucher disease point mutations generated by a single insertion mutagenesis procedure (SIMP). *Proc. Natl. Acad. Sci. USA* **95**, 2503-2508
20. Terry, R.D. and Korey, S.R. (1960) Membranous cytoplasmic granules in infantile amaurotic idiocy. *Nature* **188**, 1000-1002
21. Terry, R.D. and Weiss, M. (1963) Studies in Tay-Sachs disease II. Ultrastructure of cerebrum. *J. Neuropathol. Exp. Neurol.* **22**, 18-55
22. Gravel, R.A., Clarke, J.T.R., Kaback, M.M., Mahuran, D., Sandhoff, K., and Suzuki, K. (1995) The GM2 gangliosidosis in *The Metabolic and Molecular Basis of Inherited Disease* (Scriver, C.R., Beaudet, A.L., Sly, W.S., and Valle, D., eds.) pp. 2839-2879, McGraw-Hill, New York
23. Suzuki, K. and Suzuki, K. (1996) The gangliosidoses in *Neurodystrophies and Neurolipidoses* (Moser, H.W., ed.) pp. 247-280, Elsevier, Amsterdam
24. Yamanaka, S., Johnson, M.D., Grinberg, A., Westphal, H., Crawley, J.N., Taniike, M., Suzuki, K., and Proia, R.L. (1994) Targeted disruption of the Hexa gene results in mice with biochemical and pathologic features of Tay-Sachs disease. *Proc. Natl. Acad. Sci. USA* **91**, 9975-9979
25. Sango, K., Yamanaka, S., Hoffmann, A., Okuda, Y., Grinberg, A., Westphal, H., McDonald, M.P., Crawley, J.N., Sandhoff, K., Suzuki, K., and Proia, R.L. (1995) Mouse models of Tay-Sachs and Sandhoff diseases differ in neurologic phenotype and ganglioside metabolism. *Nature Genet.* **11**, 170-176
26. Taniike, M., Yamanaka, S., Proia, R.L., Langaman, C., Bone-Turrentine, T., and Suzuki, K. (1995) Neuropathology of mice with targeted disruption of Hexa gene, a model of Tay-Sachs disease. *Acta Neuropathol. (Berl.)* **89**, 296-304
27. Cohen-Tannoudji, M., Marchand, P., Akli, S., Sheardown, S.A., Puech, J.-P., Kress, C., Gressens, P., Nassogne, M.-C., Beccari, T., Muggleton-Harris, A.L., Evrard, P., Stirling, J.L., Poenaru, L., and Babinet, C. (1995) Disruption of murine *Hexa* gene leads to enzymatic deficiency and to neuronal lysosomal storage, similar to that observed in Tay-Sachs disease. *Mammalian Genome* **6**, 844-849
28. Liu, Y., Hoffmann, A., Grinberg, A., Westphal, H., McDonald, M.P., Miller, K.M., Crawley, J.N., Sandhoff, K., Suzuki, K., and Proia, R.L. (1997) Mouse model of GM2 activator deficiency manifests cerebellar pathology and motor impairment. *Proc. Natl. Acad. Sci. USA* **94**, 8138-8143
29. Phaneuf, D., Wakamatsu, N., Huang, J.Q., Borowski, A., Peterson, A.C., Fortunato, S.R., Ritter, G., Igdoura, S.A., Morales, C.R., Benoit, G., Akerman, B.R., Leclerc, D., Hanai, N., Marth, J.D., Trasler, J.M., and Gravel, R.A. (1996) Dramatically different phenotypes in mouse models of human Tay-Sachs and Sandhoff diseases. *Hum. Mol. Genet.* **5**, 1-14
30. Sango, K., McDonald, M.P., Crawley, J.N., Mack, M.L., Tifft, C.J., Skop, E., Starr, C.M., Hoffmann, A., Sandhoff, K., Suzuki, K., and Proia, R.L. (1996) Mice lacking both subunits of lysosomal β-hexosaminidase display gangliosidosis and mucopolysaccharidosis. *Nature Genet.* **14**, 348-352
31. Suzuki, K., Sango, K., Proia, R.L., and Langaman, C. (1997) Mice deficient in all forms of lysosomal beta-hexosaminidase show mucopolysaccharidosis-like pathology. *J. Neuropathol. Exp. Neurol.* **56**, 693-703

32. Kolodny, E.H. and Fluharty, A.L. (1995) Metachromatic leukodystrophy and multiple sulfatase deficiency: Sulfatide lipidosis in *The Metabolic and Molecular Basis of Inherited Disease* (Scriver, C.R., Beaudet, A.L., Sly, W.S., and Valle, D., eds.) pp. 2693-2739, McGraw-Hill, New York
33. Gieselmann, V., Zlotogora, J., Harris, A., Wenger, D.A., and Morris, C.P. (1994) Molecular genetics of metachromatic leukodystrophy. *Hum. Mutation* **4**, 233-242
34. Peng, L. and Suzuki, K. (1987) Ultrastructural study of neurons in metachromatic leukodystrophy. *Clin. Neuropathol.* **6**, 224-230
35. Hess, B., Saftig, P., Hartmann, D., Coenen, R., Lüllmann-Rauch, R., Goebel, H.H., Evers, M., von Figura, K., D'Hooge, R., Nagels, G., De Deyn, P., Peters, C., and Gieselmann, V. (1996) Phenotype of arylsulfatase A-deficient mice: Relationship to human metachromatic leukodystrophy. *Proc. Natl. Acad. Sci. USA* **93**, 14821-14826
36. Desnick, R.J., Ioannou, Y.A., and Eng, C.M. (1995) α-Galactosidase A deficiency: Fabry disease in *The Metabolic and Molecular Basis of Inherited Disease* (Scriver, C.R., Beaudet, A. L., Sly, W.S., and Valle, D., eds.) pp. 2741-2784, McGraw-Hill, New York
37. DeVeber, G.A., Schwarting, G.A., Kolodny, E.H., and Kowall, N.W. (1992) Fabry disease: Immunocytochemical characterization of neuronal involvement. *Ann. Neurol.* **31**, 409-415
38. Sung, J.H. (1979) Autonomic neurons affected by lipid storage in the spinal cord in Fabry's disease: distribution of autonomic neurons in the sacral cord. *J. Neuropathol. Exp. Neurol.* **38**, 87-98
39. Wang, A.M., Ioannou, Y.A., Zeidner, K.M., Gotlib, R.W., Dikman, S., Stewart, C.L., and Desnick, R.J. (1996) Fabry disease: Generation of a mouse model with α-galactosidase A deficiency. *Am. J. Hum. Genet.* **59** Suppl., A208 (abstract #1192)
40. Ohshima, T., Murray, G.J., Swaim, W.D., Longenecker, G., Quirk, J.M., Cardarelli, C.O., Sugimoto, Y., Pastan, I., Gottesman, M.M., Brady, R.O., and Kulkarni, A.B. (1997) α-Galactosidase A deficient mice: A model of Fabry disease. *Proc. Natl. Acad. Sci. USA* **94**, 2540-2544
41. Shimmoto, M., Kase, R., Itoh, K., Utsumi, K., Ishii, S., Taya, C., Yonekawa, H., and Sakuraba, H. (1997) Generation and characterization of transgenic mice expressing a human mutant α-galactosidase with an R301Q substitution causing a variant form of Fabry disease. *FEBS Lett.* **417**, 89-91
42. Schuchman, E.H. and Desnick, R.J. (1995) Niemann-Pick disease type A and B: Acid sphingomyelinase deficiencies in *The Metabolic and Molecular Basis of Inherited Disease* (Scriver, C.R., Beaudet, A.L., Sly, W.S., and Valle, D., eds.) pp. 2601-2624, McGraw-Hill, New York
43. Vanier, M.T. and Suzuki, K. (1996) Niemann-Pick diseases in *Neurodystrophies and Neurolipidoses* (Moser, H.W., ed.) pp. 133-162, Elsevier, Amsterdam
44. Kamoshita, S., Aaron, A.M., Suzuki, K., and Suzuki, K. (1969) Infantile Niemann-Pick disease: A chemical study with isolation and characterization of membranous cytoplasmic bodies and myelin. *Am. J. Dis. Child.* **117**, 379-394
45. Horinouchi, K., Erlich, S., Perl, D.P., Ferlinz, K., Bisgaier, C.L., Sandhoff, K., Desnick, R.J., Stewart, C.L., and Schuchman, E.H. (1995) Acid sphingomyelinase deficient mice: A model of types A and B Niemann-Pick disease. *Nature Genet.* **10**, 288-293
46. Otterbach, B. and Stoffel, W. (1995) Acid sphingomyelinase-deficient mice mimic the neurovisceral form of human lysosomal storage disease (Niemann-Pick disease). *Cell* **81**, 1053-1061
47. Kuemmel, T.A., Schroeder, R., and Stoffel, W. (1997) Light and electron microscopic analysis of the central and peripheral nervous system of acid sphingomyelinase-deficient mice resulting from gene targeting. *J. Neuropathol. Exp. Neurol.* **56**, 171-179
48. Suzuki, Y., Sakuraba, H., and Oshima, A. (1995) β-Galactosidase deficiency (β-galactosidosis): GM1 gangliosidosis and Morquio B disease in *The Metabolic and Molecular Basis of Inherited Disease* (Scriver, C.R., Beaudet, A.L., Sly, W.S., and Valle, D., eds.) pp. 2785-2823, McGraw-Hill, New York
49. Suzuki, K. (1968) Cerebral GM1-gangliosidosis: Chemical pathology of visceral organs. *Science* **159**, 1471-1472
50. Suzuki, K. (1991) Neuropathology of late onset gangliosidoses. A review. *Dev. Neurosci.* **13**, 205-210
51. Hahn, C.N., Martin, M.D., Schröder, M., Vanier, M.T., Hara, Y., Suzuki, K., Suzuki, K., and d'Azzo, A. (1997) Generalized CNS disease and massive G_{M1}-ganglioside accumulation in mice defective in lysosomal acid β-galactosidase. *Hum. Mol. Genet.* **6**, 205-211
52. Matsuda, J., Suzuki, O., Oshima, A., Ogura, A., Naiki, M., and Suzuki, Y. (1997) Neurological manifestations of knockout mice with β-galactosidase deficiency. *Brain Dev.* **19**, 19-20
53. Matsuda, J., Suzuki, O., Oshima, A., Ogura, A., Noguchi, Y., Yamamoto, Y., Asano, T., Takimoto, K., Sukegawa, K., Suzuki, Y., and Naiki, M. (1997) β-Galactosidase-deficient mouse as an animal model for G_{M1}-gangliosidosis. *Glycoconjugate J.* **14**, 729-736
54. Sandhoff, K., Harzer, K., and Fürst, W. (1995) Sphingolipid activator proteins in *The Metabolic and Molecular Basis of Inherited Disease* (Scriver, C.R., Beaudet, A.L., Sly, W.S., and Valle, D., eds.) pp. 2427-2441, McGraw-Hill, New York
55. Burg, J., Banerjee, A., Conzelmann, E., and Sandhoff, K. (1983) Activating proteins for ganglioside GM2 degradation by beta-hexosaminidase isoenzymes in tissue extracts from different species. *Hoppe-Seylers Z. Physiol. Chem.* **364**, 821-829
56. Mehl, E. and Jatzkewitz, H. (1964) Eine Cerebrosidsulfatase aus Schweineniere. *Hoppe-Seylers Z. Physiol. Chem.* **339**, 260-276
57. Ho, M.W. and O'Brien, J.S. (1971) Gaucher's disease: Deficiency of "acid" β-glucosidase and reconstitution of enzyme activity in vitro. *Proc. Natl. Acad. Sci. USA* **68**, 2810-2813
58. Harzer, K., Paton, B.C., Poulos, A., Kustermann-Kuhn, B., Roggendorf, W., Grisar, T., and Popp, M. (1989) Sphingolipid activator deficiency in a 16-week old atypical Gaucher disease patient and his fetal sibling: Biochemical signs of combined sphingolipidoses. *Eur. J. Pediatr.* **149**, 31-39
59. Bradová, V., Smíd, F., Ulrich-Bott, B., Roggendorf, W., Paton, B.C., and Harzer, K. (1993) Prosaposin deficiency: Further characterization of the sphingolipid activator protein-deficient sibs. Multiple glycolipid elevations (including lactosylceramidosis), partial enzyme deficiencies and ultrastructure of the skin in this generalized sphingolipid storage disease. *Hum. Genet.* **92**, 143-152
60. Schnabel, D., Schröder, M., Fürst, W., Klein, A., Hurwitz, R., Zenk, T., Weber, J., Harzer, K., Paton, B.C., Poulos, A., Suzuki, K., and Sandhoff, K. (1992) Simultaneous deficiency of sphingolipid activator proteins 1 and 2 is caused by a mutation in the initiation codon of their common gene. *J. Biol. Chem.* **267**, 3312-3315
61. Fujita, N., Suzuki, K., Vanier, M.T., Popko, B., Maeda, N., Klein, A., Henseler, M., Sandhoff, K., Nakayasu, H., and Suzuki, K. (1996) Targeted disruption of the mouse sphingolipid activator protein gene: A complex phenotype, including severe leukodystrophy and wide-spread storage of multiple sphingolipids. *Hum. Mol. Genet.* **5**, 711-725
62. Oya, Y., Nakayasu, H., Fujita, N., Suzuki, K., and Suzuki, K. (1998) Pathological study of mice with total deficiency of sphingolipid activator proteins (SAP knockout mice). *Acta Neuropathol. (Berl.)* in press
63. Bermudez, A.J., Johnson, G.C., Vanier, M.T., Schröder, M., Suzuki, K., Stogsdill, P.L., Johnson, G.S., O'Brien, D., Moore, C.P., and Fry, W.W. (1995) Gangliosidosis in emus (*Dromaius novaehollandiae*). *Avian. Dis.* **39**, 292-303
64. Freischütz, B., Tokuda, A., Ariga, T., Bermudez, A.J., and Yu, R.K. (1997) Unusual gangliosidosis in emu (*Dromaius novaehollandiae*). *J. Neurochem.* **68**, 2070-2078
65. Bansal, R. and Pfeiffer, S. (1989) Reversible inhibition of oligodendrocyte progenitor differentiation by a monoclonal antibody against surface galactolipids. *Proc. Natl. Acad. Sci. USA* **86**, 6181-6185
66. Costantino-Ceccarini, E. and Morell, P. (1972) Biosynthesis of brain sphingolipids and myelin accumulation in the mouse. *Lipids* **7**, 656-659
67. Morell, P. and Radin, N.S. (1969) Synthesis of cerebroside by brain from uridine diphosphate galactose and ceramide contain-

ing hydroxy fatty acid. *Biochemistry* **8**, 506-512
68. Schulte, S. and Stoffel, W. (1993) Ceramide UDPgalactosyltransferase from myelinating rat brain: Purification, cloning, and expression. *Proc. Natl. Acad. Sci. USA* **90**, 10265-10269
69. Stahl, N., Jurevics, H., Morell, P., Suzuki, K., and Popko, B. (1994) Isolation, characterization, and expression of cDNA clones that encode rat UDP-galactose:ceramide galactosyltransferase. *J. Neurosci. Res.* **38**, 234-242
70. Schaeren-Wiemers, N., Van der Bijl, P., and Schwab, M.E. (1995) The UDP-galactose:ceramide galactosyltransferase: Expression pattern in oligodendrocytes and schwann cells during myelination and substrate preference for hydroxyceramide. *J. Neurochem.* **65**, 2267-2278
71. Bosio, A., Binczek, E., Le Beau, M.M., Fernald, A.A., and Stoffel, W. (1996) The human gene CGT encoding the UDP-galactose ceramide galactosyltransferase(cerebroside synthase): Cloning, characterization, and assignment to human chromosome 4, band q26. *Genomics* **34**, 69-75
72. Coetzee, T., Li, X., Fujita, N., Marcus, J., Suzuki, K., Francke, U., and Popko, B. (1996) Molecular cloning, chromosomal mapping, and characterization of the mouse UDP-galactose: ceramide galactosyltransferase gene. *Genomics* **35**, 215-222
73. Coetzee, T., Fujita, N., Dupree, J., Shi, R., Blight, A., Suzuki, K., and Popko, B. (1996) Myelination in the absence of galactocerebroside and sulfatide: Normal structure with abnormal function and regional instability. *Cell* **86**, 209-219
74. Bosio, A., Binczek, E., and Stoffel, W. (1996) Functional breakdown of the lipid bilayer of the myelin membrane in central and peripheral nervous system by disrupted galactocerebroside synthesis. *Proc. Natl. Acad. Sci. USA* **93**, 13280-13285
75. Dupree, J.L., Coetzee, T., Blight, A., Suzuki, K., and Popko, B. (1998) Myelin galactolipids are essential for proper node of Ranvier formation in the CNS. *J. Neurosci.* **18**, 1642-1649
76. Bosio, A., Binczek, E., Haupt, W.F., and Stoffel, W. (1998) Composition and biophysical properties of myelin lipid define the neurological defects in galactocerebroside- and sulfatide-deficient mice. *J. Neurochem.* **70**, 308-315
77. Van der Bijl, P., Strous, G.J., Lopes-Cardozo, M., Thomas-Oates, J., and Van Meer, G. (1996) Synthesis of non-hydroxy-galactosylceramides and galactosyldiglycerides by hydroxy-ceramide galactosyltransferase. *Biochem. J.* **317**, 589-597
78. Honke, K., Tsuda, M., Hirahara, Y., Ishii, A., Makita, A., and Wada, Y. (1997) Molecular cloning and expression of cDNA encoding human 3′-phosphoadenylylsulfate:galactosylceramide 3′-sulfotransferase. *J. Biol. Chem.* **272**, 4864-4868
79. Nagata, Y., Yamashiro, S., Yodoi, J., Lloyd, K.O., Shiku, H., and Furukawa, K. (1992) Expression cloning of β1,4 *N*-acetylgalactosaminyltransferase cDNAs that determine the expression of GM2 and GD2 gangliosides. *J. Biol. Chem.* **267**, 12082 12089
80. Takamiya, K., Yamamoto, A., Furukawa, K., Yamashiro, S., Shin, M., Okada, M., Fukumoto, S., Haraguchi, M., Takeda, N., Fujimura, K., Sakae, M., Kishikawa, M., Shiku, H., and Aizawa, S. (1996) Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. *Proc. Natl. Acad. Sci. USA* **93**, 10662-10667