Exon	Sense (5′→3′)	Antisense (5′→3′)	PCR Size, bp
1	caggccgtgggtccttagtcaagt	gccagcctgtcccctagcaatg	204
2	gctactctcaaaggatcggcttctgaaa	tatcttctctccagagtgggtgttcagg	303
3	gccttctccctcttatccatgtgttagc	taaaagacacctgtgctgggtacagtcc	401
4	ccccttgtcccttgaagcttttattctt	tgtatttttagtaggggcgaggttttgc	347
5	agtttacgaatttgtgttgggccacatt	gccttcccaaatgcaattgaactaaaag	358
6	aggatctcctcatttttccctgctcttt	atgaaaaatctcaatctgcccatgacac	330
7	actaacattctgaccgtagcagggcttc	tcattcacatgtccagaatggctatgac	325
8–9	ctttacacctgtcatagatggggcattg	cacacccctcctcaaattaatcaacaga	412
10	cgtctgtgtctcccaacaagtggtttta	gtgagttcaaaagaggctctgtccaaga	312
11	gcactgttgagtctttgaccttgctttc	ttcgcagaaaaataacgaaccaattcct	301
12	gggagtagatggagaggactgaaggaga	ggatctgatgcatttgcttaccattttg	391
13	ggaggtggaggaagattttcattcctta	ctgaaaaggtgagcaaagaccccaaat	343
14	tctccttgctgaccttcttaccctcaat	tattttacccaggctggtcttgaactcc	348
15	atttcgaggttcatttcctgttggtgtt	aagtttaggcctgaattcaaacccttcc	427
16	ggggttgatggttctctgtctctctctc	gaaacctcaggtgaaaatgcacatccta	467

The PCR product, confirmed by 1% agarose gel electrophoresis, was again denatured at denatured at 95°C for 5 minutes, cooled down at room temperature for 45 minutes, and subjected to denaturing high-performance liquid chromatography (DHPLC) using the WAVE DNA Fragment Analysis System (Transgenomic, Omaha, NE). Each sample was automatically loaded on a DNAsep column (Transgenomic) and eluted with a linear acetonitrile gradient, consisting of buffer A (0.1 *M* triethylammonium acetate, pH 7.0) and buffer B (buffer A with 25 percent v/v acetonitrile; HPLC grade) at the temperature for heteroduplex detection using the Stanford Genome Technology Center software (http://insertion.stanford.edu/melt.html) or WAVEMAKER software (Transgenomic). The eluted DNA fragments were detected by a UV-C detector (Transgenomic). Any abnormal heteroduplex pattern obtained by DHPLC was reamplified and subjected to direct sequencing.

Once a mutation is established for a family with β-galactosidase deficiency, rather simple procedures can be taken for rapid genetic diagnosis. The mutation is easily detected by restriction digestion, allele-specific oligonucleotide hybridization, single-strand conformation polymorphism, or an amplification-refractory mutation system in combination with PCR amplification.

In fact, restriction analysis methods have been developed for several mutations, including the common mutations described earlier. The *Bsu*36I restriction analysis has been performed for gene diagnosis of 14 adult/chronic-form G_{M1} gangliosidosis patients from 10 different Japanese families.[63] The result showed

that all 14 patients examined had a common single-base substitution of I51T. Thirteen were homozygotes and one was a compound heterozygote of this common mutation associated with another base substitution.

Restriction analysis is possible for many β-galactosidase gene mutations, and other simple screening methods also are available (Table 151-8). They can be applied for detecting heterozygous carriers as well as affected patients.

Table 151-8: Restriction Analysis and Screening of the Mutant β-Galactosidase Gene

Restriction enzyme			Screening		
Mutation	**Site[a]**	**Enzyme**	**Ref**	**Method**	**Ref**
R49C	–	*Bbv*I/*Bso*Fi		ASO	297
I51T	+	*Bsu*361/*Sau*I	23, 58		
T82M	+	*Ms*lI		ASO	298
Y83H	+	*N/a*III			
G123R	–	*Sma*I/*Apa*I			
R201C	–	*Bsp*MI	23, 264		
R201H	–	*Hha*I		SSCP	304
R208C	N			ASO	300, 303
P263S	+	*Bam*HI			
N266S	+	*Cta*I		SSCP	304
W273L	+	*Stu*I[b]	24		
Y316C	N				
N318H	+	*Nta*III			
S434L	–	*Mbo*II			
R457X	N				
R457Q	N				
R482H	+	*Nsp*I	24		
R482C	–	*Aft*III		ASO	299
G494C	N				
W509C	+	*Rsa*I	24		
K577R	N			ASO	303
R590H	N			ASO	300
E632G	N			ASO	303

[a] + = restriction site generated; – = restriction site eliminated; N = neither generated nor eliminated.

[b] Restriction site produced by adding a sedond mutation by PCR.

ASO = allele-specific oligonucleotide hybridization (= dot blot analysis); SSCP = single-strand conformation polymorphism.

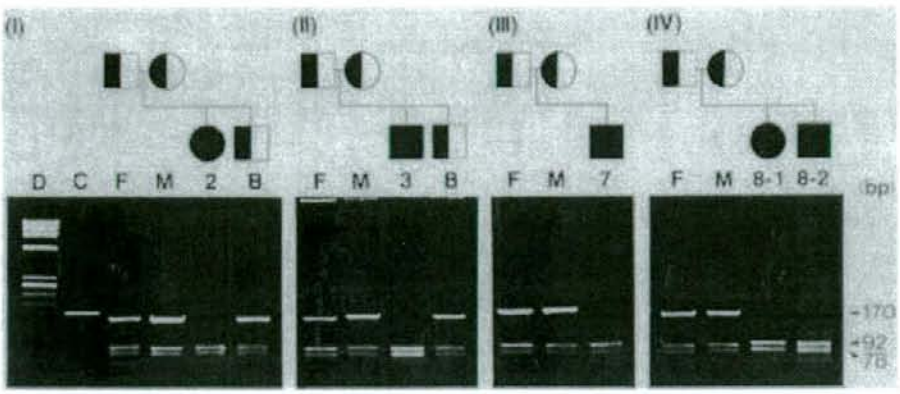
Heterozygous Carrier Diagnosis

Carrier detection is important for genetic counseling and prenatal diagnosis. Reliability of enzymatic heterozygote detection depends on two factors: (1) accuracy of assays and (2) variation of enzymatic activity among a general population and a variation in a single individual under the influence of age and physical conditions. Appropriate precautions and careful standardization of the enzyme assay are necessary.

When a sufficiently large number of specimens are examined, the average of β-galactosidase activities of known heterozygotes gives an intermediate value between normal subjects and affected patients with a high statistical significance (see Fig. 151-10 *A* and *B*). However, varying degrees of overlap occur between normal homozygotes and heterozygous carriers. It is well known that the enzyme diagnosis for heterozygous carriers is not always reliable.

Gene diagnosis will provide a more reliable method for the detection of heterozygous carriers if an appropriate method is available for a certain mutant gene. The diagnosis of I51T mutation heterozygotes was established in eight parents and two siblings from four different Japanese families with adult/chronic-form G_{M1} gangliosidosis[63] (Fig. 151-11). Heterozygous carriers are diagnosed by restriction analysis or allele-specific oligonucleotide hybridization for known gene mutations. Single-strand conformation polymorphism also is useful for screening of an unknown mutation, although it does not provide information about the mutant gene structure (see Table 151-8).

Fig. 151-11:



Restriction analysis of the family members of adult GM1 gangliosidosis patients. The lane number corresponds to the family number in Fig. 151-9. F = father; M = mother; B = brother; C = normal control; D = DNA markers (ÄÅ¸x174 phage DNA/HaeII digest). (Reproduced from Yoshida et al. 63 Used by permission.)

Prenatal Diagnosis

Assay of β-galactosidase activity in cultured amniotic fluid cells or chorionic villi is used for prenatal diagnosis, and it has been performed successfully for G_{M1} gangliosidosis.[381-386] A rapid diagnosis was made possible by microchemical assays[384]; the use of 10 to 30 freeze-dried cells for single-enzyme assays requires only a few hundred cells growing within 9 to 12 days of amniocentesis. However, special microassay methods have become unnecessary because of the development of chorionic villi sampling for rapid prenatal diagnosis with sufficient amounts of cells from fetuses. A survey was made on the first trimester prenatal diagnosis of metabolic diseases using chorionic villi in countries from the European Community.[387] As of December 1985, 258 diagnoses were made for 38 different metabolic diseases; 56 affected fetuses (22 percent) were detected. Among them, 11 fetuses were subjected to prenatal diagnosis of G_{M1} gangliosidosis: one in Denmark, six in France, one in Italy, and three in the United Kingdom. One affected patient was found in France.

An early prenatal diagnosis was accomplished by analyzing galactosyl-oligosaccharides accumulating in amniotic fluid at 14 weeks' gestation with HPLC.[375] The pattern of amniotic oligosaccharides was almost identical to that in the urine from postnatal G_{M1} gangliosidosis patients, but the concentration was about one-fifth that in urine. Accordingly, a highly sensitive assay method is necessary for prenatal diagnosis.

The diagnosis of affected fetuses was confirmed by histologic demonstration of MCBs in spinal cord motor neurons[383, 388] or basal ganglia,[385] zebra bodies in dorsal root ganglion cells,[381] pleomorphic electron-dense bodies in axons,[389] or membrane-limited vacuoles in Schwann cells.[389] Vacuolation of hepatocytes and renal glomerular and tubular epithelial cells was recorded in a fetus affected with infantile G_{M1} gangliosidosis.[389] MCBs were abundant in retinal ganglion cells.[390] Marked retinal lesions were reported in two other patients.[391, 392]

Biochemical analysis revealed an increase in G_{M1} in the fetal brain[383, 386, 392] and β-galactosidase deficiency in liver or brain.[381, 383] An affected fetus was aborted with intrauterine hypertonic saline instillation.[383] Fetal tissues were adequate for subsequent biochemical diagnosis.

ANIMAL MODELS

Animal models of G_{M1} gangliosidosis have been recorded in cats,[393-399] dogs,[400-403] sheep,[404-407] and calves.[408-410] They are domestic animals with spontaneously occurring storage diseases. Although inbred strains of laboratory rodents had not been reported, mouse models of G_{M1} gangliosidosis have been created recently through targeted disruption of the β-galactosidase gene.[411-413] All the model animals show central nervous system manifestations, with a specific deficiency of β-galactosidase.

Feline G_{M1} Gangliosidosis

A neurologic disorder with clinical, morphologic, biochemical, and genetic similarities to human G_{M1} gangliosidosis has been described in two families of Siamese cats,[359, 393, 394, 397, 398, 414, 415] in two families of short-haired domestic cats,[189, 395, 396, 416] and in a Korat cat.[399] Affected kittens appear normal at birth, and tremors of the head and hind limbs are first noted at 2 to 3 months of age, followed by generalized dysmetria and spastic quadriplegia. Affected cats show exaggerated acousticomotor response, impaired vision, and generalized convulsive seizures by 1 year of age. This disease is transmitted as an autosomal recessive trait.

A 7-month-old Korat cat was referred for a slowly progressive neurologic disease.[399] β-Galactosidase activity was very low in peripheral leukocytes, brain, and liver. Histologically, diffuse vacuolization and enlargement of neurons were observed throughout the brain, spinal cord, and peripheral ganglia, and there was severe cerebellar neuronal cell loss with moderate astrocytosis. Ultrastructurally, neuronal vacuoles were filled with concentrically whorled lamellae and small membrane-bound vesicles. A striking increase in G_{M1} was found in the brain. D. Maria and colleagues concluded that clinical onset, morphologic and histochemical features, and biochemical findings in the Korat cat G_{M1} gangliosidosis were comparable with with those in the human juvenile form, although the clinical course, survival time, and residual enzyme activity level did not completely fit those of the human disease.

A widespread neuronal vacuolation is observed microscopically in brain, retina, spinal cord, and peripheral ganglia. Numerous MCBs are observed electron microscopically, with multiple concentric lamellae simulating those in the human disease. Lesions outside the nervous system are limited to hepatocellular vacuolation and aspermatogenesis.

Neutral oligosaccharides are markedly increased in the tissues and urine of affected cats.[189] The core structure of a hexasaccharide has been identified as Galβ-GlcNAc-Man-Man-GlcNAc with an additional *N*-acetylglucosamine.[188, 190] Four major types of oligosaccharides were proposed by Barker and colleagues.[189] Their structures suggest that they arise from incomplete catabolism of *N*-glycans of glycoproteins.

A family of Siamese cats[359, 393, 414, 415] that was extensively investigated clinically and biochemically is linked to late infantile/juvenile G_{M1} gangliosidosis in humans.[190, 396, 417] The cats developed normally until the age of 4 months, and then neurologic manifestations followed. Physical appearance was normal, and no hepatosplenomegaly was observed. Electrodiagnostic tests revealed that motor and sensory nerve

conduction velocities remained within normal limits, but spinal-evoked potentials indicated slowing in conduction velocity, and brain stem auditory-evoked responses indicated prolonged latencies.[418] Some residual enzyme activity of β-galactosidase was detected in brain (15 percent of normal).[393] β-Galactosidase deficiency has been demonstrated in brain, kidney, liver, spleen, skin, cultured fibroblasts, and cultured conjunctival cells[419] associated with remarkable accumulation of ganglioside G_{M1}. The mutant enzyme has a reduced K_m for 4-methylumbelliferyl β-galactoside, high K_m for G_{M1} and asialofetuin, increased thermostability, and a shift of isoelectric point.[417]

Several investigations into the pathogenesis of neuronal dysfunction have been performed using this feline model of human disease. These showed (1) increased gangliosides, cholesterol, and phospholipids in synaptosomal membranes,[354] (2) reduced fluidity of synaptosomal membranes,[354] (3) meganeurites between the soma and initial axon segment and aberrant secondary neurites,[348, 349] suggesting a possible role for gangliosides in neuronal differentiation and synaptogenesis,[346, 348] (4) a gradient in total ganglioside and G_{M1} concentration and the proportion of docosanoate (22:0) in G_{M1}, corresponding to the morphologic gradient of meganeurite formation (cerebral cortex > caudate = thalamus > cerebellum),[353] (5) increased acetylcholine synthesis and release in cerebral cortex and hippocampal slices,[358, 359] (6) a decrease in high-affinity uptake of glutamate, g-aminobutyrate, and norepinephrine in synaptosomes,[361] (7) alteration of phospholipase C and adenyl cyclase activities,[363] (8) alteration of evoked synaptic activity patterns in cortical pyramidal neurons,[420] (9) reduced calcium flux in synaptosomes,[421] (10) quantitatively and qualitatively normal phorbol ester receptors in the brain,[422] and (11) common occurrence of glutamic acid decarboxylase–immunoreactive spheroids in many brain regions, whereas limited in distribution in other diseases.[362] These data suggest that various morphologic and metabolic aberrations occur in the presence of excessive storage of ganglioside G_{M1}.

Cox and colleagues[397] observed that thymuses from affected cats older than 7 months of age (G_{M1} mutant cats) show marked thymic reduction in size (approximately threefold) compared with age-matched normal cats. Histologic sections of lymph nodes, adrenal glands, and spleens showed no significant differences. Flow cytometric analysis showed a marked decrease in the percentage of immature CD4+CD8+ thymocytes and an increase of CD4–CD8+ cells in mutant cats. Colabeling with CD4, CD8, and CD5 indicated an increase in the percentage of CD5-high thymocytes, suggesting the presence of more mature cells. In peripheral lymphocytes there was an increase in CD4–CD8+ cells and a slight decrease in CD4+CD8– and CD4–CD8– cells. Cats younger than seven months showed no abnormalities in thymocyte or lymphocyte subpopulations. However, surface G_{M1} expression of thymocytes detected by cholera toxin B binding was increased at all ages in affected cats. Also, in situ labeling for apoptosis was increased between 3 and 7 months of age when thymic masses were within normal limits. After 7 months, the labeling was decreased. These data indicate premature thymic involution in feline G_{M1} gangliosidosis and suggest that increased surface G_{M1} alters thymocyte development in these cats.

In another experiment, incorporation of exogenous G_{M1} into feline thymocyte cell membranes produced a dose-dependent increase of apoptotic cell death.[423] Although CD4 expression on both feline thymocyte and lymph node cell membranes was abruptly decreased after introducing exogenous G_{M1}, enhanced apoptotic death was observed only in thymocytes and not in lymph node cells at the same G_{M1} concentration. Enhancement of thymocyte apoptosis appears to be age-related; the cells from cats younger than 3 months were more vulnerable.

Growth hormone (GH) and insulin-like growth factor I (IGF-I) are important hormonal peptides supporting thymic function and affecting body growth, particularly in the prepubescent period. In G_{M1} gangliosidosis cats, serum IGF-I was low by 5 months of age, and a peripubertal increase was not observed.[398] In addition, IGF-binding protein 3 was reduced and IGF-binding protein 2 was elevated after 7 months. mRNA for hepatic IGF-I and pituitary GH was significantly reduced. Stimulation by exogenous recombinant canine GH elevated serum IGF-I. These results suggested that GH/IGF-I signaling pathways in the liver remain intact and that alterations are external to the liver.

Canine G_{M1} Gangliosidosis

The canine disease has been described in mixed-breed beagle dogs,[400] English springer spaniels,[402, 424, 425] Portuguese water dogs,[403, 426] Alaskan huskies,[427, 428] and Shiba dogs.[429, 430] Two canine models, the English springer spaniel and the Portuguese water dog, have been compared.[424] The clinical course and severity of skeletal dysplasia and progressive neurologic impairment were similar in these models. The skeletal lesions are similar to those in a child with G_{M1} gangliosidosis.[431] However, dwarfism and coarse facial features were seen only in English springer spaniels; glycoproteins containing polylactosaminoglycans were found in visceral organs. Portuguese water dogs did not show these clinical or biochemical changes. It seems difficult and inappropriate to apply clinical classification of G_{M1} gangliosidosis in humans to these model animals.[424] Dysostosis multiplex, dwarfism, and orbital hypertelorism with coarse facial features were found in the cases reported by Alroy and colleagues,[402] probably representing a model of infantile G_{M1} gangliosidosis.

Pathologic and biochemical changes are similar to those in the feline cases described earlier.[432] The ultrastructural and biochemical features suggested that the disease in the first reported dog had features similar to both the infantile and juvenile forms of human G_{M1} gangliosidosis (see Table 151-1).[401] Serial MRI studies revealed an abnormal signal intensity of cerebral and cerebellar white matter observed on T_2-weighted images.[432] Some biomarkers, including aspartate aminotransferase, lactate dehydrogenase, creatine kinase, neuron-specific enolase, and myelin basic protein, as well as ganglioside G_{M1}, were significantly higher in the CSF of G_{M1} gangliosidosis dogs than of control dogs, and their changes were well related to the age and clinical course.[433] These biomarkers may be used for monitoring the effect of therapeutic trials in the near future.

Analysis of the major oligosaccharides accumulating in the dog revealed that the compounds are nearly identical to the oligosaccharides stored in human G_{M1} gangliosidosis liver, but they differ from the human compounds uniquely because they contain two GlcNAc residues at the reducing terminus instead of one, suggesting that there may be significant differences in glycoprotein metabolism or structure between mammalian species.[188] Kinetic properties of the mutant enzyme in affected dogs were identical to those from normal dogs, but the amount of CRM was markedly reduced.[388]

Antiserum raised against purified human liver β-galactosidase crossreacted with the enzyme from dog liver but not with those from cat liver or *Escherichia coli*.[434] Furthermore, 21 of the 24 tryptic peptides of the dog β-galactosidase were homologous with those of the human enzyme. A partial canine β-galactosidase cDNA was 86 percent homologous to the human counterpart, and preliminary analysis of a genomic library indicated conservation of exon number and size.[435]

The acid β-galactosidase cDNA of Portuguese water dogs was isolated and sequenced.[425] The entire coding region of the gene consists of 2004 nucleotides encoding a protein of 668 amino acids—approximately 86.5 percent identity at the nucleotide level and about 81 percent identity at the amino acid level with the human acid β-galactosidase gene. The deduced amino acid sequence contains a 24-amino-acid putative signal sequence, six possible glycosylation sites, and seven cysteine residues. A homozygous recessive mutation causing canine G_{M1} gangliosidosis was identified at nucleotide G200→A in exon 2, resulting in an amino acid substitution Arg60→His (R60H). The mutation creates a new restriction site for PmlI. Genotyping 115 dog samples for this gene mutation revealed 5 affected homozygotes, 50 heterozygous carriers, and 60 normal homozygotes. Based on these observations, it was concluded that dog and human β-galactosidases are structurally similar and that canine G_{M1} gangliosidosis is an excellent model for the human disease.

Retarded endochondral ossification and osteoporosis were observed at 2 months of age in canine G_{M1} gangliosidosis and focal cartilage necrosis within lumbar vertebral epiphyses in older puppies.[431] The lesions were characterized histologically by chondrocytic hypertrophy and lysosomal accumulation of storage materials. These changes were similar to those in a human child with G_{M1} gangliosidosis. Chondrocytes from affected dogs cultured in agarose contained numerous large vacuoles and had a reduction in mitosis and Alcian blue–staining proteoglycans.[436] This culture system may be a useful method for studying the biology of cartilage leading to skeletal abnormalities in lysosomal storage diseases.

Ovine G_{M1} Gangliosidosis

An inherited disease associated with deficiencies of β-galactosidase (5 percent normal) and α-neuraminidase (20 percent normal) has been observed in sheep.[407, 437] The neuraminidase activity was not restored after addition of leupeptin in the culture medium of fibroblasts.[404, 405] Neuronal storage was much more extensive than visceral storage. Kupffer cells and macrophages from bone marrow were affected similarly to but less severely than hepatocytes and renal epithelial cells, whereas hematopoietic cells and chondrocytes were unaffected.[437] Results of lectin histochemistry suggested that the stored material in this disease has terminal saccharide moieties consisting of β-galactose, *N*-acetylneuraminic acid, and *N*-acetylgalactosamine.[438] An interspecific genetic complementation analysis indicated that the ovine β-galactosidase deficiency is due to a mutation at the genetic locus homologous with that of human G_{M1} gangliosidosis and Morquio B disease in humans.[439] A genetic defect in lysosomal β-galactosidase may cause the deficiency of lysosomal α-neuraminidase in sheep.[404, 440]

The initial clinical sign of ovine G_{M1} gangliosidosis is ataxia around 5 months of age, and neurologic deterioration follows[404, 405]: ataxia, conscious proprioceptive deficit most severe in the hind limbs, blindness, and recumbency.[441] An ovine fetus affected with G_{M1} gangliosidosis at 4 months of gestation (normal gestation 5 months) showed marked cytoplasmic enlargement and vacuolation of central and peripheral nervous system neuronal soma and of hepatocytes and renal epithelial cells.[442] This case indicates the need for prenatal initiation of therapy. Vacuolization seems to be not the single cause of clinical signs in the ovine disease because the clinical signs do not commence until at least 5 months after vacuolization is apparent histologically.

Another form of ovine G_{M1} gangliosidosis was reported recently.[406] Neurologic signs were observed at approximately 1 month of age. A specific deficiency of β-galactosidase was found, with relatively high residual activity (10 percent) compared with that described earlier. Neuraminidase activity was normal. Storage of G_{M1} and asialo-G_{M1} was not reported for this case. In both ovine cases, molecular genetics

analysis has not been performed.

During a large neuropathologic survey of clinically normal sheep for evidence of scrapie, three adult Romney ewes from the same farm were found to have populations of distended neurons containing granular eosinophilic storage material.[443] The affected neurons were confined to the striatum. The granules had the tinctorial properties of glycolipid and the ultrastructural appearance of spherical bodies containing concentric membranous whorls. The bodies resembled those of G_{M1} gangliosidosis. The restricted neuroanatomic distribution of changes, together with the age of the sheep, suggests a similarity to human type 3 G_{M1} gangliosidosis. However, no biochemical evidence has been provided for solid diagnosis of the disease.

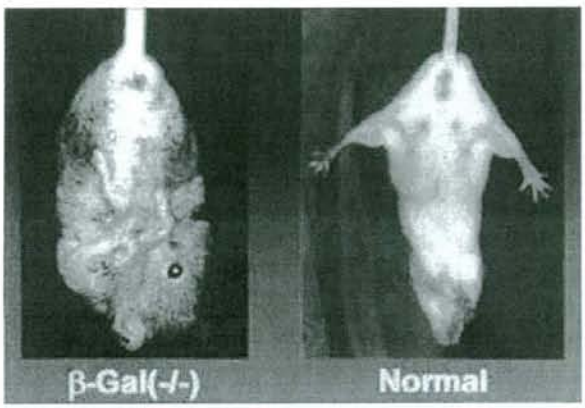
Bovine G_{M1} Gangliosidosis

Pathologic, biochemical, and enzymatic studies were performed in Friesian calves with G_{M1} gangliosidosis. G_{M1} accumulated in neurons, and β-galactosidase deficiency was confirmed.[408-410] However, morphologic hepatic changes were minimal or absent, without significant elevation of mucopolysaccharides.[444] Vision is impaired at the late stage of the disease. The ocular lesions were confined to the retina and optic nerves.[445] Retinal ganglion cells and amacrine cells in the inner nuclear layer are distended with dense aggregates of MCBs. Wallerian degeneration is present in the optic nerves.

Murine G_{M1} Gangliosidosis Generated by Gene Targeting (Knockout Mouse)

Gene targeting in embryonic stem cells generated mouse models[411-413] for conventional laboratory use. Homozygous mutants were born normally and apparently healthy until 4 months of age, although poor achievement on the water maze test was observed in some mutant mice after 2 months. Then horizontal movement became slow, and rearing or vertical climbing became less frequent. By 6 to 8 months, definite gait disturbance with mild shaking was observed, and spastic diplegia progressed. When hung vertically with the tail held upward, the mice huddled themselves with all four limbs flexed (Fig. 151-12). Normal mice always extended their limbs in the downward vertical direction. At the terminal stage, generalized and progressive paralysis appeared, and the affected mice died of extreme emaciation at 7 to 10 months of age. Hepatosplenomegaly or skeletal dysplasia is not detected. About half the homozygote pairs could produce at least one litter; thus the knockout mice could be maintained as homozygous mutants.[412]

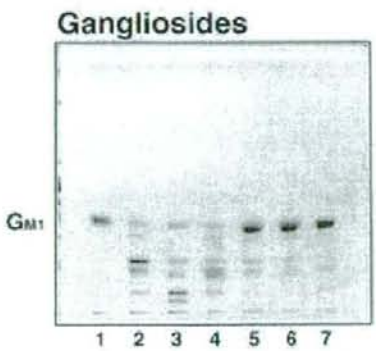
Fig. 151-12:



Abnormal posture of a β-galactosidase-deficient mouse in the downward vertical position. A, An affected β-Gal(–/–) mouse (8 months old) huddles himself with all limbs flexed and paws clenched. B, A normal β-Gal(+/+) littermate with normal reaction. (Reproduced from Matsuda et al. 411 Used by permission.)

Three biochemical markers confirmed that the affected mice are authentic models of human G_{M1} gangliosidosis: generalized β-galactosidase deficiency, abnormal storage of G_{M1} in brain and other tissues (Fig. 151-13), and hyperexcretion of urinary oligosaccharides.

Fig. 151-13:



Thin-layer chromatographic pattern of mouse brain gangliosides. Resorcinol stain. Lane 1 = GM1 (reference standard); 2 = cerebrum (wild type); 3 = brain stem (wild type); 4 = cerebellum (wild type); 5 = cerebrum (knockout); 6 = brain stem (knockout); 7 = cerebellum (knockout). A remarkable increase in GM1 is observed in three different areas of the knockout mouse. (Reproduced from Matsuda et al. 412

Used by permission.)

In a pathologic study,[446] vacuolated neurons appeared in the spinal cord 3 days after birth. The vacuolation extended to neurons in the brain stem, cerebral cortex, hippocampus, and thalamus, and ballooning neurons became prominent with age. The vacuolation also appeared in Purkinje cells without marked ballooning. Reactive astrogliosis was prominent in the entire brain at the terminal stage of the disease. Immunohistochemical study using anti-G_{M1} and anti-G_{A1} antibodies revealed extensive accumulation of G_{M1} and G_{A1} in the cerebral neurons. In the liver, however, accumulation of G_{M1} was localized to the cytoplasm of hepatocytes, whereas that of G_{A1} was localized to foamy macrophages and Kupffer cells. There were no significant abnormalities in the bone, bone marrow, or cornea at any stage. HPTLC analysis showed that the G_{M1} content of the mutant mouse brain was not pathologically increased at birth. An increase in G_{M1} and asialo-G_{M1} occurred rapidly to the same degree of storage. This remarkable accumulation of asialo-G_{M1} may be explained by a metabolic turnover of G_{M1} to asialo-G_{M1} catalyzed by a specific neuraminidase in mice. A recent report has confirmed that intact and living cells of a neurotumoral nature express a neuraminidase that hydrolyzes ganglioside G_{M1} and G_{M2}.[447] This degradative process seems to occur in lysosomes because it is blocked by the conditions preventing endocytosis or inhibiting lysosomal enzyme activities.

Skeletal dysplasia is observed in human patients with infantile G_{M1} gangliosidosis and Morquio B disease but not in mutant mice. Urinary mucopolysaccharides were normal in amount and pattern in the mutant mice. The mouse does not synthesize keratan sulfate in the skeletal system.[448] In another report, the mouse rib cartilage contained only small amounts of keratan sulfate of extremely small size.[449] This may explain an apparent lack of skeletal dysplasia in mutant mice, although more time may be necessary for development of generalized skeletal dysplasias.[412]

We further produced a transgenic (Tg) mouse line overexpressing mutant human β-galactosidase with an amino acid substitution (R201C) that causes juvenile type G_{M1} gangliosidosis in humans by injecting the DNA fragment containing β-actin promoter (CAG promoter) and human mutant β-galactosidase cDNA (R201C) into C57BL/6 fertilized eggs. Cross-breeding of the KO mice and Tg mice was performed to obtain KO-Tg mice (R201C mice). We used hemizygous Tg mice with the β-galactosidase KO background for the study decribed earlier in the experiments of chemical chaperone therapy.[339] The R201C mice, expressing the human R201C mutant but lacking the endogenous mouse β-galactosidase, had very low β-galactosidase activity in the brain (~4 percent of the wild-type activity). They exhibited an apparently normal clinical course for the first 6 months after birth, and this was followed by slowly progressive neurologic deterioration, such as tremor and gait disturbance, during the next 9 months. Death ensued around 15 months of age owing to malnutrition and emaciation. Neuropathologic examination revealed vacuolated or ballooned neurons, but they were less abundant than those in the KO mouse brain. Intracytoplasmic storage materials were present in pyramidal neurons and brain stem motor neurons but not in neurons in the other areas of the brain. There was no storage in glial cells.

TREATMENT

Therapeutic experiments using cultured cells have been reported to enhance the uptake of the exogenous enzyme and to prolong its intracellular half-life. A thiol protease inhibitor prolonged the effect of exogenous β-galactosidase in human G_{M1} gangliosidosis fibroblasts.[258] Enzyme replacement of cultured cells from cats with G_{M1} gangliosidosis was tried with the liposome-entrapped enzyme, and the storage of glycopeptides decreased.[450]

Allogeneic bone marrow transplantation was performed in an 81-day-old Portuguese water dog affected with G_{M1} gangliosidosis using a dog leukocyte antigen–identical sibling as donor.[450] Complete engraftment was achieved, and β-galactosidase activity in leukocytes of the transplanted dog was similar to that in the donor. However, neither the subsequent clinical course nor the enzyme activity was modified. It was concluded that bone marrow transplantation early in life is ineffective in canine G_{M1} gangliosidosis.

Amniotic tissue transplantation was tried on a patient with Morquio B disease.[451] No clinical improvement was observed. Only conservative therapy has been performed for human G_{M1} gangliosidosis patients. Dystonia was markedly improved dose-dependently by oral administration of trihexyphenidyl in an adult G_{M1} gangliosidosis patient.[452] No side effects were observed. The extrapyramidal signs in adults with G_{M1} gangliosidosis are probably caused by hypofunction of dopaminergic neurons or relative hyperactivity of cholinergic neurons in the basal ganglia.

In our experiment with adenovirus-mediated gene transfer to newborn knockout mice, β-galactosidase increased to 10 to 20 percent of normal activity in the brain for 60 days, and the G_{M1} storage was significantly prevented. We concluded that neonatal administration via blood vessels provided access to the central nervous system because of the incompletely formed blood-brain barrier.[453]

Chemical Chaperone Therapy

In recent years, we have tried a new molecular therapeutic approach to lysosomal diseases. Some mutant enzyme proteins were labile and rapidly degraded in somatic cells from patients with Fabry disease with genetic α-galactosidase A deficiency.[454, 455] Their catalytic activities were stabilized and restored in culture cells by addition of galactose[456] or 1-deoxygalactonojirimycin[457] in the culture medium. A short-term galactose infusion therapy was found to be clinically effective in a Fabry patient.[458] This paradoxical phenomenon was confirmed also for β-mutant galactosidasae with 1-deoxygalactonojirimycin.[459] In addition, newly synthesized in vitro enzyme inhibitors *N*-octyl-4-epi-β-valienamine (NOEV) and *N*-octyl-β-valienamine (NOV) remarkably restored mutant enzyme activities in cultured fibroblasts from some patients with β-galactosidase and β-glucosidase deficiency disorders (G_{M1} gangliosidosis and Gaucher disease), respectively.[339, 460] They are actually specific competitive inhibitors of respective lysosomal enzymes in vitro, and they serve as stabilizers of mutant proteins in the cell (chemical chaperone).[461-463]

The Tg mouse expressing the mutant β-galactosidase gene (R201C) causing juvenile G_{M1} gangliosidosis was fed orally with NOEV water solution for a week. All tissues, including the central nervous system, showed a marked increase in enzyme activity.[339] Immunohistochemical stain revealed a decrease in the amount of G_{M1} and G_{A1} in neuronal cells in the frontotemporal cerebral cortex and brain stem. Subsequently, we confirmed that the chaperone NOEV, after oral administration and intestinal absorption into the bloodstream, reached the mouse brain through the blood-brain barrier and enhanced the mutant β-galactosidase activity, resulting in substrate digestion and clinical improvement.[464] No specific adverse effects have been observed for at least 6 months of continuous oral administration in experimental mice.

This is the first achievement for developing a new therapeutic approach by oral medication directly and specifically targeting the brain pathology in lysosomal storage diseases. This new molecular therapy (chemical chaperone therapy) will be useful for certain patients with β-galactosidosis and potentially other lysosomal storage diseases with central nervous system involvement.[339]

A clinical survey using cultured fibroblasts for selection of patients for this approach indicated that NOEV chaperone therapy will be effective in 20 to 40 percent of the patients, particularly in juvenile and infantile G_{M1} gangliosidosis patients.[338] The effect is mutation-specific. Most effective mutations are R201C, R201H, and R457Q. NOEV treatment at the early stage of the disease resulted in an arrest of disease progression within a few months in R201C mice[464] based on a newly developed clinical assessment method for monitoring brain damage in experimental mice.[465]

NOV is the second compound for chemical chaperone therapy in our study. Apparently deficient β-glucosidase activity was enhanced by NOV in some Gaucher fibroblasts.[460, 466] Similar therapeutic approaches have been reported recently from other laboratories for Pompe disease using deoxynojirimycin and *N*-butyldeoxynojirimycin[467, 468] and for G_{M2} gangliosidosis using pyrimethamine[469] as promising chaperones. We expect that they also will serve as new chaperone drugs for these diseases.

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