Ⅳ．研究成果による特許権等の知的財産権の出願・登録状況

1）発明名称　カルバ糖アミン誘導体
発明者　小川誠一郎、鈴木義之
出願日　2001年9月7日
出願人　生化学工業株式会社
出願番号　特願２００１－２７２７７５号
内容の概略　ガラクトース類似化合物、N-octyl-4-epi-β-valienemine (NOEV) がβ－ガラクトシダーゼの阻害活性を持つ。出願公開中。

2）発明名称　糖脂質代謝異常症の治療薬
発明者　鈴木義之、大野耕策
出願日　2001年9月7日
出願人　生化学工業株式会社
出願番号　特願２００１－２７２７７７号
内容の概略　グルコース類似化合物、N-octyl-β-valienemineがβ－グルコシダーゼの阻害活性を持ち、細胞内で変異酵素蛋白質を安定化、活性発現を誘導する。出願公開中。

3）発明名称　糖脂質代謝異常症治療剤
発明者　鈴木義之、難波栄二、松田潤一郎
出願日　2002年9月5日
出願人　生化学工業株式会社
出願番号　特願２００２－２６０５３４号
内容の概略　ＮＯＥＶが試験管内でβ－ガラクトシダーゼ活性を阻害し、細胞内で変異分子を安定化、活性発現を誘導する。出願公開中。

4）発明名称　Carba-sugar amine derivatives and treatments for disorder of glycolipid metabolism containing the same as the active ingredient
発明者　小川誠一郎、鈴木義之、難波栄二
出願日　2002年9月2日
出願人　生化学工業株式会社
出願国　米国、カナダ、オーストラリア、ヨーロッパ（全加盟国）
出願番号　PCT/JP02/08882；米国（10/793857）、カナダ（2459887）、オーストラリア（2002328402）、ヨーロッパ（全加盟国）（02762961.7）
内容の概略　上記の３つの特許に先行するＮＯＶ国内特許申請内容を含め、２つの化合物の構造特許、機能特許申請をまとめて行った。出願公開中。

5）発明名称　Compuestos potenciadores de la actividad de glicosidasas mutantes
(変異グリコシダーゼ活性を上昇させる化合物)
発明者　Jose Garcia Fernandez, Carmen Ortiz Mellet, M. Isabel Garcia Moreno, Matilde Aguilar Moreno, 鈴木義之, 大野耕策
出願日　2008年10月23日
出願人　セビリア化学研究所、セビリア大学、国際医療福祉大学、鳥取大学
出願国　スペイン
出願番号　P0200802988
内容の概略　二環系アザ糖誘導体が試験管内でグリコシダーゼ阻害活性をもち、細胞内でシャペロン活性を発現する。出願中。

V．健康危険情報　　なし

研究成果の刊行物・別刷

Scriver's OMMBID The Online Metabolic & Molecular Bases of Inherited Disease
Editors: Valle • Beaudet • Vogelstein • Kinzler • Antonarakis • Ballabio
Editors Emeritus: Scriver • Childs • Sly
Part Editors: Bunz • Gibson • Mitchell

PART 16: LYSOSOMAL DISORDERS

Chapter 151: β-Galactosidase Deficiency (β-Galactosidosis): G_{M1} Gangliosidosis and Morquio B Disease

Yoshiyuki Suzuki, Eiji Nanba, Junichiro Matsuda, Katsumi Higaki, Akihiro Oshima

Abstract

Hereditary deficiency of lysosomal acid β-galactosidase (β-galactosidosis) is expressed clinically as two different diseases, G_{M1} gangliosidosis (OMIM 230500) and Morquio B disease (OMIM 253010). The mode of inheritance is autosomal recessive. G_{M1} gangliosidosis is a neurosomatic disease occurring mainly in early infancy (infantile form, type 1). Developmental arrest is observed a few months after birth, followed by progressive neurologic deterioration and generalized rigospasticity with sensorimotor and psychointellectual dysfunctions. Macular cherry-red spots, facial dysmorphism, hepatosplenomegaly, and generalized skeletal dysplasia are usually present in infantile cases. Cases of later onset have been described as the late infantile/juvenile form (type 2) or adult/chronic form (type 3). They are observed as progressive neurologic diseases in children or young adults. Dysmorphic changes are less prominent or absent in these clinical forms, although vertebral dysplasia is often detected by radiographic studies. No specific neurologic manifestations are known for late infantile/juvenile patients with G_{M1} gangliosidosis. Extrapyramidal signs of protracted course, mainly presenting as dystonia, are the major neurologic manifestation in adults with G_{M1} gangliosidosis.

Morquio B disease is a clinically mild phenotype of Morquio A disease. It is expressed as generalized skeletal dysplasia with corneal clouding, resulting in short stature, pectus carinatum (sternal protrusion), platyspondylia, odontoid hypoplasia, kyphoscoliosis, and genu valgum. There is no central nervous system involvement, although spinal cord compression may occur at the late stage of the disease. Intelligence is normal, and hepatosplenomegaly is not present. X-ray changes are of pathognomonic significance.

There is diffuse atrophy of the brain in patients with early-onset G_{M1} gangliosidosis. Neurons are filled with numerous membranous cytoplasmic bodies (MCBs), and inclusions of other types are observed in glial cells: pleomorphic lipid bodies, membranovesicular bodies, or large, compact oval deposits. There are histiocytes with distended cytoplasm in visceral organs. Cytoplasmic inclusions observed under electron microscopy are different from MCBs in neurons. They are vacuoles filled with fine granular, tubular, or amorphous osmiophilic material. These changes are less prominent in cases of mild phenotypic expression.

Glycoconjugates with terminal β-linked galactose are increased in tissues and urine from patients with G_{M1} gangliosidosis and Morquio B disease. Ganglioside G_{M1} and its asialo derivative G_{A1} accumulate in the G_{M1} gangliosidosis brain. High amounts of oligosaccharides derived from keratan sulfate and glycoproteins have been reported in visceral organs and urine from G_{M1}

gangliosidosis or Morquio B disease patients. Undersulfated keratan sulfate also has been described.

Two lysosomal enzymes are known for hydrolysis of terminal β-linked galactose at acidic pH in various glycoconjugates. One is an enzyme usually called β-galactosidase (EC 3.2.1.23), catabolizing ganglioside G_{M1}, galactose-containing oligosaccharides, keratan sulfate, and other β-galactose-containing glycoconjugates (G_{M1} β-galactosidase). The enzyme activity is markedly reduced or almost completely deficient in cells and body fluids from patients with β-galactosidosis. Heterogeneous kinetic or physicochemical properties have been found in the mutant enzymes. The degree of substrate storage and residual enzyme activity is correlated with the severity of each clinical phenotype; infantile G_{M1} gangliosidosis shows the highest substrate storage and the lowest residual enzyme activity compared with other, milder phenotypes. Distribution of the substrate storage in Morquio B disease is different from that in G_{M1} gangliosidosis. The major storage material in Morquio B disease is keratan sulfate and its partial degradation products. The second genetically different β-galactosidase is galactosylceramidase (galactocerebrosidase; EC 3.2.1.46), catabolizing galactosylceramide, galactosylsphingosine, and other lipid compounds. Genetic deficiency of this enzyme results in globoid cell leukodystrophy, which is another neurometabolic disease.

The human β-galactosidase gene (*GLB1*) has been mapped on chromosome 3 (3p21.33). The cDNA codes for a protein of 677 amino acids, including a putative signal sequence of 23 amino acids and 7 potential asparagine-linked glycosylation sites. The gene *GLB1* spans more than 60 kb and contains 16 exons. It produces two alternatively spliced transcripts that encode the lysosomal enzyme β-galactosidase (GLB1) and the 67-kDa enzymatically inactive elastin-binding protein (EBP). The promoter has the characteristics of a housekeeping gene, with GC-rich stretches and five SP1 transcription elements on the two strands. Mutations at the *GLB1* locus may affect either both proteins or GLB1 only. The mutation of EBP contributes to the specific features of G_{M1} gangliosidosis phenotype, such as cardiomyopathy and connective tissue abnormalities. Heterogeneous gene mutations have been found in all clinical forms of β-galactosidosis, such as missense/nonsense mutations, insertion/duplication mutations, and insertions causing splicing defects. Neither the type nor location of the mutation in the gene is correlated with the clinical phenotype. Five common mutations have been known: R482H in Italian patients with infantile G_{M1} gangliosidosis, R208C in American patients with infantile G_{M1} gangliosidosis, R201C in Japanese patients with juvenile G_{M1} gangliosidosis, I51T in Japanese patients with adult G_{M1} gangliosidosis, and W273L in Caucasian patients with Morquio B disease. Restriction analysis has been performed successfully for diagnosis of the common mutations in new patients.

Morphologic, pharmacologic, and biochemical aberrations have been found in the brains of G_{M1} gangliosidosis patients and animals. Meganeurites and ectopic dendrogenesis are observed in G_{M1} gangliosidosis, and the extent of meganeurite development is related to the onset, severity, and clinical course of the disease. Various pharmacologic abnormalities have been observed in feline G_{M1} gangliosidosis, such as cholinergic dysfunction, neuroaxonal dystrophy in GABAergic neurons, alteration of phospholipase C and adenyl cyclase activities, reduced calcium flux in synaptosomes, and alteration of evoked synaptic activity patterns in cortical pyramidal neurons. These data suggest that morphologic and metabolic effects occur in the presence of excessive storage of ganglioside G_{M1}.

G_{M1} gangliosidosis has been recorded in cats, dogs, sheep, and calves. These animals showed various central nervous system manifestations. β-Galactosidase is deficient, and storage of G_{M1} and oligosaccharides has been confirmed. Furthermore, mouse models have been generated by disruption of the β-galactosidase gene. The β-galactosidase-deficient knockout mouse presented with progressive neurologic manifestations a few months after birth. Clinical, pathologic, and biochemical analyses indicated that this also is an authentic model of human G_{M1} gangliosidosis. In addition, phenotype-specific model mice have been produced by introducing human mutant genes, resulting in various clinical forms of β-galactosidosis (knockout transgenic mice). These mice models are used for new therapeutic approaches to human β-galactosidosis patients.

The mouse model of juvenile G_{M1} gangliosidosis expressing the R201C mutation was used for a new molecular therapy using a low-molecular-weight compound, *N*-octyle-4-epi-β-valienamine (NOEV). Orally fed NOEV passed through the blood-brain barrier, enhanced the deficient β-galactosidase activity, and induced degradation of G_{M1} and G_{A1} in the central nervous system. This new molecular therapy (i.e., chemical chaperone therapy) will be useful for certain patients with β-galactosidosis and potentially other lysosomal storage diseases with central nervous system involvement.

HISTORY

In 1959, Norman and colleagues[1] reported a patient with a specific form of amaurotic idiocy: "Tay-Sachs disease with visceral involvement." Clinical and pathologic findings resembled those of Tay-Sachs disease, but lipid-laden histiocytes were observed in extraneural tissues. The stored material was ganglioside, not sphingomyelin. Craig and colleagues[2] also described an infant with clinical and radiologic features suggestive of Hurler disease: "an unusual storage disease resembling the Hurler-Hunter disease." The foam-cell histiocytes that were found in viscera did not contain mucopolysaccharides.

Subsequently, after a preliminary study of four patients with "pseudo-Hurler disease," [3] Landing and colleagues established a new disease, called *familial neurovisceral lipidosis*, as a clinicopathologic entity.[4] Their eight patients showed (1) clinical and radiologic findings suggesting those of Hurler disease (i.e., psychomotor deterioration with dysmorphism), (2) pathologic features resembling those of Niemann-Pick disease but with certain distinctive features, including involvement of glomerular epithelium, and (3) histochemical properties of the stored material differing from those seen with previously defined lipidoses. Biochemical analysis revealed generalized accumulation of ganglioside G_{M1} in brain and viscera,[5] and the term *generalized gangliosidosis* was proposed as a new inborn error of metabolism.[5] Clinical signs and symptoms developed in early infancy in all patients in these reports. The same disease was described as a biochemically special form of infantile amaurotic idiocy,[6] Tay-Sachs disease with visceral involvement,[7] familial infantile amaurotic idiocy with visceral involvement,[8] Landing disease,[9] generalized gangliosidosis of Norman-Landing type,[10] and G_{M1} gangliosidosis.[11]

Later, cases were recognized of later onset (late infantile systemic lipidosis) without distinctive clinical or radiologic features.[12, 13] Storage of G_{M1} was remarkable in brain but not in viscera. The patients with this G_{M1} storage disease were subsequently divided into two clinical forms on the basis of clinical and biochemical data.[14] Type 1 is characterized by the onset of neurologic deterioration and visceromegaly before 6 months of age, associated with dysmorphism and skeletal deformities, and type 2 is characterized by by later onset (7–14 months) without specific

physical findings.

β-Galactosidase deficiency was demonstrated first by Okada and O'Brien,[15] and then a widespread biochemical screening started. As a result, patients with later onset[16, 17] and atypical cases in adults with more protracted clinical courses[18] were found. Extrapyramidal signs and symptoms were the major manifestations in adults, starting around 10 years of age and progressing very slowly over 20 years.[18] Otherwise, there were no specific neurologic or somatic abnormalities except for slight vertebral deformities (flattening). Biochemical screening detected a specific deficiency of β-galactosidase in leukocytes and serum. This disease was classified as the adult form of G_{M1} gangliosidosis.[18]

On the other hand, spondyloepiphyseal dysplasia and somatic dysmorphism were found in a patient with β-galactosidase deficiency.[19] Intelligence was normal, and no signs of central nervous system involvement were detected. It was described as a Morquio-like syndrome in another report,[20] and a conclusion was drawn for β-galactosidase deficiency in Morquio B disease as a primary genetic defect owing to allelic mutation of the enzyme gene.[21]

The molecular basis of these phenotypic variations became evident when a cDNA for human β-galactosidase was cloned and sequenced.[22] Various mutations of the β-galactosidase gene were found in both G_{M1} gangliosidosis and Morquio B disease,[23, 24] with some overlap between them,[25] and the term *β-galactosidosis* was proposed on the basis of these molecular genetic observations.[25]

CLINICAL AND GENETIC ASPECTS

As described earlier, two diseases have been recognized for human β-galactosidase deficiency, a neurodegenerative disease with visceral involvement (G_{M1} gangliosidosis) and a generalized bone disease without central nervous system involvement (Morquio B disease). Originally, they were reported separately as different diseases, but molecular analysis has confirmed allelic mutations of the same gene in these diseases with diverse phenotypic expressions. In this chapter, however, they are described individually because of distinct clinical manifestations. Clinical, biochemical, and genetic findings of these three types are summarized in Table 151-1.

Table 151-1 Major Clinical Types of Hereditary β-Galactosidase Deficiency (β-Galactosidosis)

	G_{M1} gangliosidosis			Morquio B disease
	Infantile	Late Infantile/juvenile	Chronic/adult	
	(Type 1)	(Type 2)	(Type 3)	
Major phenotypic expression	Generalized neurosomatic	Generalized neurovertebral	Localized neurovertebral	Generalized skeletal
Onset	0–6 mo	7 m–3 yr	3–30 yr	5–10 yr
Course	<2 yr	1–5 yr	10–30 yr	>30 yr
Central nervous system	Generalized	Generalized	Localized	–
Mental	+++	++	+ or	–
Major motor	Pyramidal	Pyramidal	Extrapyramidal	–
Peripheral nervous system	–	–	–	–
Muscle	–	–	+ or –	–
Cherry-red spots	+	+ or –	–	–
Hepatosplenomegaly	+	+ or –	–	–
Dysmorphism	+ or –	+ or –	–	–
Skeletal system	Generalized	Localized	Localized	Generalized
Storage				
Ganglioside G_{M1}	+++	++	+	–
Oligosaccharides	+++	++	+	+
Keratan sulfate	+	+	ND	+++
β-Galactosidase	Deficient	Deficient	Deficient	Deficient
Gene mutation	R482H (Italian) R208C (American)	R201C (Japanese)	I51T(Japanese)	W273L (Caucasian)

ND = not described.

G_{M1} Gangliosidosis

Incidence and heredity

G_{M1} gangliosidosis is a rare disease with heterogeneous clinical manifestations. The mode of inheritance is autosomal recessive in all clinical types. Both sexes are affected equally. The incidence of the disease is not known. The infantile form of the disease is described more often than the others. Patients of various ethnic origins have been reported, including Algerian, Belgian, Dutch, English, French, German, Indian, Italian, Japanese, Jewish, Mexican, Polish, Puerto Rican, Saudi Arabian, Swedish, Swiss, and others. Among them, a high incidence has been found in some ethnic groups: 1 in 3700 live births in the Maltese Islands,[26] a common mutation p.R59H in 0.7 percent of the Gypsy population,[27] prevalence of 1 in 17,000 in southern Brazil,[28] and a high frequency (8 percent) of G_{M1} gangliosidosis carriers in a Cypriot village.[29] Adult patients have been reported most often in Japan, but the incidence is not known.

Age of onset and clinical course

In typical cases, the disease is recognized early in infancy. Psychomotor development is retarded from birth in some patients, but the age of onset is variable among patients with the infantile form (type 1). Cases of later onset have been reported less frequently and have been classified clinically as late infantile or juvenile (type 2), adult or chronic (type 3), and other variant types mainly on the basis of the age of onset and clinical course.[30] Further subclassification was proposed in some reports,[31-34] but clinical classification is not always easy for individual cases. Phenotypic variation has been observed in a family.[34] Early development is normal in the late-onset forms, and signs of central nervous system involvement appear gradually at various ages between late infancy and young adulthood. In general, the clinical course is related to the age of onset.

Signs and symptoms of the central nervous system progress rapidly in early-onset cases, and the infant becomes vegetative with generalized rigospasticity within a year of birth. The clinical course is protracted in later-onset cases. In exceptional cases, it takes 10 to 30 years before the patient is completely disabled and bedridden. Patients with type 1 or type 3 disease show relatively conspicuous clinical manifestations. Nonspecific signs and symptoms appear in childhood in cases grossly designated as type 2.

Infantile (type 1) G_{M1} gangliosidosis

Clinical signs and symptoms appear in early infancy after normal development, but some patients show physical or neurologic abnormalities immediately after birth.[10, 35-39] In severe cases, appetite is poor, sucking is weak, and weight gain is subnormal in the neonatal period. Ascites and/or edema of the extremities are sometimes observed.

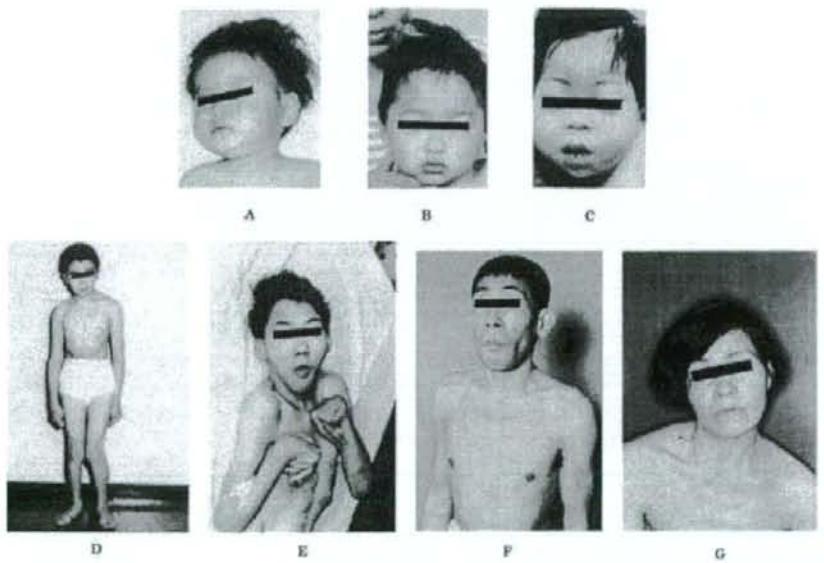
In most cases, arrest or delay in developmental milestones is observed at 3 to 6 months of age. Then the signs of severe brain damage become obvious. Definite functional deterioration of the nervous system follows within several months. The patient may show an exaggerated startle response to sounds.[36, 40, 41] Deep tendon reflexes are hyperactive, and pyramidal signs are positive. Generalized muscle hypotonia at the initial stage of the disease is gradually changed to rigospasticity later, associated with frequent convulsive seizures.

Macular cherry-red spots are of pathognomonic significance. Corneal clouding is often observed. Optic atrophy is present at the late stage of the disease, and the retina becomes edematous.[42] Hepatosplenomegaly is almost always present. Clinical signs of peripheral nerve involvement have not been described.

In typical cases, dysmorphism and generalized skeletal dysplasia are evident and progressive. The following dysmorphic changes have been described: coarse and thick skin; frontal bossing; depressed nasal bridge; large, low-set ears; increased distance between nose and upper lip; hirsutism on the forehead and neck; gingival hypertrophy; and macroglossia.[30, 36] Dorsolumbar kyphoscoliosis is present. Hands are broad, and fingers are short and stubby. Interosseous muscles are atrophic. Joints are stiff, with generalized contractures.

However, these typical dysmorphic expressions are not always obvious in infantile patients.[39] In fact, many patients have presented with neurodegenerative disease without remarkable physical changes. A number of G_{M1} gangliosidosis patients have been known in Japan, but only a few of them presented with the well-known physical changes described earlier (Fig. 151-1 *A* to *C*). It is therefore not always easy to determine the clinical type of a patient on the basis of the specific physical changes.

Fig. 151-1:



Patients with GM1 gangliosidosis. A, Infantile form, 1 year, 4 months, female; not dysmorphic. (Courtesy of Dr. Y. Tanabe, Chiba ChildrenÃ¢ÂÂs Hospital, Chiba, Japan.) B, Infantile form, 1 year, male; not dysmorphic. (Courtesy of Dr. M. Segawa, Segawa ChildrenÃ¢ÂÂs Neurological Clinic,

Tokyo, Japan.) C, Infantile form, 8 months, female; dysmorphic. (Courtesy of Dr. K. Inui, Osaka University, Osaka, Japan.) D, Juvenile form, 13 years, male. (Reproduced from Takamoto et al. 43 Used by permission.) E, Chronic form, 25 years, female. (Courtesy of Dr. A. Ishizaki, Fuchu Medical Center for the Handicapped, Tokyo, Japan.) F, Adult form, 29 years, male. 18 G, Adult form, 38 years, female. 44 (Courtesy of Dr. T. Nakano, Shinshu University, Matsumoto, Japan.)

The patient becomes vegetative with generalized rigospasticity and joint contractures at the late stage of the disease. Death ensues within a few years of disease onset.

Late infantile/juvenile (type 2) G_{M1} gangliosidosis

G_{M1} gangliosidosis of late onset has been roughly grouped into two subtypes, the late infantile/juvenile and adult/chronic forms. Clinical manifestations in adult patients are uniform, but the patients with relatively early onset (late infancy and childhood) present with heterogeneous phenotypic expressions.

The report by Gonatas and Gonatas[12] described a case of "late infantile lipidosis," but the patient's psychomotor development had been retarded in early infancy, and he smiled and watched his mother only at 8 to 10 months of age. He could never sit without support. Definite deterioration started by 14 months, and myoclonic seizures and generalized convulsions occurred for 2 months before his death at 25 months.

Two siblings in the report of Derry and colleagues[14] had almost the same clinical course after the deterioration started. However, their early development was normal up to age 1. They started walking without support at 10 to 11 months but became less responsive and had difficulty feeding at 13 months and stopped walking at 14 months. Progressive stiffness developed thereafter. By 2 years of age, they were in a state of spastic quadriplegia. Babinski reflex and tonic neck reflex were positive. Optic disks were pale, but cherry-red spots were not observed. Neither visceromegaly nor dysmorphism was described for infantile G_{M1} gangliosidosis. Bone x-ray was normal; no deformities or dysplasias were found. On the basis of the findings in these patients, Derry and colleagues[14] divided G_{M1} gangliosidosis into type 1 (infantile) and type 2 (late infantile). An almost identical clinical picture was reported by Hooft and colleagues.[45] Progressive deterioration started at 13 months, without cherry-red spots, visceromegaly, dysmorphism, or x-ray skeletal changes, although the patient's hands were short and broad.

Five patients from two families showed similar clinical courses and were reported to have "juvenile G_{M1} gangliosidosis."[16] Gait disturbance was observed at 12 to 18 months, and then psychomotor deterioration became evident. No specific general somatic abnormalities were found. Convulsive seizures occurred frequently after 2 years and were not controlled by anticonvulsants at 3 to 5 years. One of the patients died after aspiration at 57 months.

Cases of later onset and protracted course have been reported: an 11-year-old boy (onset 2 years),[46] a 10-year-old girl (onset 6 years),[47] and a 12-year-old girl (onset 6 years).[17] Cherry-red spots, visceromegaly, and dysmorphism are absent in most cases (see Fig. 151-1 *D* and *E*). Atypical cherry-red spots were described in one patient.[43] Skeletal dysplasia may not be present[45, 47, 48] but has been reported in many patients.

G_{M1} Gangliosidosis in adults (adult/chronic form; type 3)

In 1977 we reported six Japanese patients with hereditary β-galactosidase deficiency.[18] Three young adults (15–22 years) showed progressive cerebellar ataxia, action myoclonus, cherry-red spots, and dysmorphism. Intracellular β-galactosidase activity was low (10 percent of normal), but plasma enzyme activity was normal. Similar cases had been reported previously as a variant of G_{M1} gangliosidosis or a "mucolipidosis."[49-53] After a careful clinical and enzymatic study, we concluded (1) that this group had a new disease different from G_{M1} gangliosidosis or mucopolysaccharidosis and (b) that β-galactosidase deficiency is a secondary biochemical abnormality caused by another basic molecular defect (galactosialidosis; see Chap. 152).

The other two siblings, ages 34 and 30, showed different phenotypic expressions, with progressive pyramidal and extrapyramidal disease and muscle atrophy but without dysmorphism or cherry-red spots. β-Galactosidase activity was extremely low in their leukocytes, fibroblasts, and plasma. Their parents showed half-normal enzyme activity, and we concluded that they represent a new clinical type (adult type) of G_{M1} gangliosidosis caused by a genetic defect of β-galactosidase.[18] This conclusion was supported subsequently by demonstration of an increase of ganglioside G_{M1} in fibroblasts[54] and genetic complementation analysis.[55] This is a rare clinical form; less than 30 families have been reported, mainly in Japan[44, 56-65] and other areas.[66-73]

Dystonia is the major neurologic manifestation (see Fig. 151-1 *E* to *G*). Early development is normal. A study of 16 Japanese patients with adult/chronic G_{M1} gangliosidosis revealed that the disease started at 3 years in two siblings, at 4 years in one patient, and at 6 to 8 years in four patients.[63] The age of onset was variable in some siblings; it was 19, 30, and 30 years in one family; 4 and 27 years in the second family[63]; 17 and 19 years in the third family; and 3, 3, and 11 to 12 years in the fourth family. The onset was probably 4 to 7 years in the patient of Wenger and colleagues,[68] 2.5 to 3 years in the patient of Goldman and colleagues,[69] and 3 years in the patient of Nardocci and colleagues.[74] The severity of phenotypic expression was remarkably different in two sibling cases of chronic G_{M1} gangliosidosis.[75] The elder sister developed left hip joint pain and gait disturbance at age 27. Early development of the younger brother was retarded in infancy, and progressive psychomotor deterioration became manifest in childhood. Both patients were homozygous for the I51T mutation. The pathogenesis of this discordant phenotypic expression is not known.

Gait or speech disturbance is the first sign in most cases. Dystonic posture develops gradually. Severe dystonia caused masticatory impairment in one patient.[64] Pyramidal signs are present. Deterioration of intellectual activity is not remarkable. Cherry-red spots are not observed, but corneal clouding has been recorded in some patients. Dysmorphism is not obvious. Slight vertebral changes are usually described. Clinical data on the Japanese patients are summarized in Table 151-2.

Table 151-2 Clinical Summary of Japanese Patients with Adult/Chronic G_{M1} Gangliosidosis

Family	1	2	3	4			5	6	7	8		9		10		
Case				1	2	3				1	2	1	2	1	2	3
Sex	F	F	M	F	M	M	M	M	M	F	M	M	F	M	M	M
Age (yr) at onset	7	13	10	19	30	30	8	6	7	27	4	17	19	11	3	3
Age (yr) at diagnosis	25	22	37	38	45	43	46	53	32	29	27	40	34	33	31	28
Clinical manifestations[a]																
Initial sign	G/S	S	S	G/S	G	G	G/S	S	S	G	S	S	H	S	G/S	G/S
MR/MD	+	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+
Speech	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gait	9	+	+	+	+	+	+	49	29	+	+	+	+	+	22	26
Pyr/ExPyr	–/+	–/+	–/+	–/+	–/+	–/+	–/+	–/+	–/+	–/+	–/+	–/+	–/+	–/+	–/+	–/+
Muscle	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+
Cornea	–	+	–	–	–	–	–	–	+	–	–	–	–	–	+	–
Bone	+	+	+	+	–	–	+	+	+	+	+	–	–	+	+	+
Laboratory data[b]																
CT/MRI	Ab	N	Ab	Ab	N		Ab	Ab	Ab	N		Ab	Ab	Ab	Ab	Ab
Bone marrow		–		–					+							+
β-Galactosidase	4.2	7.0	5.7	7.3	8.5	4.4	5.3	7.0	7.9	8.9	5.4	4.7	2.7	5.0	5.0	5.0

[a] Initial signs and symptoms: G = gait disturbance; S = speech disturbance; H = disturbance of hand movements; MR/MD = mental retardation/deterioration; Speech = speech disturbance; Gait = gait disturbance (numbers denote age at onset of gait disturbance); Pyr/ExPyr = pyramidal and extrapyramidal signs; Muscle = muscle atrophy; Cornea = corneal opacity; Bone = vertebral dysplasia (mainly flattening of vertebral bodies). [b] CT/MRI = neuroimaging (computed tomography and/or magnetic resonance imaging [N = normal; Ab = abnormal]); Bone marrow = foam cells in bone marrow; β-Galactosidase = enzyme activity expressed as percent of normal in fibroblasts (substrate: 4-methylumbelliferyl b-galactoside).

SOURCE: Data from reference 63.

In some "chronic" cases, progressive neurologic signs and symptoms appeared at age 2 to 3, but the course was protracted with long survival. Postmortem examinations confirmed the diagnosis in two patients neuropathologically and biochemically.[69, 76, 77] One patient[76] was spastic, decerebrate, and underweight by age 7 and died at 17 years. Ocular signs (i.e., cherry-red spots and corneal clouding) and visceromegaly were never observed, but there was mild spondylodysplasia. Her features became coarse, her nasal bridge was slightly depressed, and her forehead developed bossing in the last few years of her life. Autofluorescent material accumulated strikingly in cerebral neurons, as seen in neuronal ceroid lipofuscinosis, in addition to storage of ganglioside G_{M1}. The phenotype of this patient was originally classified as the late

infantile/juvenile form with protracted clinical course.

The patient reported by Takamoto and colleagues[43] is phenotypically similar to adult patients, presenting mainly with dystonia and dysostosis, but atypical cherry-red spots were present. Subsequent clinical follow-up was not possible.

Atypical clinical manifestations

Numerous telangiectasias, isolated or in groups, were recorded on the abdomen, pubis, thighs, and face of an infantile patient[40]; a small dilated vessel was surrounded by stellar-ranged distended capillaries, which faded easily under pressure. Angiokeratoma was one of the prominent clinical signs in a few infants with G_{M1} gangliosidosis.[78, 79] β-Galactosidase was deficient, but neuraminidase was normal. The clinical course and manifestations were typical of infantile G_{M1} gangliosidosis with dysmorphism, visceromegaly, and neurologic signs and symptoms in early infancy. The lesions were pinhead-sized, purplish, slightly raised, and scattered on the chest, abdomen, thighs, and forearm. They did not blanch with pressure. Foamy endothelial cells were observed in the cutaneous blood vessels in one patient.[80] Extensive Mongolian spots have been described in some cases of infantile G_{M1} gangliosidosis.[79-83]

Cardiac involvement has been another striking manifestation in some patients.[84-88] Cardiomyopathy was observed in an infant with dysmorphism, skeletal dysplasia, and visceromegaly.[84] Corneal opacities or cherry-red spots were not present. A grade 2/6 systolic murmur was heard along the left sternal border. An electrocardiogram (ECG) showed incomplete bundle branch block. At autopsy, the myocardium was pale brown and waxy. Microscopically, myofibers were vacuolated and hypertrophied. The mitral valve leaflets were thick and nodular with vacuolated histiocytes and fibrous tissue. The right coronary artery was partially occluded by a large intimal atherosclerotic plaque containing ballooned cells.

Congenital cardiomyopathy, muscular weakness, and hypotonia were the major symptoms in a male infant who subsequently developed hepatosplenomegaly and died from heart failure at 8 months of age.[86] His clinical condition resembled that of Pompe disease (infantile glycogenosis II); lymphocytes were vacuolated, and vacuolar inclusions were finely granular and membranous. Axons of intradermal nerve fascicles often were tightly packed with mitochondria and dense bodies, and Schwann cells contained membrane-bound lamellar bodies. β-Galactosidase deficiency was found in plasma, leukocytes, and fibroblasts from the patient.

In another female patient, progressive cardiomyopathy (i.e., congestive heart failure) and skeletal myopathy (i.e., hypotonia and weakness) developed at 4 months of age.[88] α-Glucosidase was normal, but β-galactosidase was found to be deficient. Progressive neurologic manifestations appeared at 9 months, and the patient died at 12 months. Bone dysplasia was not found in this case.

Involvement of the respiratory tract was remarkable in two infants.[89, 90] Repeated respiratory infections occurred in one patient, and the patient died at 10.5 months from respiratory failure.[89] Alveolar lumina often were completely filled with phagocytic cells.

Hydrops fetalis or ascites in the neonatal period has been reported.[91-93] Venoportography and portal vein pressure were normal.[91] Heavy infiltration of the capillary endothelial cells may have been the cause of the ascites. Administration of salt-free albumin, resulting in better colloid osmotic pressure, temporarily improved the ascites in these patients. In a normal-appearing infant born to a previously unaffected family, progressive third-trimester oligohydramnios and fetal growth retardation had been documented by ultrasonography. Routine placental examination revealed vacuolization of syncytiotrophoblasts, intermediate trophoblasts, and stromal Hofbauer cells.[94] Subsequent enzyme assays confirmed the diagnosis of G_{M1} gangliosidosis.

Laboratory findings

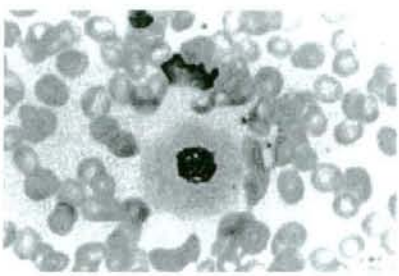
Peripheral Blood

Blood counts are normal. Vacuolation of lymphocytes (up to 80 percent) has been recorded in many cases[10, 36, 40-42] but is less frequent in late-onset cases, particularly in adults. Vacuoles appear empty on electron microscopy. Lymphocytes and neutrophils show basophilic cytoplasmic granules that are slightly positive for toluidine blue stain.[40] Routine blood chemistry is normal. Type IV hyperlipidemia was recorded in two unrelated families; triglycerides and pro-β-lipoprotein were increased.[95] Serum alkaline phosphatase activity was increased in five patients in three reports.[93, 96, 97] Scattered accumulation of osteoblasts and osteoclasts was found in bone marrow aspirates from one patient.[96] However, the osteoblastosis as a source of hyperphosphatasemia was not supported in the second report.[91] This biochemical abnormality could be an early biologic marker of the disease.

Bone Marrow

Large foam cells or ballooned cells (Fig. 151-2) are present in the bone marrow,[40] but in fewer numbers than in Gaucher disease or Niemann-Pick disease. The cytoplasm is finely vacuolated but does not stain with Sudan III. Lymphocytes are highly vacuolated, as in the peripheral blood. Vacuolation is found less frequently in adult patients[62] and may not be observed in late infantile/juvenile[40] or adult patients.[69] Sea-blue histiocytes were present in two siblings with juvenile G_{M1} gangliosidosis.[98] Osteoblastosis was observed in one patient, as described earlier.[97]

Fig. 151-2:



Ballooned histiocyte in the bone marrow of a patient with the chronic form of GM1 gangliosidosis

(17 years, male). (Courtesy of Dr. M. Yanagisawa, Jichi Medical School, Tochigi, Japan.)

Urinalysis

Routine urinalysis is normal. Foamy mononuclear cells may be found in the urinary sediment.[36] Cetylpyridinium chloride (CPC)–precipitable urinary mucopolysaccharides are normal or only slightly elevated.

Cerebrospinal Fluid (CSF)

Cell counts and protein are normal. G_{M1} was increased in CSF by radioassay in adult patients as well as in earlier-onset patients.[69] On the other hand, G_{M1} was not detected in plasma or CSF from an adult patient by an immunostaining method, but an increase was found in infantile and juvenile patients.[62, 99] An increase of G_{M1} was confirmed in patients with G_{M1} gangliosidosis using high-performance liquid chromatography (HPLC).[100] G_{M1} in CSF may be helpful in the diagnosis and monitoring of G_{M1} gangliosidosis.

Electrocardiography and Echocardiography

Signs of ventricular hypertrophy were found in the patients with cardiomyopathy.[84-88] Prominent left ventricular forces, with a normal PR interval and tall QRS complexes, were present in one patient,[88] and left-axis deviation in the frontal plane was present in another patient.[86] Echocardiography showed dilatation of the left ventricle with poor contractility.[86, 88] A negative T wave was observed by ECG at precordial leads in one patient,[43] and myocardial damage was suspected.

Electromyography

No detectable signs of denervation have been recorded. The number, duration, and amplitude of muscle excitability potentials are normal. H-reflex was demonstrable, but weak and inconsistent, in one patient.[41] Nerve conduction velocities are normal.

Electroencephalography (EEG) and Electroretinography

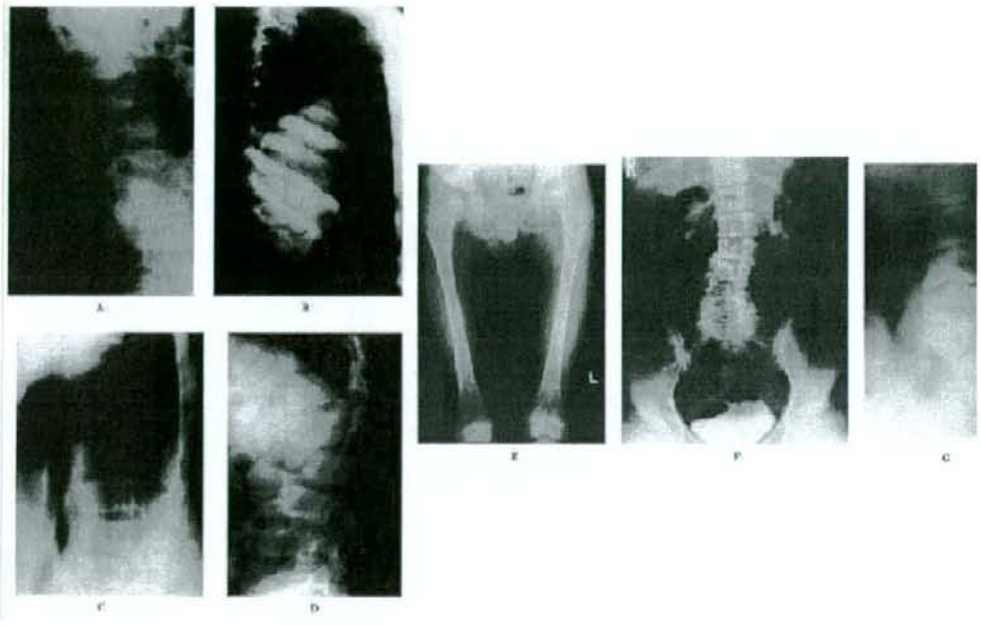
EEG may be normal at the initial stage of disease.[13, 40] Generalized dysrhythmias with irregular slow activity at the initial stages[41, 42] become increasingly pronounced around 2 to 3 years of age in infantile-form patients.[101] Fluctuating 4 to 5 cycles per second, rhythmic activity often was prominent in the temporal region in type 2 patients.[101] Paroxysmal activity was not a conspicuous feature,[102] but epileptogenic foci are observed in many patients.[42] A normal EEG has been recorded in a patient with chronic G_{M1} gangliosidosis.[66]

Electroretinogram was normal in all patients studied, but visual-evoked potential was variably altered.[101]

Bone X-Ray Imaging

Bone changes generally are prominent in patients with clinically severe disease, but there are cases of rapid psychomotor deterioration in early infancy with minimal bone dysplasia.[39] No detectable bone changes were reported in a patient with of late infantile G_{M1} gangliosidosis.[45] Bones generally are rarefied with coarse trabeculation. Vertebral deformities are remarkable; hypoplasia and anterior beaking at the thoracolumbar region[10, 17, 36, 40] and anterior notch deformity occur (Fig. 151-3 *A* and *B*).[103] The pathogenesis of the thoracolumbar junction deformity has been explained to be the result of an anteriorly herniated intervertebral disk following prolonged kyphosis[104] or as the effect of long-standing gravitational pressure and stress on growth and development of the vertebral body.[105]

Fig. 151-3:



Bone x-ray film of GM1 gangliosidosis patients. A, Infantile form, 2 years, 5 months. (Courtesy of Dr. Y. Kubota, Mito Saiseikai Hospital, Mito, Japan.) B, Juvenile form, 7 years, male (Courtesy of Dr. Y. Koizumi, Hitachi General Hospital, Hitachi, Japan.) C, Juvenile form, 13 years, male. 43 (Courtesy of K. Takamoto, Tokyo Metropolitan Neurological Hospital, Tokyo, Japan.) D and E, Chronic form, 17 years, male. (Courtesy of Dr. M. Yanagisawa, Jichi Medical School, Tochigi, Japan.) F, Adult form, 22 years, female. (Courtesy of Dr. M. Ushiyama, Shinshu University, Matsumoto, Japan.) G, Chronic form, 38 years, female. 44 (Courtesy of Dr. T. Nakano, Shinshu University, Matsumoto, Japan.)

Bone age is retarded.[13] Long bones are short, and the midshaft region is wider, tapering both proximally and distally. Metacarpal bones become wedge-shaped with a constriction proximally[10, 36]; the fifth metacarpal is frequently the most expanded and deformed. Cloaking of the humerus occurs owing to subperiosteal new bone formation. With increasing age, the externally thickened cortical wall is removed ("reamed out") by expansion of the medullary cavity, resulting in a synchronous thinning of the cortical wall.[36]

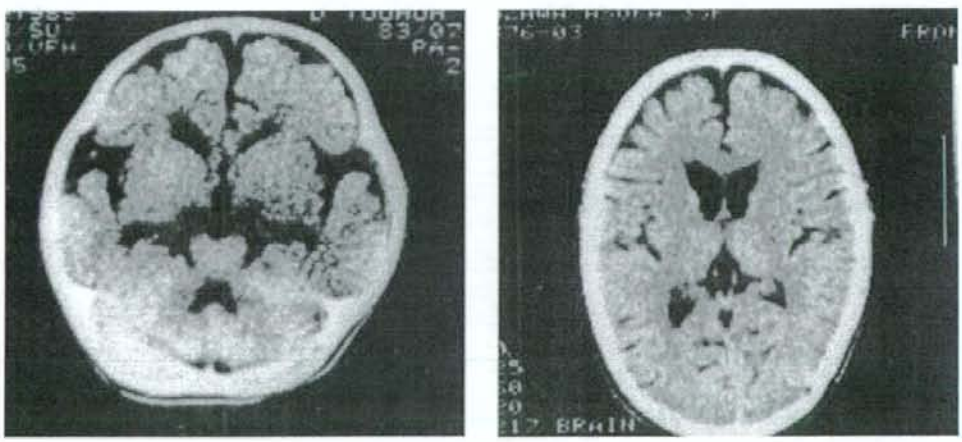
Other bones are also involved: thick skull,[17] shallow and elongated pituitary fossa (shoe-shaped sella),[10] widened spatulate ribs,[10] flared ilia,[4] acetabular dysplasia and flattened femoral heads with irregular ossification,[17, 43, 106] and dislocation of bilateral hip joints.[10]

Bone changes in adult/chronic patients are characterized by mild anterior beaking of lumbar vertebral bodies (see Fig. 151-3 *D*), platyspondylia (see Fig. 151-3 *C* and *G*),[44, 61] diminished intervertebral spaces, scoliosis (see Fig. 151-3 *F*),[44] acetabular hypoplasia, and flattened femoral heads[44, 57, 61, 62, 67] (see Fig. 151-3 *E*).

Neuroimaging

Cranial CT scan and MRI show diffuse atrophy of the central nervous system (generalized cortical atrophy and enlargement of the ventricular system) and features of myelin loss in the cerebral white matter in early-onset patients (Fig. 151-4 *A*). CT scan revealed an increasing white matter involvement in one patient,[107] with generalized areas of reduced density early in the course of the disease.

Fig. 151-4:



A B

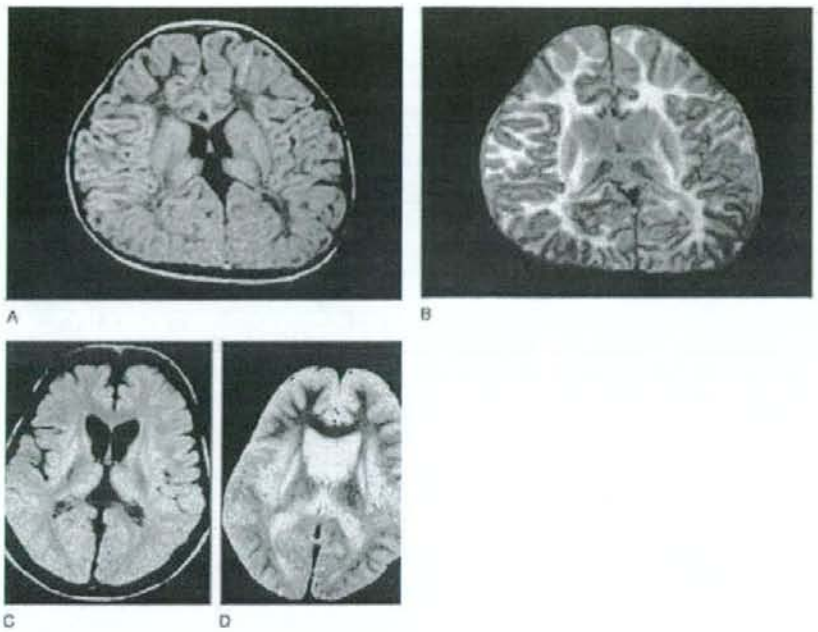
Cranial CT scans of GM1 gangliosidosis. A, Infantile form, 1 year, male. (Courtesy of Dr. M. Segawa, Segawa Children's Neurological Clinic, Tokyo, Japan.) B, Adult form, 38 years, female.

(Reproduced from Nakano et al. 44 Used by permission.)

In adult patients, CT scan shows diffuse but mild cerebral atrophy[61, 62] or localized atrophy of the head of the caudate nucleus.[69] In some other patients, CT scan was normal,[44, 58] or the frontal horns of the lateral ventricles were dilated only slightly,[56, 59] suggesting a slight atrophy of the head of the caudate nucleus (see Fig. 151-4 *B*).

A patient with infantile G_{M1} gangliosidosis at age 34 months showed high signal intensity in the basal ganglia and low signal intensity in the white matter on T_1-weighted MRI and low signal intensity in the basal ganglia and high signal intensity in the white matter on T_2-weighted MRI (see Fig. 151-5 *A*).[108] Similar changes were observed in an 11-year-old patient with chronic G_{M1} gangliosidosis,[109] with bilaterally symmetric low intensity in the putamen and globus pallidus on T_2-weighted images. In this case, single-photon-emission computed tomography (SPECT) using ^{99}Tc-HMPAO showed bilateral hyperperfusion in the basal ganglia that decreased gradually during 1 year of observation. In contrast, proton-density and T_2-weighted MRI showed symmetric hyperintense lesions of both putamina in adult patients (Fig. 151-5 *B*).[61, 62] Symmetric hyperintensity was seen in both putamina. Calcification in the basal ganglia was demonstrated by cranial plain x-ray imaging in a patient with juvenile G_{M1} gangliosidosis.[17] Persistent hyperintensity was observed in the thalamus, brain stem, and deep cerebellum bilaterally by T_1-weighted MRI in one patient with infantile G_{M1} gangliosidosis with typical clinical manifestations, indicating myelination arrest in these areas at ages 14 and 18 months.[110] In this case, there was no myelination in the basal ganglia, and diffuse leukomalacia was present in the cerebral hemispheres at the newborn stage. In another 2-year-old Saudi patient, cranial MRI revealed similar findings of dysmyelination/demyelination.[111] Furthermore, functional changes were indicated in this patient by cerebral fluorine-18-labeled 2-fluoro-2-deoxyglucose positron-emission tomography (FDG PET): a mild decrease of glucose metabolism in the basal ganglia and a moderate to severe decrease in the thalamic and visual cortex. There was an area of increased glucose uptake in the lower aspect of the left frontal lobe that possibly represented an active seizure focus.[111] The FDG PET and MRI findings were mutually complementary in imaging evaluation in this patient.

Fig. 151-5:



MRI of patients with GM1 gangliosidosis. A, B, Infantile form, 34 months, male. A, T1-weighted image (TR/RE: 560/26). Symmetric hyperintensity in the thalami, caudate nuclei, and lenticular nuclei, with wave-shaped hyperintensity in the cerebral cortex (particularly in the temporal lobe) and decreased signal intensity in the entire white matter. B, T2-weighted image (TR/TE: 2000/100). High signal intensity in the white matter and low intensity in the basal ganglia and thalami (particularly in the medial regions). (Reproduced from Kobayashi and Takashima. 108 Used by permission.) C, D. Adult form, 32 years, male. C, Proton-density image (TR/TE: 2000/25). D, T2-weighted image (TR/TE: 2000/100). Symmetric hyperintensity in both putamina. (Reproduced from Inui et al. 62 Used by permission.)

In one patient with infantile G_{M1} gangliosidosis, proton magnetic resonance spectroscopy (^{1}H-MRS) revealed a mild reduction in *N*-acetylaspartate, suggesting a relative paucity of axons and neurons; an increase in myoinositol, suggesting gliotic white matter changes; and an additional compound representing either guanidinoacetate or Galβ1-6Galβ1-4GlcNac.[112] ^{1}H-MRS may be useful for monitoring the efficacy of new treatments in the near future.