

Overexpression of β -galactosidase has been achieved in the baculovirus-Sf9 (*Spodoptera frugiperda*) system³¹³⁻³¹⁵ and the baculovirus-TN 368 (*Trichoplusia ni*) system.³¹⁵ TN 368 cells produced β -galactosidase more efficiently than Sf9 cells on a per-cell basis. However, the Sf9 cell density varied markedly, and more enzyme activity was recovered at the highest density.

β -Galactosidase gene

The human β -galactosidase gene spans more than 62.5 kb and contains 16 exons.³¹⁶ The ATG translation initiation was numbered as position 1. The promoter activity is located on the 236-bp *Pst* fragment (–261 to –26). The 851-bp *Pst* fragment (–1112 to –262) negatively regulates the initiation of transcription. The promoter has the characteristics of a housekeeping gene, with GC-rich stretches and five SP1 transcription elements on the two strands. The major multiple cap site of mRNA is –53. The mouse β -galactosidase gene spans more than 80 kb, but intron-exon organization is well conserved.³¹⁷

The human β -galactosidase gene produces two alternatively spliced transcripts that encode the lysosomal enzyme β -galactosidase (GLB1) and the elastin-binding protein (EBP). GLB1 is catalytically active in its precursor form. EBP does not display β -galactosidase activity. The sequence encoded by exons 3, 4, and 6 is missing, a frameshift occurs in exon 5 (unique 32-amino-acid sequence), and the reading frame is restored at the start of exon 7.³⁰⁸ Except for this frameshift-unique sequence, the primary structure of EBP is identical to catalytically active β -galactosidase. EBP is localized to the cell surface but not to lysosomes.³¹⁸

Mutations at the GLB1 locus may affect either both proteins or GLB1 only. The mutation of EBP contributes to the specific features of the G_{M1} gangliosidosis phenotype, such as cardiomyopathy and connective tissue abnormalities.³¹⁹⁻³²¹ Real-time polymerase chain reaction (PCR) was used for assessing the levels of GLB1 and EBP transcripts in nine patients with G_{M1} gangliosidosis.³²² Comparative analysis of the patients' phenotypes enabled a clear correlation between GLB1 mutations and specific clinical manifestations. Both GLB1 and EBP mRNA levels were reduced in three patients carrying splicing defects. Impaired elastogenesis also was observed in juvenile-form patients despite mutations affecting only β -galactosidase, suggesting secondary EBP deficiency by keratan sulfate accumulation.³¹⁹⁻³²¹ Careful assessment of EBP in G_{M1} gangliosidosis will reveal more insight into the pathogenesis of the disease.

Molecular Pathology of β -Galactosidase Deficiency

Analysis of mutant genes

Mutations are spread throughout the gene. In 70 percent of them, both GLB1 and EBP are involved simultaneously. Mutant genes in various clinical forms of human β -galactosidase deficiency have been analyzed. Southern blot analysis did not detect gross gene rearrangements. mRNA was not detected or reduced by northern blotting^{308, 323} or abnormally large in size²⁰ in patients with infantile G_{M1} gangliosidosis, but no abnormality was observed in patients with other clinical subtypes. For patients with normal amounts of mRNA, mutant genes were identified by reverse-transcriptase treatment of mRNA, PCR amplification of cDNA, cloning into m13 bacteriophage, and sequencing. The mutation was confirmed, in some cases, by restriction analysis of the PCR-amplified exon comprising the mutation site.

Gene mutations identified in G_{M1} gangliosidosis and Morquio B disease are summarized in Table 151-6. They include missense/nonsense mutations,^{23, 24, 127, 323-327} duplications/insertions,³²⁷⁻³²⁹ deletions,^{322, 330} and insertions causing splicing defects.^{121, 331, 332} Neither the type nor location of

mutation in the gene was correlated with the phenotype of the patients with β-galactosidosis. However, five common mutations have been found in different clinical phenotypes: R482H in Italian patients with infantile GM₁ gangliosidosis,³²⁴ R208C in American patients with infantile GM₁ gangliosidosis,³²⁵ R201C in Japanese patients with juvenile GM₁ gangliosidosis,^{23, 323} I51T in Japanese patients with adult GM₁ gangliosidosis,²³ and W273L in Caucasian patients with Morquio B disease.²⁴ The R208C mutation has been suggested to have originated from Puerto Rican ancestry.³²⁵ The R482H mutation also had been identified in Morquio B disease as well as in infantile GM₁ gangliosidosis.^{24, 324} There is no clear genetic distinction between the two clinical phenotypes, one with generalized neurosomatic manifestations and the other with generalized skeletal dysplasia without central nervous system involvement. This observation supports our nomenclature of β-galactosidosis.²⁵ The mutant gene products had almost normal enzyme activity (L10P polymorphism; 10Leu → Pro / CTT → CCT), about 10 percent normal activity (i.e., 51T, R201C, and W273L), or less than 1 percent other activity.

Table 151-6 β-Galactosidase Gene Mutations in β-Galactosidosis

Exon	Mutation	Amino acid/mRNA	Nucleotide ^b	Phenotype ^a	Ethnic Origin	Ref.
Intron 1	r.75_76ins75+1_75+20	ins20bp;ex1/2	c.75+2-3insT	Adult GM1	Caucasian	331
2	p.R49C	49Arg>Cys	c.145C>T	Inf GM1	Japanese	323
2	p.I51T	51Ile>Thr	c.152T>C	Adult GM1	Japanese	23, 63
2	p.S54I	54Ser>Ile	c.161G>T	GM1	Caucasian	New ^a
2	p.S54N	54Ser>Asn	c.161G>A	Inf GM1	Caucasian	320
2	p.Y57X	57Try>Ter	c.171C>G	Inf GM1	Caucasian	333
2	p.R59C	59Arg>Cys	c.175C>T	Inf GM1	Caucasian	320
2	p.R59H	59Arg>His	c.176G>A	Inf GM1	Brazilian	329
2	p.R68W	68Arg>Trp	c.202C>T	Adult GM1	Caucasian	322
Intron 2	p.76_245del (exon 2 skipping)	Exon 2 skipping	c.76+1G>A	Inf GM1	Caucasian	27
Intron 2	r.245_246ins246-32_246-1 ^d	ins32bp;ex2/3	c.246-1G>A	MB	South African	127
2-3	p.T82M	82Thr>Met	c.245C>T	Adult GM1	Caucasian	331, 332
3	p.Y83C	83Try>Cys	c.248A>G	MB	Caucasian	27
3	p.Y83H	83Tyr>His	c.247T>C	MB	Japanese	121
3	p.W92fsX32	Duplication	c.254_276dup	Inf GM1	Japanese	328
3	p.W92X	92Trp>Ter	c.276G>A	Inf GM1	Indian	New ^a
3	p.P93fs	Frame shift+stop codon	c.276_277insG	Inf GM1	Japanese	New ^a

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3	p.F107L	107Phe>Leu	c.319T>C	Juv GM1	Caucasian	New ^a
3	p.R121S	121Arg>Ser	c.363G>T	Inf GM1	Brazilian	329
3	p.G123R	123Gly>Arg	c.367G>A	Inf GM1	Japanese	23
3	p.E131K	131Glu>Lys	c.391G>A	Inf GM1	Caucasian	New ^a
4	p.G134V	134Gly>Val	c.401G>T	Inf GM1	Italian/French	330
4	p.P136S	136Pro>Ser	c.406C>T	Inf GM1	Caucasian	27
4	p.L146del	L146del	c.436_438delCTT	Inf GM1	Japanese	New ^a
4	p.L147del	L147del	c.435_440delTCT	Inf GM1	Argentine	330
4	p.R148C	148Arg>Cys	c.442C>T	Inf GM1	Caucasian	322
4	p.R148S	148Arg>Ser	c.442C>A	Inf GM1		233
4	p.R148T	148Arg>Thr	c.443G>A	Inf GM1	Caucasian	New ^a
4	p.D151V	151Asp>Val	c.451G>C c.452A>T	Inf GM1 Inf GM1	Arab African	333 27
5	p.458-401_552+1033del1529	ex5del	c.458-401_552+1033del1529	Inf G _{M1}	Argentine	330
5	p.L155R	155Leu>Arg	c.464T>G	Adult G _{M1}	Argentine	330
5	p.W161G	161Tyr>Gly	c.481T>G	Inf G _{M1}	Caucasian	GM03589
5	p.L162S	162Leu>Ser	c.485T>C	Inf G _{M1}	Spanish	330
5	p.L173P	173Leu>Pro	c.518T>C		Caucasian	27
5	p.G178R	178Gly>Arg	c.532G>A	Inf G _{M1}	Caucasian	New ^a
5	p.I181K	181Ile > Leu	c.542T > A	Inf G _{M1}	Turkish	New ^a
6	p.190D	190Gly>Asp	c.569G>A	Inf G _{M1}	Turkish	New ^a
6	p.D198X	198Asp>Ter	φ.588_591insT	Inf G _{M1}	Brazilian	329
6	p.Y199C	199Try>Cys	c.596A>G	Inf G _{M1}	Caucasian	27
6	p.R201C	201Arg>Cys	c.601C>T	Juv G _{M1}	Japanese	23
6	p.R201H	201Arg>His	c.602G>A	Adult G _{M1} MB	Caucasian Caucasian	127 127
6	p.R201Y	201Arg>Tyr	c.601-602CG>TA			New ^a
6	p.R208C	208Arg>Cys	c. 622C>T	Inf G _{M1}	American ^c Puerto Rican	325 326
6	p.D214Y	214Asp>Tyr	c.640G>T			334

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6	p.V216A	216Val>Ala	c.647T>C			334
6	p.H210_L211insENF	insGluAsnPhe	c.730_731insCAGAATTTT	Adult G _{M1}	American ^c	327
6	p.C230Y	230Cys>Tyr	c.689G>A	Juv G _{M1}	Caucasian	320
6	p.V240M	240Val>Met	c.718G>A	Inf G _{M1}	Brazilian	329
Intron 6	Aberrant splicing (intron 6)	ins9bp;ex6/7	c.731C>G	Juv G _{M1}	Japanese	335
7	p.Q255H	255Gln>His	c.765G>C	Inf G _{M1}	Caucasian	GM05335
7	p.P263S	263Pro>Ser	c.787C>T	Adult G _{M1}	Unknown	127
7	p.L264S	264Leu>Ser	c.791T>C	Juv G _{M1}	Caucasian	27
8	p.N266S	266Asn>Ser	c.797A>G	Adult G _{M1}	American ^c	327
8	p.Y270D	270Tyr>Asp	c.808T>G	MB	German	122
8	p.G272D	272Gly>Asp	c.815G>A		Caucasian	27
8	p.W273L	273Trp>Leu	c.818G>T	MB	Caucasian	24
8	p.W273R	273Trp>Arg	c.817T>C	MB	Macedonian	125
8	p.H281Y	281His>Tyr	c.841C>T	MB	German	122
8	p.T283QfsX21	Frameshift + stop codon	c.845_846delC	Inf G _{M1}	Uruguay	330
8	p.T283QfsX12	Frameshift + stop codon	c.845_846insC	Inf G _{M1}	Brazilian	329
8	p.901-914 del	901-914 del	c.902C>T	Inf G _{M1}	Caucasian	27
Intron 8	(Exon 8 skipping)	Aberrant splicing	c.914+2T>C	Inf G _{M1}	Caucasian	319
Intron 8	(Aberrant splicing intron 8)	Aberrant splicing	c.914+4G>A	Inf G _{M1}	Arabian	333
9	p.Y316C	316Tyr>Cys	c.947A>G	Inf G _{M1}	Japanese	23
9	p.N318H	318Asn>His	c.952A>C	Juv G _{M1} -MB	Greek	127
9	p.Y324C	324Tyr>Cys	c.971A>G	Inf G _{M1}	Caucasian	New ^a
10	p.T329A	329Thr>Ala	c.985A>G	Adult G _{M1}	Caucasian	320
10	p.D332N	332Asp>Asn	c.994G>A	Inf G _{M1}		233
10	p.D332E	332Asp>Glu	c.996C>G	Inf G _{M1}	Caucasian	New ^a
10	p.Y333LfsX	Frameshift + stop codon	c.996_997insC	Inf G _{M1}	Japanese	New ^a

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10	p.Y333H	333Tyr>His	c.999T>C	Inf G _{M1}	Japanese	New ^a
10	p.K346N	346Lys>Asn	c.1038G>C	Inf G _{M1}	Caucasian	27
10	p.Y347C	347Tyr>Cys	c.1040A>G	Inf G _{M1}	Caucasian	27
10	p.R351X	351Arg>Ter	c.1051C>T	Inf G _{M1}		336
Intron 10	(Aberrant splicing intron 10)	Aberrant splicing	c.1068+1G>T	Inf G _{M1}	Argentina	330
11–12	p.H412fsX	Duplication	c.1069_1233dup	Inf G _{M1}	Japanese	23
11	p.V377_K381del	V377_K381del	c.1131_1145del15	Inf G _{M1}	Argentine	330
12	p.Q408P	408Gln>Pro	c.1223A>G	MB	French	122
13	p.T420P	420Thr>Pro	c.1258A>C	Inf G _{M1}	Caucasian	27
13	p.T420K	420Thr>Lys	c.1259C>A	Adult G _{M1}	Caucasian	27
13	p.L422R	422Leu>Arg	c.1265T>G	Inf G _{M1}	Caucasian	27
13	p.S434L	434Ser>Leu	c.1301C>T	Inf G _{M1}	English#	330
13	p.G438E	438Gly>Glu	c.1313G>A	MB		336
13	p.V439G	439Val>Gly	c.1316T>G	G _{M1}	?German	New ^a
13	p.D441N	441Asp>Asn	c.1321G>A	Inf G _{M1}	Caucasian	27
13	p.R442Q	442Arg>Gln	c.1325G>A	Adult G _{M1}	Caucasian	320
13	p.Y444C	444Tyr>Cys	c.1331A>G	?MB	Caucasian	27
13	p.D448V	448Asp>Val	c.1343A>T	G _{M1}	Japanese	New ^a
14	p.R457X	457Arg>Ter	c.1369C>T	Inf G _{M1}	Japanese	323
14	p.R457Q	457Arg>Gln	c.1370G>A	Adult G _{M1}	Japanese	23
14	p.M480V	480Met>Val	c.1438A>G	Inf G _{M1}	?	New ^a
14	p.G481X	481Gly>Ter	c.1441G>T	Inf G _{M1}	Caucasian	27
14	p.R482H	482Arg>His	c.1445G>A	MB Inf G _{M1}	Caucasian Italian	24 324
14	p.R482C	482Arg>Cys	c.1444C>T	MB	Japanese	127
14	p.N484K	484Asn>Lys	c.1452C>A	MB		336
14	p.D491N	491Asp>Asn	c.1471G>A	Inf G _{M1}	Brazilian	329
14	p.D491Y	491Asp>Tyr	c.1471G>T	Inf G _{M1}	Argentine	330

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Intron 14	p.1348_1479del (exon 14 skipping)	Exon 14 skipping	c.1479+1G>T	Inf G _{M1}	Caucasian	27
Intron 14	r.1480-28_1480-1 ins	Aberrant splicing	c.1480-2A>G	Inf G _{M1}	Caucasian	27
15	p.G494C	494Gly>Cys	c.1480G>T	Inf G _{M1}	Japanese	23
15	p.G494S	494Gly>Ser	c.1480GT>A	?MB	Caucasian	27
15	p.T500A	500Thr>Ala	c.1498A>G	MB		336
15	p.W509C	509Trp>Cys	c.1527G>T	MB	Caucasian	24
15	p.E517X	517Glu>Ter	c.1549G>T	Inf G _{M1}	Caucasian	27
15	p.W527LfsX	Frameshift + stop codon	c.1572_1577insG	Inf G _{M1}	Brazilian	329
15	p.W527X	527Trp>Ter	c.1580G>A	Inf G _{M1}	Caucasian	27
15	p.P549L	549Pro>Leu	c.1646C>T	Inf G _{M1}	Uruguayan	330
15	p.G554E	554Gly>Glu	c.1661G>A		Argentine/ Paraguayan	330
15	p.F570LfsX30	Frameshift + stop codon	c.1706_1707delC	Inf G _{M1}	Italian/ French	330
15	p.W576X	576Trp>Ter	c.1728G>A	Inf G _{M1}	Argentine	330
15	p.K578R	578Lys>Arg	c.1733A>G	Inf G _{M1}	Caucasian	325
15	r.1439_1440ins1440-28_1440-1	Aberrant splicing (intron 14)	c.1440-2A>G	Inf G _{M1}	Italian	319
16	p.G579D	579Gly>Asp	c.1736G>A	Juv G _{M1}	Italian	319
16	p.W582X	582Trp>Ter	c.1746G>A	G _{M1}	Japanese	New ^a
16	p.R590C	590Arg>Cys	c.1768C>T	Inf G _{M1}	Caucasian	27
16	p.R590H	590Arg>His	c.1769G>A	Juv G _{M1}	Caucasian	325
16	p.Y591N	591Tyr>Asn	c.1771T>A	Inf G _{M1}	Italian	319
16	p.Y591C	591Tyr>Cys	c.1772A>G	Inf G _{M1}	Italian	319
16	p.N614PfsX	Frameshift + stop codon	c.1835_1836delCC	Inf G _{M1}	Caucasian	322
16	p.E632G	632Glu>Gly	c.1895A>G	Juv G _{M1}	Caucasian	325
16	p.D640E	640Asp>Glu	c.1920C>G	Inf G _{M1}	?	new*

^a **GM1 = G_{M1} gangliosidosis; Inf = infantile form; Juv = juvenile form; Adult = adult/chronic form; MB = Morquio B disease.**

^b **For cDNA sequences, the A of ATG of the initiator methionine codon is denoted nucleotide +1.**³³⁷

^c **Ethnic origin not specified. Higaki and colleagues, unpublished data.**

^d **Defined as Spl2 in the original report.**¹²⁷

Table 151-6

Six novel mutations were found in a study of 19 Brazilian patients and 1 Uruguayan patient with infantile G_{M1} gangliosidosis: p.R121S, p.V240M, p.D491N, 588_591insT, 845_846insC, and 1572_1577insG.³²⁹ Two previously described point mutations also were identified: p.R59H and p.R208C. Together they accounted for 90 percent of the disease alleles of the patients. Among them, 1572_1577insG and p.R59H were present in 18 of 20 patients. In addition, four polymorphisms were identified: p.L10P, p.L12L, p.R521C, and p.S532G.

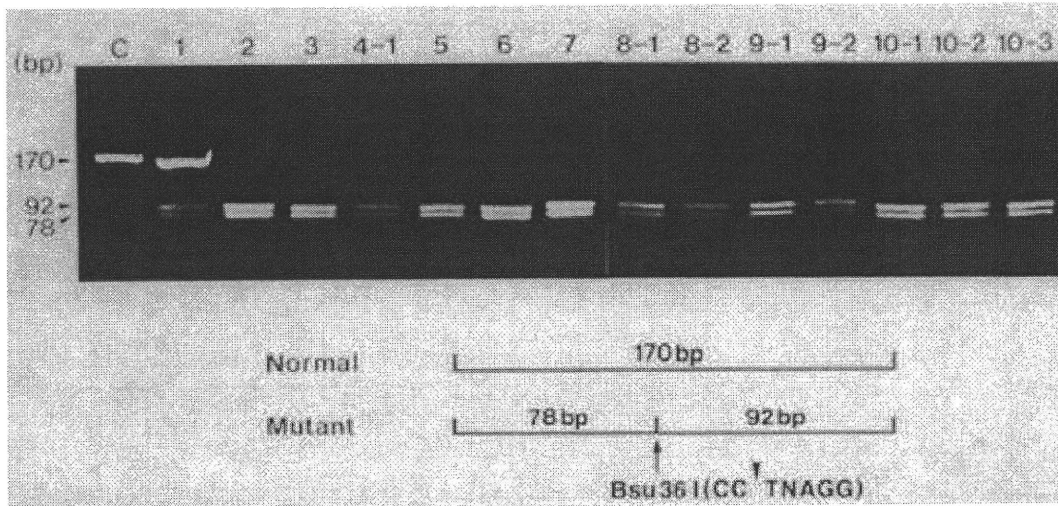
In one patient with infantile G_{M1} gangliosidosis, three missense mutations were found²³³; p.D332N (G994>A in exon 10) expressed catalytically inactive protein, suggesting a role in the active site. P.R148S (C442>A in exon 4) introduced a major conformational change into the protein, but there was no effect on catalytic activity. p.S532G (1594>G in exon 15) was found to be a polymorphism. Both mutants expressed immunoreactive protein in the perinuclear area consistent with localization in the endoplasmic reticulum (ER), but a low amount was detected in lysosomes. These results suggest that many of the missense mutations described in G_{M1} gangliosidosis to date have little effect on catalytic activity but do affect protein conformation such that the resulting protein cannot be transported out of the ER and fails to arrive in the lysosome. This accounts for the minimal amounts of enzyme protein and activity seen in most G_{M1} gangliosidosis patients.

Paschke and colleagues¹²² reported the results of mutation analysis in 17 juvenile and adult patients from various European regions with β-galactosidase deficiency and skeletal abnormalities: 15 with the Morquio phenotype and 2 with psychomotor retardation of juvenile onset. P.W273L was present in 14 of the 15 Morquio B patients. The Morquio phenotype was expressed in heterozygotes for W273L and alleles typically found in infantile G_{M1} gangliosidosis. A single French patient had a novel missense point mutation (p.Q408P) together with a known mutation (p.T500A). Mentally retarded patients were both heterozygous for two mutations known in chronic G_{M1} gangliosidosis together with two novel missense point mutations (p.Y270D and p.H281Y) in the vicinity of p.W273L. These results suggest a high impact of Trp-273 on the function of β-galactosidase and expression of the Morquio phenotype. In addition, a second domain around amino acids 400 to 500 also may be of significance.

Morrone and colleagues³¹⁹ reported five new β -galactosidase gene mutations in nine Italian patients and one fetus from seven unrelated families. Six of the eight patients with infantile-form GM₁ gangliosidosis presented with cardiac involvement, a feature only rarely associated with this disease. Two new RNA splicing defects were identified, together with three previously described amino acid substitutions. All patients with cardiac involvement were homozygous for one of the mutations, p.R59H, p.Y591C, p.Y591N, or IVS14-2A>G (c.1480-2A>G). All others were compound heterozygotes for one of the following mutations: p.R201H, p.R482H, p.G579D, and IVS8+2T>C (914+2T>C).

In Fig. 151-9, the results of restriction analysis on families with adult GM₁ gangliosidosis are presented. The combination of exon 2 amplification and restriction analysis is useful for diagnosis of the p.I51T mutation, expressing adult GM₁ gangliosidosis, which is common among the Japanese population; 16 patients were homozygotes, and 1 was a compound heterozygote for this mutation.⁶³

Fig. 151-9:



Restriction analysis of genomic DNA from 14 patients with adult/chronic GM1 gangliosidosis. A 170-bp fragment was PCR-amplified, digested with Bsu36I, electrophoresed, and stained with ethidium bromide. The lane number corresponds to the family number in Table 151-2. The p.I51T mutant allele is digested to produce two smaller fragments, 92 and 78 bp. All patients in this study were homozygous for the p.I51T mutation (genotype: p.I51T/p.I51T), except patient 1 (compound heterozygote: p.I51T/p.R457Q). (Reproduced from Yoshida et al. 63 Used by permission.)

Genotype-Phenotype Correlation

As shown in Table 151-5, gene mutations are heterogeneous in the clinical phenotypes defined as GM₁ gangliosidosis and Morquio B disease. In general, the mutant gene locus or mutant protein is not directly related to any specific phenotype. Infantile GM1 gangliosidosis has been the most common phenotype among the human β -galactosidase deficiencies. Apparently most β -galactosidase gene mutations express no or extremely low residual enzyme activity. The mRNA amount is reduced or not detectable. Most of the patients with infantile GM1 gangliosidosis are compound heterozygotes without consanguinity. However,

recent investigations revealed that the mutant enzyme proteins in some patients with various clinical phenotypes are often unstable owing to molecular misfolding after biosynthesis, and they are rapidly degraded without expressing the catalytic activity (see “Chemical Chaperone Therapy” below).

For other phenotypes with milder clinical manifestations, these characteristics were observed in our studies^{23, 24, 63}: (1) three gene mutations, p.R201C, p.I51T, and p.W273L, were always found for late infantile/juvenile G_{M1} gangliosidosis, adult/chronic G_{M1} gangliosidosis, and Morquio B disease, respectively, in specific ethnic populations, and (2) patients are either homozygotes or compound heterozygotes, but the expression products of counterpart mutant genes (p.R457Q, p.R482H, and p.W509C) showed no enzyme activity; any combination of these second mutant genes results in infantile G_{M1} gangliosidosis.

The three common mutant genes produce reduced but active enzymes. Partial breakdown of natural substrates, e.g., ganglioside G_{M1}, oligosaccharide, and keratan sulfate, probably results in mild phenotypic expression in different ways. For late infantile/juvenile G_{M1} gangliosidosis, no obvious difference was observed in clinical manifestations between homozygotes and compound heterozygotes. For adult/chronic G_{M1} gangliosidosis, the age of onset and clinical signs were similar, but a compound heterozygote p.I51T/p.R457Q had a relatively rapid clinical course compared with homozygotes p.I51T/p.I51T.⁶³

Among these common mutations, p.R201C and p.R457Q, together with some sporadic mutations, responded effectively to a chaperone compound to express catalytic enzyme activity in cultured fibroblasts and experimental mouse tissues, including the brain.^{338, 339} This finding prompted us to develop a new therapeutic approach to β-galactosidosis and other lysosomal diseases by oral administration of various chaperone compounds (see “Chemical Chaperone Therapy” below).

PATHOGENESIS

G_{M1} has been suggested to have antineurotoxic, neuroprotective, and neurorestorative effects on various central transmitter systems.³⁴⁰ It may be used for treating neuroinjury and a variety of degenerative conditions, including aging. On the other hand, as already described in this chapter, morphologic, pharmacologic, and biochemical changes have been demonstrated in human and animal diseases involving catabolism of ganglioside G_{M1} and related complex carbohydrates, resulting in excessive storage of these compounds. Neuronal apoptosis is found in G_{M1} gangliosidosis.³⁴¹ A loss of oligodendrocytes and abnormal axoplasmic transport were observed, resulting in myelin deficit, in two patients with G_{M1} gangliosidosis with massive neuronal storage of G_{M1}.³⁴² Furthermore, inflammatory responses may contribute to the pathogenesis of disease progression.^{343, 344}

Ganglioside G_{M1} stimulates neurite outgrowth as a morphologic expression of neuronal differentiation and enhances the action of nerve growth factor. Golgi and electron-microscopic studies of cortical neurons demonstrated large neural processes (meganeurites) in several lysosomal storage diseases (i.e., G_{M2} gangliosidosis, Hurler disease, and neuronal ceroid lipofuscinosis).³⁴⁵ Meganeurites develop between the base of the perikaryon and the initial portion of the axon, particularly in pyramidal neurons. Dendritic growth also occurs at aberrant sites (ectopic dendrogenesis).³⁴⁶ The extent of meganeurite development is related to the onset, severity, and clinical course of the disease. Inhibition of neurite initiation occurred in Neuro-2a neuroblastoma cells when the cell surface G_{M1} was blocked by the B subunit of cholera toxin

or anti-G_{M1} antibody, but no effect was observed on preformed neurites.³⁴⁷ Neuronal G_{M1} storage was shown to activate an unfolded protein response, leading to neuronal apoptosis in the G_{M1} gangliosidosis model knockout mouse. This activation did not occur in mice double deficient for β-galactosidase and ganglioside synthase, βgal^{-/-}:GalNAc^{-/-}, which do not accumulate G_{M1}. It was concluded that accumulation of G_{M1} may cause neuronal apoptosis in G_{M1}-gangliosidosis.³⁴¹

Similar morphologic changes were observed subsequently for feline G_{M1} gangliosidosis.^{348, 349} MCBs were densely packed in meganeurites, except near their peripheral area. Meganeurites were present on pyramidal neurons, granule cells of the fascia dentata, and spiny neurons of the caudate nucleus. Neurite outgrowth from meganeurites is particularly prominent in this feline mutant. The onset of ectopic neurite growth occurred after the elaboration of dendrites on cortical pyramidal neurons.³⁵⁰ There was a greater tendency toward formation of meganeurites in sheep with G_{M1} gangliosidosis, whereas the growth of ectopic axon hillock neurites without meganeurites predominated in cats with G_{M2} gangliosidosis.³⁵⁰ There are probably two separate driving forces behind somadendritic abnormalities of pyramidal neurons in the two types of gangliosidoses. The analytical results in cats are summarized later (see “Feline G_{M1} Gangliosidosis” below).

In another study, significant changes in neuronal connectivity were found in the cerebral cortex, such as degenerative changes in axons and synapses of inhibitory neurons and regrowth of dendrites and new synapse formation (“rewiring”) involving pyramidal neurons.³⁵¹ In addition, G_{M1} was found in the nuclear membranes of neuroblastoma cells and cerebellar granule cells in association with neurite outgrowth.³⁵² The total ganglioside and G_{M1} concentrations correspond with the extent of the meganeurite formation in the feline disease,³⁵³ and a reduced fluidity of synaptosomal membranes was found.³⁵⁴ These data suggest that storage of G_{M1} is closely related to abnormal synaptogenesis in this disease.

G_{M1} also associated tightly with Trk, the high-affinity tyrosine kinase-type receptor for nerve growth factor (NGF), enhancing neurite outgrowth and neurofilament expression in rat PC12 cells³⁵⁵ and C6trk⁺ cells, a derivative of C6-2B glioma cells.³⁵⁶ In the latter, tyrosine phosphorylation of trkA target proteins, such as extracellular signal-regulated kinases and *suc*-associated neurotrophic factor-induced tyrosine-phosphorylated target, also were activated. However, administration of G_{M1} had no effect on the Trk protein, although it partially restored NGF and NGF mRNA in frontal cortex and hippocampus in the brains of aged rats, which showed moderate decreases in both Trk and NGF compared with the brains of young rats.^{355, 357}

Cholinergic function was altered in the brains of cats with G_{M1} gangliosidosis.^{358, 359} Acetylcholine synthesis and its K⁺-stimulated release were increased in cerebral cortex and hippocampal slices. Choline acetyltransferase activity was not significantly different from that in controls. However, the decreased activity of choline acetyltransferase in aged rats was enhanced by treatment with G_{M1} in the striatum and frontal cortex but not in the hippocampus.³⁶⁰ In another experiment, a decrease in high-affinity uptake of glutamate, γ-aminobutyrate, and norepinephrine was observed in synaptosomes in feline G_{M1} gangliosidosis.³⁶¹ Neuroaxonal dystrophy was observed in GABAergic neurons in feline models of lysosomal storage disorders (i.e., G_{M1} gangliosidosis, G_{M2} gangliosidosis, α-mannosidosis, and mucopolysaccharidosis I).³⁶² A resulting defect in neurotransmission in inhibitory circuits may be an important factor underlying brain dysfunction in them.

Phosphoinositol-specific phospholipase C and adenylyl cyclase activities were altered in the membranes of cerebral cortex from cats with GM₁ gangliosidosis.³⁶³ Phospholipase C did not respond to stimulation by GTP γ S, carbachol, or fluoroaluminate but was normally activated by calcium. Basal adenylyl cyclase activity was increased threefold in the brains of affected cats. Plasma membrane-localized GM₁ modulates prostaglandin E₁-induced cAMP in Neuro-2a neuroblastoma cells³⁶⁴; treatment with neuraminidase, an enzyme that increases cell surface GM₁, or with nanomolar concentrations of GM₁ elevated cAMP in these cells. However, higher concentrations of exogenous GM₁ progressively inhibited adenylyl cyclase and induced a dose-responsive fall-off in cAMP elevation.

The B subunit of cholera toxin, which binds specifically to ganglioside GM₁ in the outer leaflet of the cell membrane, was found to induce an increase in calcium and manganese influx in N18 cells.³⁶⁵ Activation of an L-type voltage-dependent calcium channel has been suggested. This channel modulation by GM₁ has important implications for its role in neural development, differentiation, regeneration, and electrical excitability of neurons, possibly under pathologic conditions owing to an abnormal storage of GM₁. On the other hand, GM₁ reduced the ethanol-induced activation of phospholipase A₂ in synaptosomal preparations from rat brain.³⁶⁶ This neuroprotective effect against ethanol on the nervous system is probably induced by influencing deacylation/reacylation of membrane phospholipids. An increase in autophagy was found in the brain of a β -galactosidase-deficient knockout mouse, as evidenced by elevation of LC3-II and beclin-1 levels. It was accompanied by enhanced Akt-mTOR and Erk signaling. In addition, the mitochondrial cytochrome C oxidase activity was significantly decreased in the brain and cultured astrocytes. Mitochondria isolated from astrocytes were morphologically abnormal and had a decreased membrane potential. These cells were more sensitive to oxidative stress than wild-type cells, and this sensitivity was suppressed by ATP, an autophagy inhibitor, 3-methyladenine, and a pan-caspase inhibitor, z-VAD-fmk. These results suggest activation of autophagy leading to mitochondrial dysfunction in the brains of GM₁ gangliosidosis patients and animals.³⁶⁷

These data suggest that various morphologic and metabolic aberrations occur in the brains of GM₁ gangliosidosis patients and animals. However, the pathogenesis of localized encephalopathy is not known in adults with GM₁ gangliosidosis. Furthermore, the pathogenetic role of oligosaccharides or keratan sulfate has not been investigated for the development of morphologic abnormalities occurring mainly in mesodermal tissues. Understanding the information-processing system induced by ganglioside GM₁ and possibly other galactose-containing glycoconjugates may provide further insight into the precise mechanisms of brain dysfunction in β -galactosidase-deficient disorders and also of dendritic elaboration and synapse formation in the developing nervous system.

The role of EBP also is important for the pathogenesis of phenotypic variations of GM₁ gangliosidosis. Cultured skin fibroblasts from β -galactosidosis patients were subjected to elastic fiber assembly study.³³⁶ The cells from infantile- or adult-form GM₁ gangliosidosis patients carrying missense mutations affecting GLB1 but not EBP (or S-Gal) assembled elastic fibers. In contrast, the cells from two infantile GM₁ gangliosidosis patients with nonsense mutations and from Morquio B patients with mutations causing deficiency in both proteins did not, and transfection of EBP in these cells improved elastogenesis. This result indicated an association between impaired elastogenesis and the development of connective tissue disorders in infantile GM₁ gangliosidosis and Morquio B disease. The mutations identified in patients with cardiomyopathy fell in the GLB1 cDNA region common to the enzyme and EBP.³¹⁹ Both molecules are affected by the mutations. The site of gene mutation may contribute differently to the occurrence of specific clinical manifestations.

DIAGNOSIS

Clinical Diagnosis

The diagnostic procedure starts with careful clinical evaluation of a patient with neurosomatic manifestations of unknown origin. The possibility of G_{M1} gangliosidosis always should be considered in patients with progressive neurologic deterioration or developmental retardation with signs of general somatic changes, particularly of the skeletal system and connective tissue, with or without visceromegaly, in infancy. It should be remembered, however, that many cases of G_{M1} gangliosidosis do not present with specific somatic manifestations such as facial dysmorphism, visceromegaly, or remarkable bone changes. Clinical diagnosis of patients with the onset after infancy is more difficult because no specific signs or symptoms have been described, except for deteriorating clinical course and slight vertebral deformities common to disorders of complex carbohydrates (i.e., mucopolysaccharides and glycoprotein-derived oligosaccharides).

Dysmorphism and skeletal dysplasias are important clinical signs for differential diagnosis in infancy, late infancy, and childhood. Biochemical screening of urinary mucopolysaccharides and oligosaccharides, as well as enzyme studies, should be performed for excluding mucopolysaccharidoses, multiple sulfatase deficiency, α -mannosidosis, β -mannosidosis, fucosidosis, aspartylglucosaminuria, I-cell disease, sialidosis, galactosialidosis, and other related diseases.

Macular cherry-red spots, if present, are important for consideration of lysosomal storage diseases. Several diseases have been recognized with this unique finding in the optic fundi: G_{M2} gangliosidosis (Tay-Sachs disease, Sandhoff disease, and AB variant), G_{M1} gangliosidosis, Niemann-Pick disease, sialidosis, and galactosialidosis show typical macular changes. Similar but not typical changes have been described in patients with other lysosomal diseases, such as metachromatic leukodystrophy, globoid cell leukodystrophy, and Gaucher disease.

G_{M1} gangliosidosis in adults is considered a discrete clinical entity. Progressive extrapyramidal disease, mainly presenting as dystonia, associated with vertebral dysplasia, is an important diagnostic point. Any adult patient with this combination of neuroskeletal signs, unless associated with other specific manifestations indicating diagnosis, should be considered for enzymatic screening of β -galactosidase deficiency.

Rectal biopsy can be of diagnostic help; the neuronal cytoplasm is ballooned with storage material.^{58, 368} Skin biopsy may afford a clue to diagnosis if sweat glands are studied carefully; the cells of secretory coils are swollen and vacuolated with faintly visible small granules.³⁶⁹ Cytoplasmic vesicles were observed in the endothelial cells of conjunctiva, producing a mechanical narrowing of the lumen.³⁷⁰ The storage material was similar to that seen in fibroblasts from skin or of visceral organs in G_{M1} gangliosidosis. The conjunctiva provides a ready source for biopsy and diagnostic evaluation. Various lectin stains indicate the presence of acidic glycoconjugates in histiocytes containing abundant terminal β -galactose residues.⁵⁸

Clinical diagnosis of Morquio disease is not difficult. Skeletal deformities are unique, and enzyme assays will confirm the diagnosis of type B disease. However, bone changes on x-ray films are not specific to G_{M1} gangliosidosis; mucopolysaccharidoses and glycoprotein disorders can show almost the same picture. Further biochemical analysis is necessary for the final diagnosis.

Biochemical Diagnosis

Analysis of storage compounds

The analytical hallmark of G_{M1} gangliosidosis is the abnormal accumulation of ganglioside G_{M1} in tissues. Because the levels of gangliosides in extraneural tissues are very low, lipid analyses for diagnostic purposes are usually limited to the brain. The total concentration of brain gangliosides may be three- to fivefold normal in the gray matter and may be as high as 10 times in the white matter.¹⁵⁰ About 90 to 95 molar percent of the total ganglioside is G_{M1} , in contrast to 22 to 25 molar percent in normal brain; the actual increase of G_{M1} in the gray matter is up to 20-fold normal.

The determination of G_{M1} accumulation in the brain by means of thin-layer chromatography³⁷¹ is convenient, but its applicability is usually restricted to postmortem examination. An immunologic method for determination of gangliosides in CSF using enzyme immunostaining and densitometry has been reported,³⁷² and an increase in G_{M1} was confirmed in the CSF of infantile-form patients.^{62, 99, 100} G_{M1} was not detected in adult patients.

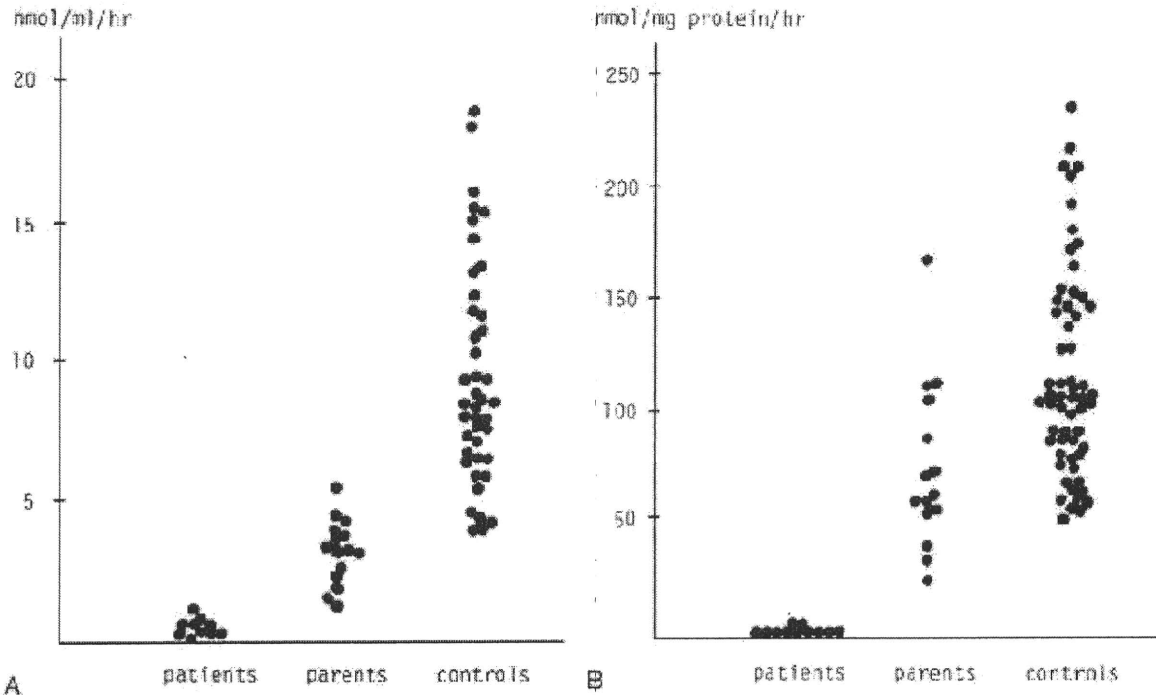
The postmortem diagnosis of lysosomal storage diseases can be confounded by the unavailability of suitable material. Whitfield and colleagues³⁷³ reported a new diagnostic method for G_{M1} gangliosidosis in a cross-bred dog, in which only formalin-fixed brain was available, by a combination of electron microscopy and the detection of elevated levels of ganglioside G_{M1} within the tissue using electrospray ionization tandem mass spectrometry. Electron microscopic examination of resin-embedded tissue revealed cytoplasmic inclusions (membranous cytoplasmic and zebra bodies) in brain stem and cerebellar neurons. Glycolipids were extracted from the fixed tissue and analyzed by tandem mass spectrometry. Two major ions were detected, corresponding to G_{M1} (d18:1-C18:0) and G_{A1} (d20:1-C18:0). Their identity was confirmed by comparison of their fragmentation patterns with those of authentic standards. G_{M1} concentration in the brain was approximately sixfold higher on a wet-weight basis than in a normal control dog, confirming the diagnosis of G_{M1} gangliosidosis.

Galactose-containing oligosaccharides are excreted in the urine of patients with G_{M1} gangliosidosis and Morquio B disease.^{175- 177, 180, 183, 374, 375} Thin-layer chromatographic or HPLC analysis of the urinary oligosaccharides is employed as an ancillary diagnostic test. The concentration of the urinary oligosaccharides correlates with the severity of the disease.^{177, 375} Analysis of urinary mucopolysaccharides and demonstration of the excessive excretion of keratan sulfate are the earliest biochemical procedures available for the diagnosis of Morquio B disease.

Enzyme diagnosis

The most common biochemical parameter is the activity of β -galactosidase. For enzyme diagnosis of G_{M1} gangliosidosis and Morquio B disease, leukocytes, fibroblasts, and solid tissues usually can be used as enzyme sources (Fig. 151-10 A and B). Serum potentially might be useful, but it is known that β -galactosidase activity in serum changes contingent on the preparation conditions such as the time allowed for clotting.²⁷⁵ In fact, the data using serum have not been reliable or reproducible, when serum was used as the sole enzyme source for diagnostic assay for G_{M1} gangliosidosis. Plasma gives more reliable enzymatic results for the diagnosis of G_{M1} gangliosidosis.

Fig. 151-10:



β -Galactosidase activity in plasma and leukocytes from patients with infantile GM1 gangliosidosis. A, Plasma. B, Leukocytes. Enzyme assays give a clear diagnosis of the patients. However, there is more overlap between the normal individual group and obligate heterozygotes when leukocytes (a mixture of granulocytes and lymphocytes) are used for enzyme assay. (Y. Suzuki, unpublished data.)

β -Galactosidase activity is also present in urine, but there is an enormous range of variation in this enzyme activity in normal urine. The ratio of β -hexosaminidase to β -galactosidase should be used for diagnosis. In view of the ready availability of other enzyme sources, the use of urine is not recommended. Tears may be used for diagnostic enzyme assays.³⁷⁶

Artificial substrates such as *p*-nitrophenyl β -galactopyranoside or 4-methylumbelliferyl β -galactopyranoside are readily available; the latter is fluorogenic, sensitive, and widely used. In infantile and juvenile GM1 gangliosidosis, β -galactosidase activity is almost completely deficient, but a considerable residual enzyme activity is detected in adult GM1 gangliosidosis and Morquio B disease by enzyme assays in leukocytes or fibroblasts using artificial substrates. In addition, a recent report suggested that new fluorogenic substrates, i.e., 4-fluoromethylumbelliferyl derivatives, were convenient for revealing glycosidase activities directly in tissue samples³⁷⁷ because 4-fluoromethylumbelliferone exhibits more contrast yellow fluorescence under the ultraviolet (UV) light than the blue light of 4-methylumbelliferone on exposure of the enzyme activity on solid supports.

However, these substrates also can be degraded by galactosylceramidase, which is commonly increased in G_{M1} gangliosidosis, and ambiguous results sometimes are obtained with the white matter, which is normally rich in galactosylceramidase. Hydrolysis of 4-methylumbelliferyl β-galactopyranoside by the white matter from G_{M1} gangliosidosis patients ranged from 20 to 25 percent of normal values.^{241, 245} Use of the natural substrate—radiolabeled G_{M1} or G_{A1} is recommended when the results with the artificial substrates are equivocal.²¹⁵

A newly developed fluorophor may overcome this problem.

O-[4-(1-Imidazolyl)butyl]2,3-dicyano-1,4-hydroquinonyl β-D-galactopyranoside (Im-DCH-β-Gal)³⁷⁸ was shown to be a specific substrate for human lysosomal β-galactosidase in cell homogenates. Furthermore, its tetraacetate derivative, Im-DCH-β-Gal(OAc)₄, was taken up and hydrolyzed by normal fibroblasts but not hydrolyzed in the enzyme-deficient cells. The rates of uptake and deacetylation were similar for normal and mutant cells. Another fluorogenic substrate, 5-chloromethylfluorescein di-α-D-galactopyranoside, was used for monitoring *lac Z* gene expression in cultured cells.³⁷⁹ It is far more sensitive than the conventional X-gal cytochemistry and may be useful for monitoring gene expression of human α-galactosidase after gene therapy or protein therapy in the future. Another staining method based on tetranitroblue tetrazolium (TNBT) reduction and precipitation has been proposed as a sensitive procedure for immunohistochemistry and in situ hybridization of β-galactosidase and its mRNA.³⁸⁰ These compounds will be useful as fluorescent markers for detecting genetic β-galactosidase mutants.

It is also important to determine other enzyme activities, including neuraminidase, for the differential diagnosis of secondary β-galactosidase deficiency, such as galactosialidosis, I-cell disease, mucopolipidosis III, and mucopolysaccharidoses other than Morquio B disease.

Gene Diagnosis

Direct Sequence Analysis

We established an analytical system for detection of β-galactosidase gene mutations. PCR primers were designed to include the entire β-Gal coding sequence and all intron-exon boundaries in 15 separate PCR amplification products (Table 151-7). The PCR mixture contained 0.2 μg DNA, 2 μL 10× PCR buffer (15 mM MgCl₂, 2.5 μL 2 mM dNTP mix, and 20 pmol of each primer), and 1 unit of *Taq* DNA polymerase. The cycling started first with denaturation at 95°C for 5 minutes, then 35 cycles of denaturation at 95°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes.

Table 151-7 The Oligonucleotide Primers for PCR Amplification

Exon	Sense (5'→3')	Antisense (5'→3')	PCR Size, bp
1	caggccgtgggtccttagtcaagt	gccagcctgtcccctagcaatg	204
2	gctactctcaaaggatcggtctgaaa	tatcttctccagagtgggtgtcagg	303
3	gccttctccctctatccatgtgttagc	taaaagacacctgtgctgggtacagtcc	401
4	cccctgtccctgaagcttttattctt	tgtatttttagtagggcgaggtttgc	347
5	agtttacgaattgtgtgggccacatt	gccttcccaaatgcaattgaactaaaag	358
6	aggatctctcattttccctgctcttt	atgaaaaatctcaatctgccatgacac	330
7	actaacattctgacctagcagggttc	tattcacatgtccagaatggctatgac	325
8–9	ctttacacctgtcatagatggggcattg	cacaccctctcaaattaatcaacaga	412
10	cgctgtgtctccaacaagtggtttta	gtgagttcaaagaggctctgtccaaga	312
11	gcactgttgagtcttgacctgtcttc	ttcgagaaaaataacgaaccaattctt	301
12	gggagtagatggagaggactgaaggaga	ggatctgatgcatttctaccattttg	391
13	ggaggtggaggaagattttcattcctta	ctgaaaaggtagcaaagaccccaaat	343
14	tctcctgtctgaccttctaccctcaat	tatttaccaggctgttctgaactcc	348
15	atttcgaggtcatttctgttggtgtt	aagtttaggcctgaattcaaacccttc	427
16	ggggtgatggttctctgtctctctc	gaaacctcaggtgaaaatgcacatccta	467

The PCR product, confirmed by 1% agarose gel electrophoresis, was again 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*361 restriction analysis has been performed for gene diagnosis of 14 adult/chronic-form GM1 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	-	<i>BbvI/BsoFI</i>		ASO	297
I51T	+	<i>Bsu361/SauI</i>	23, 58		
T82M	+	<i>MslI</i>		ASO	298
Y83H	+	<i>N/aIII</i>			
G123R	-	<i>SmaI/ApaI</i>			
R201C	-	<i>BspMI</i>	23, 264		
R201H	-	<i>HhaI</i>		SSCP	304
R208C	N			ASO	300, 303
P263S	+	<i>BamHI</i>			
N266S	+	<i>CtaI</i>		SSCP	304
W273L	+	<i>StuI^b</i>	24		
Y316C	N				
N318H	+	<i>NtaIII</i>			
S434L	-	<i>MboII</i>			
R457X	N				
R457Q	N				
R482H	+	<i>NspI</i>	24		
R482C	-	<i>AflIII</i>		ASO	299
G494C	N				
W509C	+	<i>RsaI</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 second mutation by PCR.*

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

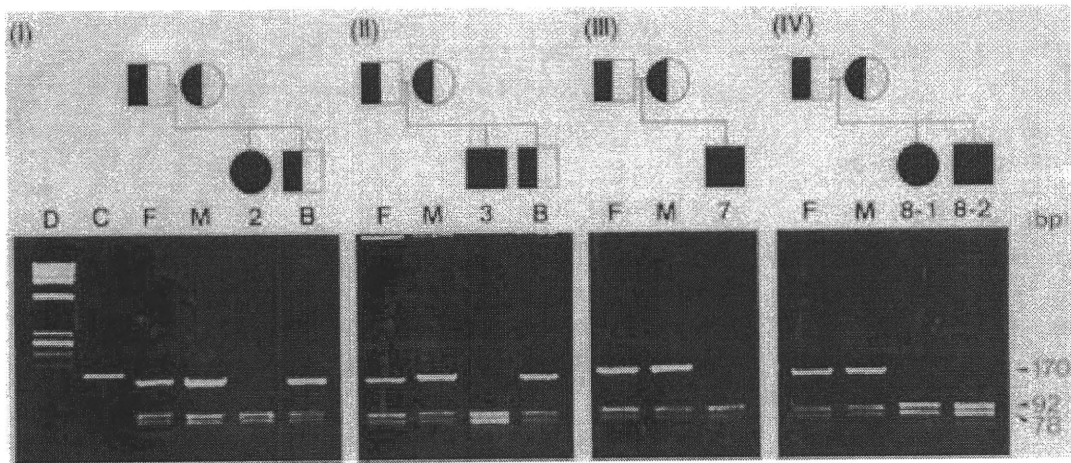
Heterozygous Carrier Diagnosis

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 GM1 gangliosidosis⁶³ (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 (λ 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 GM₁ gangliosidosis.³⁸¹⁻³⁸⁶ A rapid diagnosis was made possible by microchemical assays³⁸⁴; 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 GM₁ 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 GM₁ 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 GM₁ gangliosidosis.³⁸⁹ MCBs were abundant in retinal ganglion cells.³⁹⁰ Marked retinal lesions were reported in two other patients.^{391, 392}