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B_1 Variant of G_{M2} Gangliosidosis in a 12-Year-Old Patient[1]

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ABSTRACT. A girl aged 12 y, 9 mo, suffered from a progressive neurodegenerative disorder marked by ataxia, extrapyramidal symptoms, and convulsions. A skin biopsy showed axonal pathology that emphasized axonal segments enlarged by mitochondria, dense bodies, and lysosomal residual bodies of the membranous cytoplasmic body type. This ultrastructural pathology suggested G_{M2} gangliosidosis which was shown to be a B_1 variant by specific biochemical studies, although conventional techniques had failed to detect G_{M2} gangliosidosis. The B_1 variant is marked by a deficient activity of β-hexosaminidase A towards one substrate, and by an almost normal activity towards another. Both parents showed a diminished activity towards the sulfated substrate, suggesting a heterozygous state, and almost normal activity with the second substrate type. (*Pediatr Res* 25:89–93, 1989)

Abbreviations

MCB, membranous cytoplasmic bodies

To date, more than 10 variants with different (allelic or non-allelic) molecular defects of G_{M2} ganglioside catabolism have been characterized. Recent molecular studies have revealed the underlying mutations in patients with various β-hexosaminidase A deficiency (1–3). Four variants of G_{M2} gangliosidoses are known (4) with fundamentally different molecular defects: *1*) variant B; Tay-Sachs disease, marked by a total deficiency of β-hexosaminidase A activity due to a missing α-subunit (defective maturation of mRNA in the Ashkenazi (2), or gene deletion in the French Canadian type (1)); *2*) variant 0; Sandhoff's disease, marked by defective β-subunits of hexosaminidase enzyme proteins resulting in deficient β-hexosaminidase A and B activities; *3*) variant AB; showing intact β-hexosaminidases A and B, but marked by a defect of the activator protein necessary for the degradation of G_{M2} ganglioside; *4*) variant B_1 marked by a mutant but not absent β-hexosaminidase A; the mutation in the α-subunit gene has been characterized in one patient as a single base substitution (3).

The mutant enzyme in variant B_1 has an almost normal activity towards substrates that are split preferentially at the active site located on the β-subunit. It is virtually inactive towards those substrates that are exclusively or preferentially cleaved at the other active site on the α-subunit [G_{M2} ganglioside and synthetic substrates containing a sulfate group at their 6-position; (5)] in normal individuals.

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Screening for G_{M2} gangliosidoses by β-hexosaminidase determinations will miss the AB and B_1 variants, if conventional assay methods are used. The use of the 6-sulfated synthetic substrates (5, 6) will detect the B_1 variant during the screening procedure, but screening for the AB variant is possible in only a few specialized laboratories.

Morphologic investigation of dermal and conjunctival cells in biopsy tissue enables a recognition of numerous lysosomal diseases (7–10) and, in other instances, pinpoint an area for specialized biochemical investigation. In G_{M2} gangliosidosis, it has previously (11) been shown that studying axons, particularly axonal terminals, in biopsied skin specimens may be particularly rewarding in that axonal enlargement due to accumulation of mitochondria, dense bodies, and not infrequently of rather disease-specific lysosomal residual bodies may be present. This approach has previously resulted in the demonstration of a B subvariant of G_{M2} gangliosidosis in a young Turkish child (12). In this article we report a 12-y-old patient with the B_1 variant of G_{M2} gangliosidosis.

Clinical data. This girl was born after an uneventful pregnancy (breech position, 3290 g, 54 cm) to nonconsanguineous parents who came from the same Spanish village. No family member had abnormal neurologic symptoms. Her postnatal development was normal. She started to walk at 14 mo and began to talk when 2 y old. From early in her infancy, the parents had noted feeding and sleeping problems and an unsteady gait. She was first examined at 4½ y because of increasing gait and behavioral abnormalities. At that time, she showed truncal ataxia when standing and walking. She was unable to stand or hop on one leg or to walk on tiptoes or heels; she fell frequently. The muscle tone was decreased; the muscle reflexes were hyperactive, and Babinski's sign was absent. Her mental status was described as unconcentrated, but she was still able to follow simple commands.

During the following years, her neurologic and mental state deteriorated, and grand mal and partial seizures with complex symptomatology appeared which were treated with valproate and later with phenobarbital.

When hospitalized at the age of 6½ y, she was demented, spoke only a few words, could not feed herself, and was incontinent. She could only walk with assistance, and developed choreoathetoid movements and general restlessness, suggesting extrapyramidal involvement. Muscle tone and reflexes were unchanged; plantar reflexes were absent.

She was bedridden from the age of 9 y and mute from the age of 11 y. Physical symptoms were severe kyphoscoliosis and severe weight loss (from the 50th percentile at the age of 6 y to less than the 3rd percentile when 11 y old). Persistent feeding problems, vomiting, and finally equivocal signs of intestinal obstruction resulted in a duodenojejunal bypass operation, but intestinal obstruction could not be verified.

When last seen at the age of 12⁹/₁₂ y, she weighed 30 kg (3rd percentile), was 159 cm tall (90th percentile), and had a head

circumference of 54 cm (75th percentile). She had no contractures and no hepatosplenomegaly. Her arms and legs were held in flexed or extended positions. Her hands and face showed continuous involuntary movements with occasional choreoathetoid appearance. She hardly responded to external stimuli except for questionable following of her eyes to known objects and slight reaction to familiar sounds. Muscle tone had remained decreased, muscle reflexes were exaggerated without Babinski's signs. For the first time, macular cherry red spots were noticed. She died at the age of 13 y, 2 mo.

Ancillary data. Routine laboratory data including those of bone marrow were negative. Serum lactate and pyruvate, blood gases, ammonia, amino acids in serum and urine, ceruloplasmin, and serum copper showed normal values. The urinary copper excretion was first elevated, but was nearly normal on repeat analysis. Cerebrospinal fluid protein and cell counts, nerve conduction velocities, electroretinogram, and visual evoked potentials were also normal. Several EEGs recorded during the course of the disease showed increasingly pronounced slowing of basal activity; generalized and focal epileptic activities were always present. Three computerized tomographic scans showed cortical, subcortical, and cerebellar atrophy, associated with enlargment of lateral ventricles. Two separate skin biopsies were taken at the age of 11 y for routine electron microscopic processing and fibroblast culturing.

MATERIALS AND METHODS

Biochemical methods. The β-hexosaminidase activities toward the synthetic substrates *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide and *p*-nitrophenyl-6-sulfo-*N*-acetyl-β-D-glucosaminide (which is almost specific for β-hexosaminidase A activity) were assayed as described by Fuchs *et al.* (6) but modified in that plasma, white blood cell, or fibroblast homogenates (0.3–1 mg protein/ml) were used instead of serum. The sulfate-containing substrate was synthesized and purified also according to Fuchs *et al.* (6). The β-hexosaminidase A and B activities toward 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide after cellogel electrophoresis of white blood cell and fibroblast extracts were assayed as described (13).

Protein content was measured according to Lowry *et al.* (14) with bovine serum albumin as standard. The protein content of the highly diluted fibroblast enzyme extract used for the G_{M2} ganglioside β-hexosaminidase assay was determined more precisely by calculating the difference between starting fibroblast homogenate and centrifugation pellet protein contents from a blank assay without activator protein.

Assay of G_{M2} ganglioside β-hexosaminidase in cultured fibroblasts. G_{M2} ganglioside labeled with tritium in the *N*-acetylgalactosamine moiety was prepared as described (15); 24 nmol ganglioside (1000 dps) were dissolved in 0.15 ml sodium citrate buffer (pH 4.0; 0.007 M) by shaking for 10 min at 37°C. A 0.15-ml fibroblast homogenate (40–60 μg protein) in sodium citrate buffer (pH 4.0; 0.022 M) and a small volume with 10 μg activator protein preparation (see below) were adjusted with water to a total volume of 0.8 ml, thoroughly mixed, and centrifuged for 10 min at 44,000 × g. The supernatant was added to the ganglioside solution. The mixed supernate and ganglioside solution were immediately loaded on a molecular filter equipment (Amicon, 581 Witten, FRG; no. 4010 and no. 40420 YMT membrane) and centrifuged for 15 min at 1510 × g at 37°C (first incubation period). The filter was then washed with another 0.8 ml sodium citrate buffer (pH 4.0; 0.007 M) by centrifugation in the same manner (second incubation period; it was confirmed that primary incubation at 37°C previous to both centrifugation steps did not result in substantial G_{M2} hydrolysis). The combined filtrates containing the enzymatically released radioactive *N*-acetylgalactosamine and trace amounts of percolated G_{M2} ganglioside were mixed with 0.1 mg unlabeled *N*-acetylgalactosamine and dried. Two consecutive methanol extracts (each 75 μl) of the dry residue were combined and subjected to thin layer chromatography as described (15). The iodine-visualized *N*-acetylgalactosamine spot was cut out and counted for radioactivity.

Preparation of G_{M2} ganglioside β-hexosaminidase activator. Deep-frozen human kidney was homogenized with 9 vol (wt:vol) phosphate buffer (pH 6.0; 0.02 M), stirred for 10 min at 20°C, and centrifuged for 30 min at 13,000 × g. The supernate was loaded onto a DEAE Sephadex A 50 column equilibrated and eluted with 3 column vol of the same buffer. When β-hexosaminidase B was eluted, further elution of β-hexosaminidase A was done with a 0.2 to 0.5 M NaCl gradient in the same buffer (8 column vol). The fractions containing β-hexosaminidase A also contained the activator; they were pooled, dialyzed for 3 h against distilled water, concentrated, and loaded onto a Sephadex G 200 column equilibrated with water. Elution with water revealed the peak of activator activity directly after the β-hexosaminidase A peak. The activator peak fractions were pooled, concentrated, mixed with 1/4 vol of sodium citrate buffer (pH 4.0; 0.022 M) so that 10 μg protein were contained in about 0.1 ml, heated for 2 h at 60°C, stored deep-frozen, and used for the G_{M2} ganglioside β-hexosaminidase assays.

RESULTS

Biochemical findings. The β-hexosaminidase activities of our patient were profoundly deficient only when the 6-sulfated substrate and G_{M2} ganglioside were used (Table 1). These findings indicated the B_1 variant of G_{M2} gangliosidosis. Different from classical Tay-Sachs disease (one 1.5-y-old patient studied for Table 1) our patient still showed a small residual β-hexosaminidase A activity of about 1 to 5% as compared to the controls with both the 6-sulfated synthetic and the ganglioside substrate. This residual activity may be significantly different from 0, but its level may be described better as 3 ± 2%, indicating a possible range of error.

When several additional fibroblast cultures from our patient were studied using the 6-sulfated substrate, higher residual activities were found in some cultures (not shown). These exhibited very high hexosaminidase B and A (β-subunit) activities with the nonsulfated synthetic substrate possibly enhancing the background effect with the 6-sulfated substrate. Independently, the diagnosis of the B_1 variant with the 6-sulfated synthetic substrates (including the 4-methylumbelliferyl type with its increased sensitivity) should always be unequivocal when white blood cells are used. These also allow heterozygote studies as shown for our patient's parents in Table 2.

Minor abnormalities in the patient and her father were that they both had an approximately 50% reduced total β-hexosaminidase activities toward the nonsulfated synthetic substrate in their white blood cells (Table 2). Only in the patient was the percentage of electrophoretically separated hexosaminidase A slightly reduced with this substrate. The biochemical data on fibroblast hexosaminidase A and B activities were independently confirmed (E. Conzelmann, unpublished data). These confirmatory experiments included the assay of β-hexosaminidase A activity with the 6-sulfated 4-methylumbelliferyl substrate. However, the results shown in Table 1 for the 6-sulfated *p*-nitrophenyl substrate were fully confirmed.

Morphologic findings. Although mesenchymal cells, such as fibroblasts, mural cells of dermal vessels, or smooth muscle cells, and epithelial cells, such as epidermis and glandular cells, were thoroughly examined, ultrastructural pathology was confined to neuroectodermal cells, both axons and Schwann cells.

Large lamellar-like inclusions resembling MCB and dense bodies were seen in myelinated axons and unmyelinated axon-Schwann cell complexes (Fig. 1). Terminal axons around eccrine sweat glands or among smooth muscle cells (Fig. 2) were laden with mitochondria and dense bodies. The terminal character of these unmyelinated axons was also demonstrated by the presence of clear and dense-core vesicles within the axoplasm (Fig. 2).

Table 1. *Patient's β-hexosaminidase activities*

Enzyme source	β-hexosaminidase substrate*	U of activity	Present patient (variant B_1)	Controls ($n = 8$) mean ± SD	Classical Tay-Sachs disease (variant B)
Plasma	PGNac*	$nmol \cdot h^{-1} \cdot ml^{-1}$	645	852 ± 218	790
	$PGNacSO_4$*	$nmol \cdot h^{-1} \cdot ml^{-1}$	2	38 ± 9	<1.5†
	PGNac	% heat labile (2 h, 50°C)	64	52 ± 12	1.5
White blood cells	PGNac	$nmol \cdot h^{-1} \cdot mg^{-1}$ protein	499	1071 ± 264	422
	MUGNac*	% hexA‡ electrophoresis	45	65 ± 6	<2†
	$PGNacSO_4$	$nmol \cdot h^{-1} \cdot mg^{-1}$ protein	2.1	177 ± 58	<1.5†
Cultured skin fibroblasts	PGNac	$nmol \cdot h^{-1} \cdot mg^{-1}$ protein	2880	4429 ± 945	1513
	MUGNac	% hexA‡ electrophoresis	42	57 ± 6	<2†
	$PGNacSO_4$	$nmol^{-1} \cdot h^{-1} \cdot mg^{-1}$ protein	32.4; 39	690 ± 144	14
	GM_2 ganglioside with 10 μg activator protein preparation	$nmol^{-1} \cdot h^{-1} \cdot mg^{-1}$ enzyme extract protein	0.12; 0.14	3.61 ± 1.56	<0.1†

* PGNac, *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide; MUGNac, 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide; $PGNacSO_4$, *p*-nitrophenyl-6-sulfo-*N*-acetyl-β-D-glucosaminide.

† Lower limit of detection.

‡ hexA, β-hexosaminidase A activity as % of total β-hexosaminidase activity on cellogel electrophoresis.

Table 2. *Relative β-hexosaminidase activities in blood**

	Plasma			White blood cells		
	PGNac	PGNac % heat labile (2h, 50°C)	$PGNacSO_4$	PGNac	MUGNac hexA electrophoresis	$PGNacSO_4$
Patient	76	64	5.3	47	45	1.2
Father	78	70	63	50	60	32
Mother	62	59	39	81	66	54
Patient's 4-y-old healthy brother	127	70	113	124	72	102
Controls ($n = 8$; mean ± SD)	100 ± 26	52 ± 12	100 ± 24	100 ± 25	65 ± 6	100 ± 33

* See Table 1 for abbreviations and for specific activities of controls.

Other membranous bodies also occupied the axoplasm of unmyelinated axons. Occasionally, pi and lipopigment granules were seen in Schwann cells, the latter consisting of a granular matrix with haphazardly embedded membranes and a lipid droplet.

DISCUSSION

As confirmed by two independent laboratories, our patient's progressive neurodegenerative disorder proved to be a B_1 variant of G_{M2} gangliosidosis. The clinical symptomatology is compatible with other instances of juvenile or adolescent and even adult forms of G_{M2} gangliosidoses (16, 17). The late appearance of a cherry red spot, an early fundoscopic hallmark in infantile forms of gangliosidoses, is probably in keeping with the protracted clinical course of our patient's disease.

The biochemical defect in the B_1 variant was initially characterized by Li *et al.* (18). Goldman *et al.* (19) gave an earlier clinical, biochemical, and pathologic description of the B_1 variant, but remained unaware of the biochemical defect. The B_1 variant was further studied by Kytzia and Sandhoff (5) and has now been elucidated at the gene level in one patient (3). Two patients with this variant have been previously described (20). One patient died at the age of 4.7 y and the other one probably did not live much longer. One patient with the B_1 variant was diagnosed prenatally (21). The present patient was alive at the age of 13 y, 2 mo, and may have represented a milder subtype. The residual β-hexosaminidase A activity toward *p*-nitrophenyl-6-sulfo-*N*-acetyl-β-D-glucosaminide and G_{M2} ganglioside (Table 1) of about 3 ± 2% as compared to controls is higher than that in classical Tay-Sachs disease (variant B). This relatively high residual activity may have allowed the long survival of our patient. Nevertheless, it cannot be excluded that the biologic activity effective in her neural tissues differs from the level found in her cultured skin fibroblasts. Her parents showed reduced levels of enzyme activity (using the 6-sulfated substrate), suggesting their heterozygote status, but showed normal values when using the classical heat inactivation method for β-hexosaminidase A (Table 2). Thus, our patient probably was not a compound heterozygote for the variant B_1 and classical B (Tay-Sachs) allele, but both parents may carry alleles of the B_1 variant.

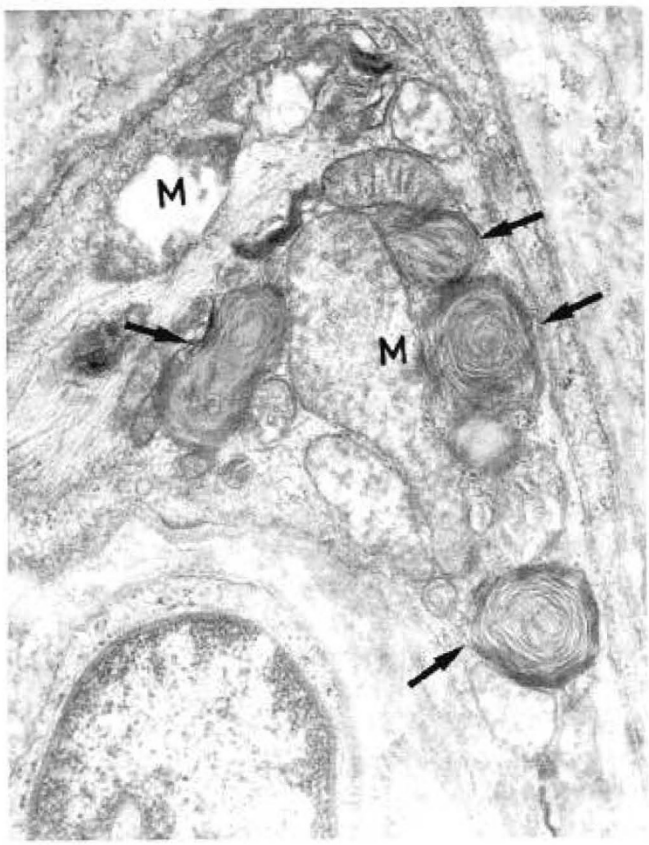

Fig. 1. First skin biopsy specimen: MCB-like cytosomes (*arrows*) in an unmyelinated axon-Schwann cell complex, ×23,650.

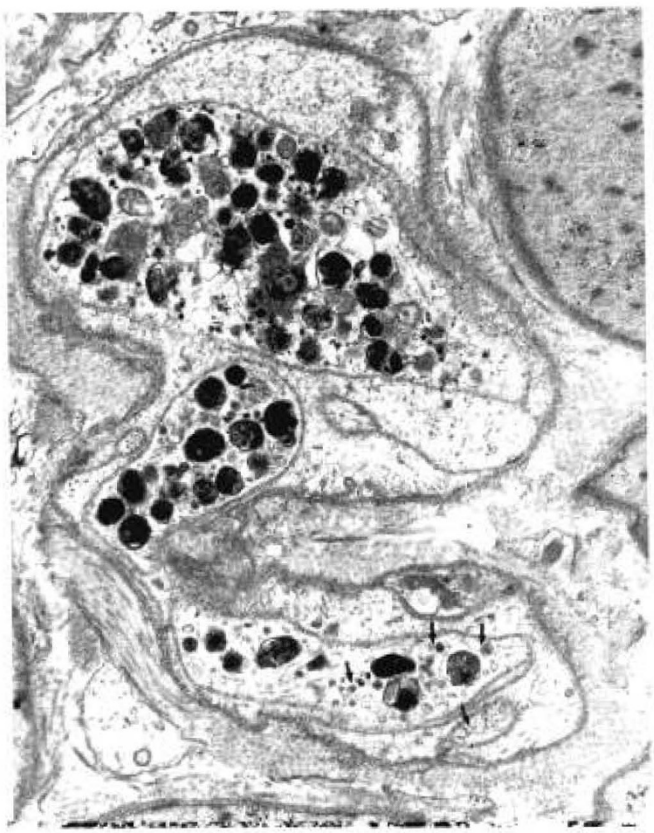

Fig. 2. First skin biopsy specimen: numerous axons enlarged by dense bodies among smooth muscle cells. Their terminal character is evident from clear and dense core vesicles (*arrows*), ×19,030.

Biochemical investigations were initiated on account of the electron microscopic findings documented in the biopsied skin. The unmyelinated and myelinated axons were enlarged by numerous dense bodies and some membranous inclusions, among them several of the MCB type. In this respect, the diagnostic procedures to clarify our patient's disorder resembled that followed in a previous similar patient (12). Nonspecific enlargement of axons, particularly of their terminal segments, is not confined to the G_{M1} or G_{M2} gangliosidoses, but is seen rather in a broad variety of lysosomal disorders. The presence of more specific lysosomal residual bodies such as MCB seems to be limited to the gangliosidoses, especially the G_{M2} forms, and to mucolipidosis IV (22).

In this context, it may be of diagnostic value to biopsy both muscle and skin when exploring atypical or juvenile/adult forms of G_{M1} and G_{M2} gangliosidoses associated with extrapyramidal symptoms, ataxia, muscle weakness, muscle atrophy, or signs suggesting spinocerebellar degeneration (23–27) for a thorough ultrastructural examination. Endplate regions are usually absent from biopsied muscle specimens when not obtained from the motor point region.

Cause and pathogenesis of both the nonspecific dense bodies, and the rather disease-specific lysosomal residual bodies are obscure. However, it is intriguing to speculate, but currently impossible to prove, that these intraaxonal inclusions are related to an impaired metabolism of the parent perikaryon. As organelles are transported from and to the perikaryon it is conceivable that the disease-specific lysosomal residual bodies, in our patient the MCB, may have originated in the parent perikaryon marked by lysosomal storage.

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