

Corneal clouding in GM₁-generalized gangliosidosis

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There are several clinical and chemical features shared by GM₁-generalized gangliosidosis, Hurler's syndrome, and some of the other mucopolysaccharidoses. We report a child with biochemically confirmed GM₁-generalized gangliosidosis with corneal clouding, which is a characteristic feature of some mucopolysaccharidoses and mucopolipidoses. GM₁-generalized gangliosidosis has an autosomal recessive mode of inheritance and is characterized by a severe deficiency of lysosomal β -galactosidase (Okada and O'Brien, 1968) leading to an accumulation of the monosialoganglioside GM₁ and its asialo derivative GA₁ in neurones, histiocytes, hepatocytes, and renal glomerular epithelial cells (Ledeen, Salsman, Gonatas, and Taghavy, 1965; O'Brien, 1972).

Patient and methods

CASE REPORT

Two siblings aged nine and eight years are alive and well but a third sibling died from a chest infection when aged three months.

Case 1, aged 14 months, is the fourth child of Yemeni parents who are first cousins. She was born after a normal pregnancy and delivery at full term, the birth weight was not known but was assessed as being normal. She did not smile until she was four months old and had made no further progress when examined at 14 months.

After an attack of *P. falciparum* malaria at four months, she remained unwell and soon after had feeding difficulties with choking attacks, grunting respirations, repeated generalized convulsions, and failing vision.

At 14 months her length was 74 cm (10th centile), weight 6.3 kg (under 3rd centile) (Tanner, Whitehouse, and Takaishi, 1966). She looked ill and had noisy breathing with lower costal recession. She was generally unresponsive and severely retarded, being unable to sit up and with a general developmental level of under three months. She had coarse facial features characteristic of GM₁-generalized gangliosidosis (O'Brien, 1972) and a doughy skin which pitted on pressure.

The ocular findings included definite corneal clouding, bilateral cherry-red spots, slight conjunctival injection, and pendular nystagmus of both eyes. There was no response to visual stimuli but both pupils contracted in response to light.

The liver was smooth, firm, and enlarged to 3.0 cm below the costal margin, but only the tip of the spleen

was palpable. No abnormality was detected in the cardiovascular system. The limbs were markedly hypotonic and the tendon reflexes depressed. There was striking 'mongolian' blue spot pigmentation over the front and back of the trunk. Vesicular lesions were present on the soles and palms.

Radiographs revealed early bilateral periosteal thickening of the humerus, slight beaking of the first two lumbar vertebrae, and patchy consolidation of the right upper pulmonary lobe.

The electroencephalogram showed gross bilateral abnormalities. Histological examination of a skin biopsy showed cytoplasmic vacuolation in upper dermal cells. Similar vacuolation could be seen in lymphocytes and peripheral blood, and bone marrow.

BIOCHEMICAL STUDIES

Leucocytes from 10 ml of heparinized blood were isolated by sedimentation in dextran (Mosser, 1972), freed from contaminating red cells by hypotonic lysis (Bertino, Silber, Freeman, Alenty, Albrecht, Gabrio, and Huennekens, 1963) and disrupted by alternate freezing and thawing three times.

Skin fibroblasts were cultured and disrupted before assays as previously described (Babarik, Benson, Dean, and Muir, 1974). Aliquots of leucocyte or fibroblast homogenates were taken for assay of β -galactosidase, using 4-methylumbelliferyl- β -D-galactoside as substrate (Benson, Babarik, Brown, and Mann, 1976), β -D-hexosaminidase A and B (Kaback, 1972) and protein (Lowry, Rosebrough, Farr, and Randall, 1951).

Urinary polymeric glycosaminoglycans were assayed as uronic acid after precipitation with cetyl pyridinium chloride (CPC) (Di Ferrante, 1967).

In the patient, there was a marked reduction of β -galactosidase activities both in leucocytes (0.8 per cent of control mean) and in cultured fibroblasts (0.7 per cent of control mean). A partial defect of the leucocyte enzyme was observed in both parents, but was particularly severe in the mother. Fibroblast enzyme levels in the parents were also below the control mean value but within the control range.

Total β -hexosaminidase activities in fibroblasts were normal in the patient (10 500 nmol of 4-methylumbelliferone released/h/mg protein), father (9650 units) and mother (7930 units), the A and B components being within normal limits. Total β -hexosaminidase activities, however, were raised in leucocytes from the patient (1260 units; father, 968 units; mother, 821 units) (control range 295.1 to 1007.2 units; n = 51; Kaback and Zeiger, 1972).

Urinary glycosaminoglycan excretion was within normal limits (2.59 mg of CPC-precipitable uronic acid/24h).

Discussion

Of 27 children with probable GM₁-generalized gangliosidosis in the literature (Craig, Clarke, and Banker, 1959; Norman, Urich, Tingey, and Goodbody, 1959; Landing, Silverman, Craig, Jacoby, Lahey, and Chadwick, 1964; O'Brien, Stern, Landing, O'Brien, and O'Donnell, 1965; Gonatas and Gonatas, 1965; Attal, Farkas-Bargeton, Edgar, Pham-Huu-Trung, Girard, and Mozziconacci, 1967; Sacrez, Juif, Gigonnet, and Gruner, 1962; Scott, Lagunoff, and Trump, 1967; Grossman and Danes, 1968; Seringe, Plainfosse, Lautmann, Lorilloux, Calamy, Berry, and Watchi, 1968; Emery, Green, Wyllie, and Howell, 1971; O'Brien, 1972; Benson and others, 1976) the diagnosis had been confirmed by demonstration of β -galactosidase deficiency in 11 and by identification of GM₁ accumulation in a further six. Of these 17 patients in whom the diagnosis has been confirmed biochemically, one had mild corneal clouding (Emery and others, 1971). This patient may have had a variant form of the disease since leucocyte β -galactosidase was reduced to 30 per cent of normal—a level nearly twice as high as that reported in eight other patients (0–17 per cent) (Singer, Nankervis, and Schafer, 1972; Young, Ellis, and Patrick, 1972; Benson and others, 1976). Clouding was present at 14 months of age in a child (Case 7 of Landing and others, 1964) who had cherry-red macular spots and in a nine-month old infant (Case 1 of Norman and others, 1959) with cherry-red spots who died at 17 months of age with a diagnosis of Tay-Sachs disease. Biochemical confirmation of the diagnosis was lacking for both these patients.

Corneal clouding is a characteristic finding in the Hurler, Scheie, Maroteaux-Lamy, and Morquio syndromes (McKusick, 1972a), and some of the mucopolysaccharidoses (Merrin, Livni, Berman, and Yatziv, 1975), but its pathogenesis is not clear. It always occurs in the Hurler syndrome which is characterized by the accumulation of glycosaminoglycan (GAG) molecules with terminal desulphated iduronide residues because of α -L-iduronidase deficiency (Bach, Friedman, Weissman, and Neufeld, 1972; Matalon and Dorfman, 1972) but it does not occur in the mild or severe forms of Hunter's syndrome, where the stored GAG has terminal sulphated iduronide residues owing to sulphiduronate sulphatase deficiency. One possible explanation is that corneal GAGs lack sulphiduronate residues and may therefore be degraded even in the absence of the sulphatase (Bach, Eisenberg, Cantz, and Neufeld, 1973).

It is not clear from the literature whether all corneal grafts become opacified in the mucopolysaccharidoses. Opacification occurred in a patient

with Scheie's syndrome (Scheie, Hambrick, and Barness, 1962) and in a patient who may have had the Scheie or Maroteaux-Lamy syndrome (Lahdensuu, 1943). However, corneal transplants remained clear for two and three years in two patients who may have had the Maroteaux-Lamy syndrome (Rosen, Haust, Yamashite, and Bryans, 1968). McKusick (1972b) points out that opacification may be a graft reaction. It should be noted that besides corneal clouding, there are several other features shared by GM₁-generalized gangliosidosis, the Hurler syndrome (and some of the other mucopolysaccharidoses). Clinically they both have dorsolumbar kyphosis with radiological beaking of the first two lumbar vertebrae, hepatosplenomegaly, spatulate ribs, and enlargement of the sella. In GM₁-generalized gangliosidosis, visceral histiocytosis is due to storage of GAG rather than ganglioside (O'Brien, 1969; Suzuki, Suzuki, and Kamoshita, 1969) while in the viscera and in bone deformities in addition to ganglioside accumulation there is storage of GAG, structurally related to keratan sulphate (Suzuki and others, 1969) in similar amounts to dermatan sulphate and heparan sulphate in the Hurler syndrome (Suzuki, Suzuki, Rapin, Suzuki, and Ishii, 1970). One may speculate, therefore, that accumulation of keratan sulphate-like GAG might have been responsible for corneal clouding in our patient, and furthermore, that clouding might be noted more often in generalized gangliosidosis if specifically sought. Alternatively, different variants of the disease may be due to different mutations of β -galactosidase (resulting in differing substrate specificities), some of which may predispose to accumulation of corneal GAG more than others.

In the Hurler syndrome, there is considerable increase in brain GM₁, GM₂, and GM₃ (Ledecken and others, 1965; Wallace, Kaplan, Adachi, Schneck, and Volk, 1966; Ledeen, 1966; Taketomi and Yamakawa, 1967) and in visceral gangliosides (Brante, 1952; Borri, Hooghwinkel, and Edgar, 1966). It appears, therefore, that the metabolism of both GAG and gangliosides are disturbed in GM₁-generalized gangliosidosis and in the mucopolysaccharidoses. It should further be noted that in the Hurler, Hunter, and Sanfilippo syndromes there is a partial deficiency of β -galactosidase (Ho and O'Brien, 1969; Hultberg and Ockerman, 1969)—the enzyme which has markedly reduced activity in GM₁-generalized gangliosidosis.

Our patient is the first reported case of Yemeni origin but the fifth out of 27 possible cases to have consanguineous parents. As noted in the results, partial enzyme defects were present in the parents' leucocytes, particularly in the mother in whom the severe deficiency (4.0 per cent of control mean) approached homozygote mutant

levels. However, her fibroblast level (57.6 per cent of control mean) suggested heterozygosis.

Summary

Corneal clouding is added to the list of clinical and chemical abnormalities which occur both in GM₁-generalized gangliosidosis and in Hurler's

syndrome (and some other mucopolysaccharidoses).

The parents of our patient were first cousin Yemeni and had partial β -galactosidase deficiency in their leucocytes and cultured fibroblasts.

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