Morquio’s disease type B (β-galactosidase deficiency) in three siblings

M. BECK, E. M. PETERSEN, J. SPRANGER, P. BEIGHTON

Summary
The clinical and biochemical findings in 3 siblings with Morquio’s disease type B (mucopolysaccharidosis (MPS) IV B) are presented. Their phenotype is characterised by short trunk dwarfism with kyphoscoliosis and thoracic deformity. Radiographic findings include general platyspondyly, dysplasia of the pelvis and epiphyseal abnormalities. The patients are of normal intelligence. In the urine of all 3 affected children abnormal oligosaccharide excretion was found by thin-layer chromatography and in 1 of them keratosulphaturia was detected. The clinical diagnosis was confirmed biochemically by demonstration of a profound deficiency of β-galactosidase activity in cultured fibroblasts. The clinical picture is compared with that of other cases in the literature and the possible molecular basis of the different phenotypes of β-galactosidase deficiency (variants of monosialoganglioside-1 (GM1)-gangliosidosis, Morquio’s disease type B) is discussed.

Clinical heterogeneity in Morquio’s disease has been recognised for many years.1 A defect of N-acetyl-galactosamine-6-sulphate (GalNac-6-S) sulphatase (EC 3.1.6.-) is responsible for ‘classic’ Morquio’s disease.2 Phenotypic variations in this disorder can be explained by different biochemical properties of the residual sulphatase3 and/or additional neuraminidase defect.4 A different enzyme defect produces a variant type of Morquio’s disease. In 1976 O’Brien et al.5 described a 14-year-old girl with severe spondyo-epiphyseal dysplasia, clouding of the cornea and normal intelligence. This clinical picture was produced by a deficiency of β-galactosidase. Since the phenotype clearly differed from monosialoganglioside-1 (GM1)-gangliosidosis and closely resembled Morquio’s disease, the disorder was designated ‘Morquio’s disease type B’ (mucopolysaccharidosis (MPS) IV B).6

Since the first description, several additional patients with MPS IV B have been reported.7,8 These individuals differed from the classic Morquio’s disease form by way of milder skeletal involvement, taller stature and absence of joint laxity. Biochemically they are characterised by an increased urinary excretion of keratan sulphate and abnormal oligosaccharides. The clinical and biochemical findings in 3 South African siblings with MPS IV B are reported and the possible molecular basis of the different phenotypes of β-galactosidase deficiency are discussed.

Case report
The 3 siblings, 2 brothers and a sister, were hospitalised in Cape Town in 1980 for evaluation of their short stature and orthopaedic disabilities. The eldest boy (case 1) was 15 years old at the time, his brother (case 2) was 12 years old and their sister (case 3) 13 years old. The parents and 3 other siblings were normal and no relatives were known to be affected. There was no admitted consanguinity but the parents came from the same remote area and it is not unlikely that they had a common progenitor.

All 3 patients had similar clinical features, notably short trunk dwarfism with variable kyphoscoliosis and thoracic distortion (Fig. 1). The limbs were ostensibly uninvolved and the joints were neither lax nor stiff. The elder boy was paraplegic due to cord compression consequent upon severe spinal malalignment. The 3 siblings had protruding jaws but their facies were otherwise normal. In particular the hair, eyes and teeth were uninvolved. Intelligence was unimpaired and there was no evidence of any systemic ramification. Radiographically the skulls were normal but the spines showed generalised platyspondyly (Fig. 2). In each child the spinal malalignment was associated with marked wedging of a single vertebral body at the apex of the curve. The pelvis showed marked acetabular dysplasia with flattening and fragmentation of femoral heads (Fig. 3). The hands were virtually normal apart from minor dysplastic changes with a suggestion of relative shortening of the metacarpals.

Laboratory studies
Skin biopsies of the affected siblings and of normal controls were performed and fibroblast cultures initiated and maintained by standard methods. Beta-galactosidase and other lysosomal enzymes were assayed by using 4-methylumbelliferyl substrates9 and radiosulphate incorporation measured as described by Cantz et al.10 Random and pooled urine specimens were collected from all 3 patients when they were 14, 13 and 12 years of age, respectively. Mucopolysaccharide excretion was quantified by determining hexuronic acid after precipitation with cetylpyridinium chloride (CPC) and also by the Alcian blue method.11 Subsequent qualitative analysis of the CPC precipitate was performed by thin-layer chromatography on cellulose.12 Keratan sulphate was measured as galactose equivalents.12 After ion-exchange chromatography (Dowex 1 x 2 resin) of the precipitate the keratan sulphate-rich fraction was hydrolysed with 0.5 M hydrochloric acid and after neutralisation with sodium bicarbonate, galactose was determined using a commercial kit. Urinary oligosaccharide thin-layer chromatography was performed as described by Sewell.13

Results
A striking deficiency of β-galactosidase activity was found in cultured fibroblasts from all 3 siblings. Enzyme activity averaged 0.2776 nmol/min/mg protein, less than 5% of the mean control value of 7.173 nmol/min/mg protein (n=6). Other lysosomal enzyme activities (β-hexosaminidase, β-glucuronidase) were normal.

Department of Paediatrics, University of Mainz, FRG
M. BECK, M.D.
J. SPRANGER, M.D.

Department of Human Genetics, University of Cape Town
P. BEIGHTON, M.D., PH.D., F.R.C.P., D.C.H.
E. M. PETERSEN, B.SC. HONS

Reprint requests to: Professor P. Beighton, Dept of Human Genetics, University of Cape Town, Observatory, 7925, RSA.
Fig. 1. Patients at age 13, 15 and 12 years respectively.

Fig. 2. Lateral view of spine of case 1.

Fig. 3. Anteroposterior view of pelvis of case 3.

Fig. 4. pH activity profile of β-galactosidase of normal fibroblasts from individuals with GM₃-gangliosidosis and from the youngest of the siblings. As in normal fibroblasts, the highest activity is seen at pH 4.0 and 4.5. In contrast with the findings of Groebe et al. we did not find a shift of the pH-optimum to the more alkaline side. Radiosulphate incorporation by cultured fibroblasts from cases 1 and 2 showed no excess accumulation of the sulphate-35 label (Fig. 5).

Fig. 4. pH activity profile of β-galactosidase of normal fibroblasts (O—O) and of β-galactosidase deficient fibroblasts (O---O). 1: Normal control. 2: MPS IV B. 3: GM₃-gangliosidosis type 2. 4: GM₃-gangliosidosis type 1.
Discussion

Acid-β-galactosidase (EC 3.2.1.23) is a heterocatalytic enzyme that hydrolyses a broad spectrum of natural substrates such as lactose, N-acetyl-lactosamine, GM₁, and asialo-GM₁-ganglioside, lactosylceramide, asialofetuin, red cell stroma glycoprotein and keratan sulphate. Recent biochemical investigations have revealed details of the molecular nature of this enzyme and its intracellular processing.

The gene for β-galactosidase is located on chromosome 3. The enzyme is synthesised as an 85 kd precursor molecule and then processed in a number of steps to the 64 kd mature enzyme. A so-called 'protective protein' is required for the aggregation of monomeric β-galactosidase into high molecular weight multimers and to prevent rapid proteolytic degradation of β-galactosidase in the lysosome. An isolated deficiency of β-galactosidase (as opposed to the coexistent deficiencies of β-galactosidase and sialidase in galactosialodosis) leads to a broad phenotypic spectrum: GM₁-gangliosidosis type I presents clinically in the first months of life with hepatosplenomegaly, coarse facies, intellectual deterioration and radiographic signs consistent with dysostosis multiplex. Patients with GM₁-gangliosidosis type 2 normally present at the age of 2 - 4 years. A chronic GM₁-gangliosidosis presenting as dystonia has been described by Goldman et al. In some patients with reduced β-galactosidase activity, cardiomyopathy is the most prominent clinical feature.

In addition to the foregoing, β-galactosidase deficiency can also produce MPS IV B. Whereas the mild and severe forms of GM₁-gangliosidosis are characterised by progressive psychomotor deterioration, central nervous system abnormalities are absent in MPS IV B. By complementation studies it has been shown that the various types of GM₁-gangliosidosis and MPS IV B are caused by allelic mutations at the same gene locus. Hoogeveen et al. found that in MPS IV B the mutation did not interfere with the normal processing of β-galactosidase whereas in cells from infantile and adult GM₁-gangliosidosis the precursor β-galactosidase was degraded at early steps in post-translational processing. Kinetic studies of the mutant β-galactosidase in MPS IV B suggest that differences in affinity towards the various natural substrates may influence the clinical phenotype. A further possible explanation for the absence of neurological symptoms in MPS IV B patients is enhancement
of the residual activity of β-galactosidase towards GM₃-ganglioside in the presence of an activator protein. ²⁷

The results of Groebe et al. ²⁸ who found differences in the pH-dependence between normal β-galactosidase and the mutant enzyme of MPS IV B fibroblasts could not be confirmed by our investigations (Fig. 4). Andria et al. ²⁹ described atypical expression of β-galactosidase deficiency in a patient with Hurler-like features but without neurological abnormalities; the urine of this child was MPS-negative but his cultured fibroblasts showed ³⁵SO₄ incorporation slightly higher than that of control cells and comparable with that found in a case of adult GM₁-ganglosidosis. Fibroblasts from 2 of the siblings described here, however, incorporated radiosulphate at a rate indistinguishable from normal control cells.

After the first description by O'Brien et al. ³⁰ it was assumed that type B represents the mild form of Morquio's disease. However, the clinical course of MPS IV B can be severe with precocious presentation. ³¹, ³² Our patients appear to represent an intermediate form in which joint laxity and deafness are absent, while their radiographic signs are milder than those of classic Morquio's disease type A. However, the eldest of the siblings described here has developed neurological symptoms due to spinal cord compression, a complication not hitherto reported in MPS IV B.

Since there are mild variants of Morquio's disease type A, ³³, ³⁴ diagnostic confirmation by biochemical methods is warranted in patients with stigmata of MPS IV. The presence of keratosulphaturia is not obligatory in Morquio's disease type A; it seems to depend on the age of the patient and on the severity of clinical involvement. In MPS IV B, however, it has been shown that there is no activity of β-galactosidase towards keratan sulphate ³⁵ and consequently we were surprised to find abnormal excretion of keratan sulphate only in the youngest of the 3 siblings described here. The simplest method to differentiate between Morquio's disease type A and type B is the examination of a urine specimen for the abnormal oligosacchariduria that is observed in β-galactosidase deficiency, but not in the GaINac-6-S sulphatase defect. Establishing the exact diagnosis of Morquio's disease type A or B by enzymatic assay on cultured fibroblasts is essential for accurate genetic counselling and makes prenatal diagnosis possible in subsequent pregnancies.

We thank Gillian Shapley for typing the manuscript.

This research was supported by grants from the South African Medical Research Council, the Mauerberger Foundation, the Harry Crossley Foundation and the University of Cape Town Staff Research Fund.

REFERENCES