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Leukocyte beta-glucosidase in a child with Gaucher's disease and his kinship

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Beta-glucosidase was determined in leukocyte homogenates of a male child with Gaucher's disease and members of his family. An important difference in enzyme activity was found in the heterozygous gene carriers; in one obligate heterozygote, the mother, a high residual enzyme capacity was detected. Various methods of enzyme determination using various substrates are recommended for the detection of the gene carrier condition.

Gaucher's disease (Gd) is an autosomal recessive hereditary lipidosis with cerebroside storage in the reticulohistiocyte cells of the liver, spleen, bone marrow and lymph nodes, due to glucocerebroside beta glucosidase (bG = beta-glucocerebrosidase) deficiency. Three forms of it are known, infantile progressive neuropathy, juvenile subacute neuropathy and an adult form with chronic visceral manifestations. Neurological symptoms occur in both the rare acute infantile form and the chronic adult form. Because of the infiltration of G cells, hypersplenism and bone marrow depression may be present in both types of the disease. On account of their cerebroside content, the G cells are PAS positive; their demonstration from bone marrow and spleen biopsy is of diagnostic value.

We have performed diagnostic bG examinations for the purpose of detecting heterozygotes among the members of the family with Gd gene.

Report of a Case

Z. S., a 2 1/2-year-old male child was admitted with hepatosplenomegaly and the suspicion of haematological disease. Growth of the abdomen and the intensified appearance of the venous network on the abdominal wall had been noted several months previously. In the last period before admission the child had become fatigable and lost his appetite.

Physical examination revealed an extremely enlarged spleen extending below the navel and the liver that was 5 cm larger than normal. The lymph nodes were not enlarged and bone marrow biopsy excluded an acute leukosis. Large, excentric, often multinuclear cells with light foamy cytoplasm raised the suspicion of a lipidosis. Liver biopsy proved the presence of PAS positive G cells. It was not the hepatocytes but the lipid-negative reticulo-histiocyte cells that were responsible for the storage, therefore Gd was suspected.

Liver and spleen scintigraphy showed hepatosplenomegaly; the RES function of the liver was reduced. A spacerestricting invasive process could be excluded.

In the blood smear there were signs of hypersplenism, first of all thrombocytopenia (thrombocyte count 45 000)

TABLE I

Leukocyte beta-glucosidase activity (μmol/g protein/h) in a patient with Gaucher's disease and his kinship

Sign	Relation	Beta-glucosidase	Per cent	Genotype
Controls				
(n=12)		14.4	100	Healthy subjects
IV/2	Patient	3.2	22	Homozygote
IV/1	Brother	12.8	89	Heterozygote? Healthy?
II/3	Paternal grandmother	5.5	38	Heterozygote
II/1	Maternal grandmother	15.2	101	Healthy
III/2	Mother	12.8	89	Obligate heterozygote
I/1	Paternal great-grandmother	13.3	92	Healthy
III/1	Maternal aunt	14.5	100	Healthy
III/4	Paternal uncle	14.5	100	Healthy
III/3	Father	7.4	51	Heterozygote

Heterozygotes (IV/2, II/3, III/2, III/3) 9.62 ± 3.7 *vs* controls $p < 0.04$

and moderate pancytopenia with WBC 3700 and a haematocrit of 28%. Beside periodic nose-bleeding, no other bleeding was detectable.

The laboratory findings showed and ESR of 25 mm/h, negative urine, normal acid-base values, increased total protein (80.5 g/l with 43.5 g/l albumin) thymol: 3.6 U, SGOT: 10 U/l, SGPT: 5.5 U/l, alkaline phosphatase: 74 U/l, total cholesterol: 4.1 mmol/l, triglyceride: 1.12 mmol/l.

bG was estimated according to Daniels and Glew [6] from leukocyte homogenates of the homozygous patient with Gd and the members of his family (Table I). The substrate was 4-methylumbelliferyl-

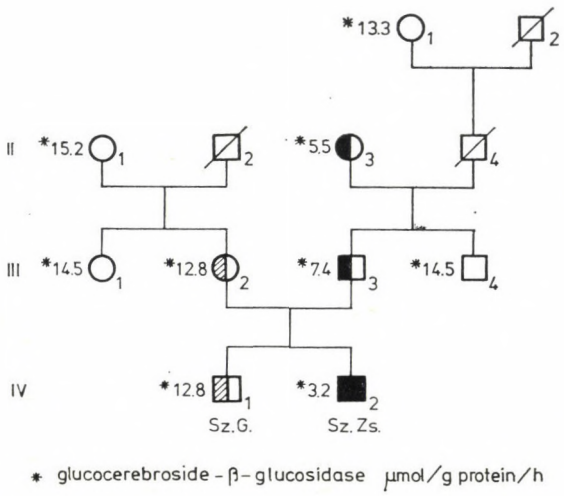

FIG. 1. Pedigree of family with Gaucher's disease

beta-D-glucopyranoside pH 5.8; non-specific isoenzymes were blocked with Na-taurocholate. The enzyme activity values as well as the genotypes suggested by these, and the pedigree are shown in Table I. The line of inheritance was autosomal recessive (Fig. 1). Taking for 100% the enzyme activity value of healthy control children, we determined the level of the family members. In the homozygous patient the bG level was 22%; the paternal grandmother (II/3) and the father (II/3) proved to be heterozygotes with low enzyme activity (38% and 51%, respectively). The high residual enzyme activity in the obligate heterozygous mother (III/2) was unexpected. The brother of the patient (IV/1) was probably also heterozygous because his enzyme level agreed with that of the mother. The paternal great-grandmother (I/1), the maternal grandmother (II/1), the mother's sister (II/1) and the father's brother (III/4) could be considered healthy homozygotes.

Discussion

Typical clinical and laboratory abnormalities facilitate the recognition of Gd patients or guide the diagnosis in the right direction, and on the basis of the presence of G cells the diagnosis can reasonably be suspected. The suspicion is further supported by the finding of a high serum phosphatase and, above all, of isoenzyme 4 [10, 12] together with an elevated activity of lysosomal hydrolases [10] and deficiency of betaxylosidase [2].

The final diagnosis rests on demonstration of the intracellular bG deficiency. Knowledge of the enzyme activity is necessary for correct genetic advice as the only therapeutic possibility; enzyme substitution is still the subject of research. Prevention will only be effective when reliable diagnosis in utero has become possible.

The estimation of enzyme activity gives rise to new problems. In the patients (diseased homozygotes) it differed in every case from normal and heterozygous values, no matter whether the substrate used was natural or artificial. Between heterozygotes and normal homozygotes there is, however, a certain overlapping, the extent of which depends first of all on the method used.

The isoenzymes of bG are known, their distribution in the organs has also been partly explored, but in the patients with Gd there is an unidentified residual enzyme activity, dependent on the type and often the severity of the clinical picture and the time of manifestation. Avoidance of overlapping will become possible in routine laboratory diagnostics only when we shall exactly know the isoenzymes and their activators. At present, blocking of the Triton X 100-treated, non-specific bG with Na taurocholate and their determination at the optimum pH of the specific enzyme is considered the best method, still, as shown also by the pedigree of our patient, the method's specificity is not adequate, since it gave a false high value for the certainly heterozygous mother.

In genetic councelling and prevention, first of all the adult forms manifesting themselves after reaching reproductive age require careful biochemical analysis since in these

cases the residual enzyme activity is often high and the risk of recurrence is between 0 and 50%.

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