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STUDY OF FIBROBLAST AND LEUCOCYTE BETA-GALACTOSIDASE ACTIVITY IN A FAMILY WITH MULTIPLE CASES OF GM1 GANGLIOSIDOSIS

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ABSTRACT

In this study we investigated a family with multiple cases of GM1 gangliosidosis in order to identify heterozygous subjects through the measurement of the activity of beta-galactosidase in cultured fibroblasts and to test the possibility of performing this detection also in leucocytes.

From the results obtained in fibroblasts, and taking into account the genealogical details, it was possible to forecast the probable genotype of each subject studied. It was also possible to estimate the mean value and the range of the activity of beta-galactosidase in heterozygous subjects in this family. The activity of beta-galactosidase in leucocytes showed a wide variation and no correlation with the results obtained in fibroblasts.

We conclude that the assay of beta-galactosidase in fibroblasts can be a viable method for the identification of heterozygotes in an appropriate familial context, a fact that does not appear to be true for leucocytes, though more data are needed from the study of a larger number of normal individuals and of obligate carriers from unrelated families.

INTRODUCTION

GM1 Gangliosidosis is an inborn error of sphingolipid metabolism of recessive autosomal inheritance. The defect is caused by the deficiency of the enzyme

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beta-galactosidase, leading to GM1 ganglioside accumulation in different tissues. There are three clinical forms of the disease (infantile, juvenile and adult), which are differentiated by the age of manifestation and by the severity of the clinical picture. Retarded neuropsychomotor development and hepato- and/or splenomegaly are the major clinical findings. Progressive neurological deterioration leads to death which, in the most severe form (infantile), occurs before two years of age. A detailed description of the clinical picture has been presented by O'Brien (1983, 1989).

Several lines of evidence have indicated that in patients with GM1 Gangliosidosis the 84 kd beta-galactosidase precursor molecule is not processed to the adult 64 kd form, a fact that prevents its compartmentalization into lysosomes, with consequent inactivity (Hoogeveen *et al.*, 1984, 1986; Galjaard *et al.*, 1987; Nanba *et al.*, 1988). Studies on the localization of the structural gene for beta-galactosidase have permitted its mapping on chromosome 3 (Shows *et al.*, 1978; Bruns *et al.*, 1978; De Wit *et al.*, 1979; Sips *et al.*, 1985).

The severity of the disease and the lack of effective treatment strongly motivate the identification of carrier couples for prevention of the disease through genetic counseling and/or prenatal diagnosis. In the present study we investigated a genealogy with several individuals affected by GM1 Gangliosidosis in order to identify heterozygous individuals by measuring beta-galactosidase activity in cultured fibroblasts, and to test the possibility of performing this detection also in peripheral blood leucocytes, a potentially simpler, rapid and economic method.

MATERIAL AND METHODS

Family studied

The propositus (D.Z.) appeared to be normal at birth, but during the very first months of life he started to show symptoms indicating that his neuromotor development was inadequate. Progressive hepatosplenomegaly was also detected and several tests showed alterations. Particularly outstanding were an EEG with a grossly abnormal tracing and the presence of cells suggesting storage disease in peripheral blood lymphocytes and in bone marrow. Skull computerized tomography and ophtalmological examination performed at about six months of age were considered normal. The patients was first examined by one of us (WPJ) at the age of nine months and the hypothesis of GM1 Gangliosidosis was raised. The diagnosis was confirmed by the finding of undetectable beta-galactosidase activity in leycocytes.

Family history showed that the parents were not consanguineous but several individuals (three boys and one girl) in the sibship of the patient's mother were found to

have died with clinical signs and symptoms very similar to those of the propositus (Figure 1). A careful reevaluation of the data available for these cases, including a detailed examination of photographs, permitted us to conclude that these persons were affected by GM1 Gangliosidosis.

The finding of multiple cases of GM1 Gangliosidosis as well as different obligate heterozygotes in one family made this family an ideal group for the study of methods for carrier detection, which was the objective of the present investigation.

Cell culture

Fibroblasts from a skin biopsy obtained from each individual studied were cultured in Ham F-10 medium supplemented with 20% and 10% fetal calf serum in primary and secondary cultures, respectively (culture medium and fetal calf serum obtained from CULTILAB). Cultures were performed in 25 cm^2 plastic flasks and maintained until the cells reached the subconfluent stage, after which they were harvested. For enzymatic analysis, the fibroblasts were diluted with 0.02% Triton X-100. Lysosomal enzymes were released by four cycles of freezing in liquid nitrogen and thawing under running water.

Leucocyte preparation

Heparinized peripheral blood (10 ml) was obtained from each individual studied. Leucocytes were fractionated by the technique of Skoog and Beck (1956) with minor modifications, and diluted in 0.02% Triton X-100 for enzymatic analysis. Lysosomal enzymes were released as described above.

Protein measurement

Protein was measured by the colorimetric technique of Lowry *et al.* (1951) both in fibroblasts and leucocytes, using a standard curve prepared with bovine albumin as reference.

Beta-galactosidase measurement

Beta-galactosidase activity was assayed by the fluorimetric technique of Suzuky (1977) using 4-methylumbelliferyl-beta-D-galactoside as substrate and a standard methylumbelliferone curve as reference. Fluorescence readings were taken with a 7-60 primary filter and 2A, 48 and 10% secondary filters. The results are expressed as nanomoles of substrate hydrolyzed per hour per mg protein.

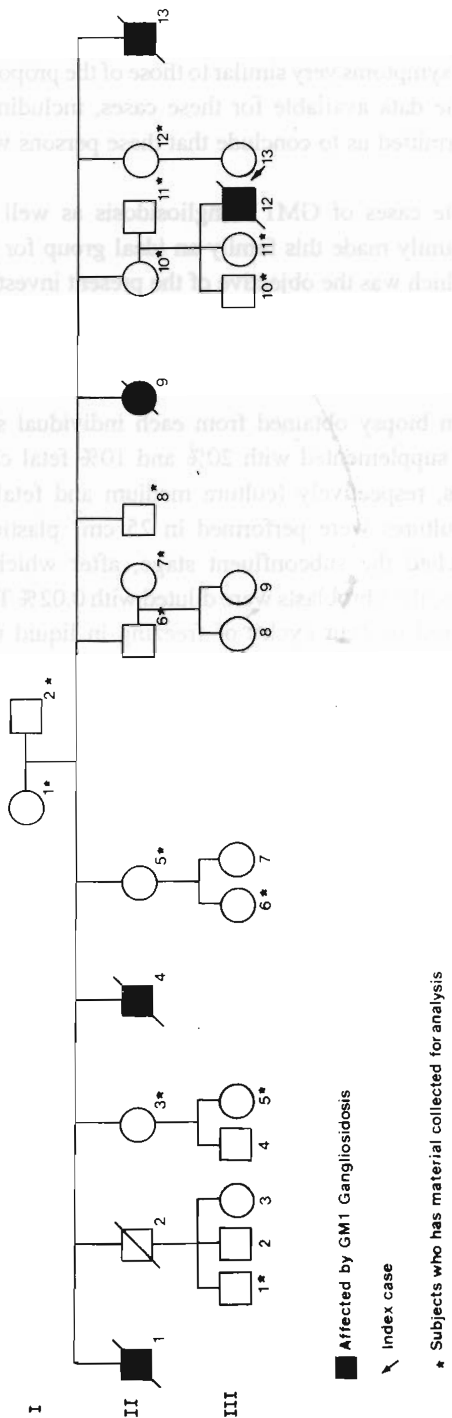

Figure 1 - Pedigree of the affected family.

Total hexosaminidase measurement

Total hexosaminidase activity was measured in order to check the viability of the material. The enzymatic assay was performed by the fluorimetric technique described by Singer *et al.* (1973), using 4-methylumbelliferyl-N-acetyl-beta-D-Glucosaminide as substrate. The reference standards, reading conditions and way of expressing results were the same as those described for beta-galactosidase.

RESULTS AND DISCUSSION

Since sphingolipid hydrolases (such as beta-galactosidase) are more active in cultured fibroblasts than in leucocytes or other tissues (Kolodny, 1977), a distinct interpretation should be made for each type of material.

The present study permitted us to determine that there was no correlation between leucocytes and fibroblasts with respect to this parameter (Figure 2) when the values for beta-galactosidase activity were compared in the individuals studied (Table I).

Table I - Results of the beta-galactosidase assay in fibroblasts and leucocytes from the 15 individuals studied.

Samples	Beta-galactosidase activity (nmol/hour/mg protein)	
	Fibroblasts	Leucocytes
I.1	166.20	4.32
I.2	185.09	46.60
II.3	149.55	66.14
II.5	278.05	5.86
II.6	209.97	11.65
II.7	243.59	19.01
II.8	170.29	34.73
II.10	182.02	100.23
II.11	69.29	218.77
II.12	182.04	18.73
III.1	125.00	43.59
III.5	319.19	149.41
III.6	229.81	46.47
III.10	189.30	27.64
III.11	221.40	67.37

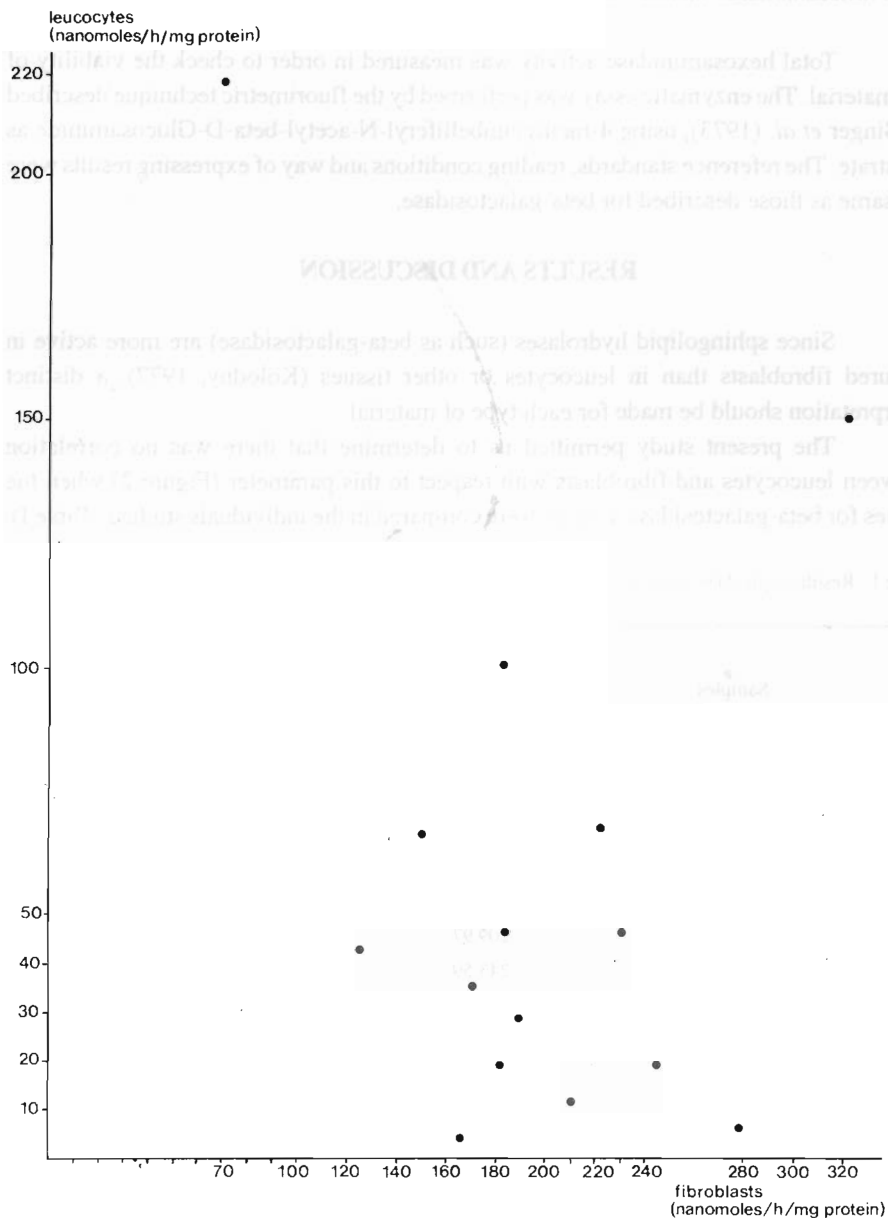

Figure 2 - Beta-galactosidase activity in fibroblasts/leucocytes.

The values for beta-galactosidase activity in leucocytes do not appear to be discriminant, since individuals known to be heterozygotes (II.10 and II.11) presented greater activity than the others. Similarly, individual II.7 - who does not genetically belong to the family and therefore has a high chance of being a normal homozygote - presented a very low enzyme activity when compared with obligate heterozygotes (Figure 3). Singer and Schafer (1970), in one of the very few reports which demonstrated intermediate values of beta-galactosidase activity in leucocytes from heterozygotes, made it quite clear that this result should be confirmed by more precise studies. The precision of enzymatic assays and the natural variation of enzyme activity among individuals and even within a single individual are critical points for the reliability of a method for heterozygote determination (Suzuki, 1977). Thus, on the basis of our experience, the measurement of beta-galactosidase activity in leucocytes did not prove to be a reliable determinant of the heterozygous condition for GM1 Gangliosidosis.

The determination of beta-galactosidase activity in fibroblasts was used for genotypic evaluation of the genealogy. Always taking as a parameter those individuals whose genotypes could be deduced from their family condition - specifically II.10 and II.11, parents of the proband and therefore heterozygotes, and II.7, not genetically belonging to the family and therefore a normal homozygote - we evaluated the data as follows:

For a better visualization and interpretation of the data, we grouped the values by intervals (Figure 4), obtaining a curve similar to a normal one. Considering the data reported by Fensom (unpublished data) for fibroblasts, where the overlap of heterozygotes and normal individuals is clear, and since this overlap usually exists as a function of the coefficient of variation imposed by the methodology (Beaudet *et al.*, 1989), we assume that this curve corresponds to the distribution of the values for heterozygotes. At the right end of this curve there must be an overlap with the left end of a hypothetical normal curve.

The value of 200 nmol/hour/mg protein, corresponding to the mean between the value obtained for II.10 (higher than the values for obligate heterozygotes) and that for II.7 (lowest value among the normal individuals in the sample) was taken as the intercept between the two curves, thus corresponding to a presumed threshold between heterozygotes and normal individuals for this family.

On this basis, we qualified individuals II.3, II.8, II.12, III.1, III.10, I.1 and I.2 as heterozygotes. It should be pointed out that the last two already showed indications of this condition because of the four cases of early death among their progeny, and the present study confirmed this fact. It is important to note that, since III.1 was qualified as a heterozygote, his father (II.1) must also have been a heterozygote, although he died in an accident and therefore could not be included in the study. We believe that the recessive allele of III.1 was not maternally inherited since there was no consanguinity.

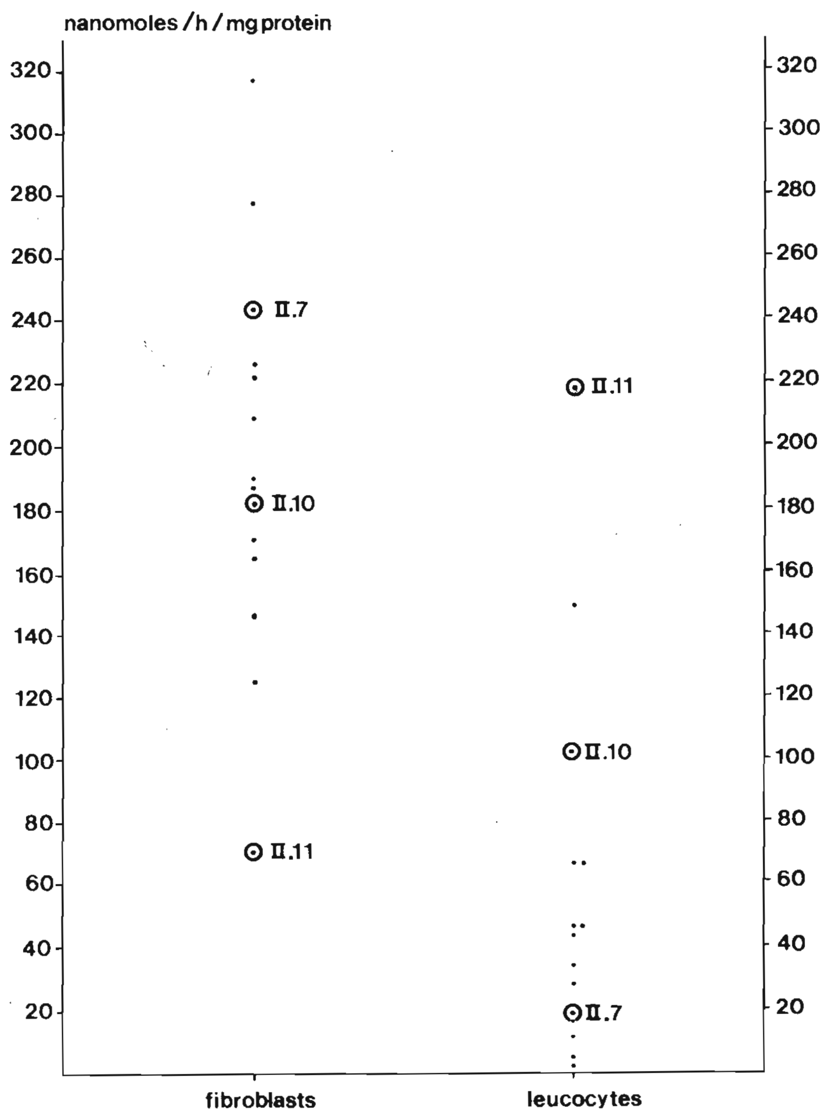

Figure 3 - Dispersal of beta-galactosidase activity in fibroblasts and leucocytes.

Individuals II.5, III.5, III.6 and III.11 could be qualified as dominant (normal) homozygotes. In the case of individual II.6, even though he could be classified as a heterozygote because of his level of beta-galactosidase activity, his contextual situation

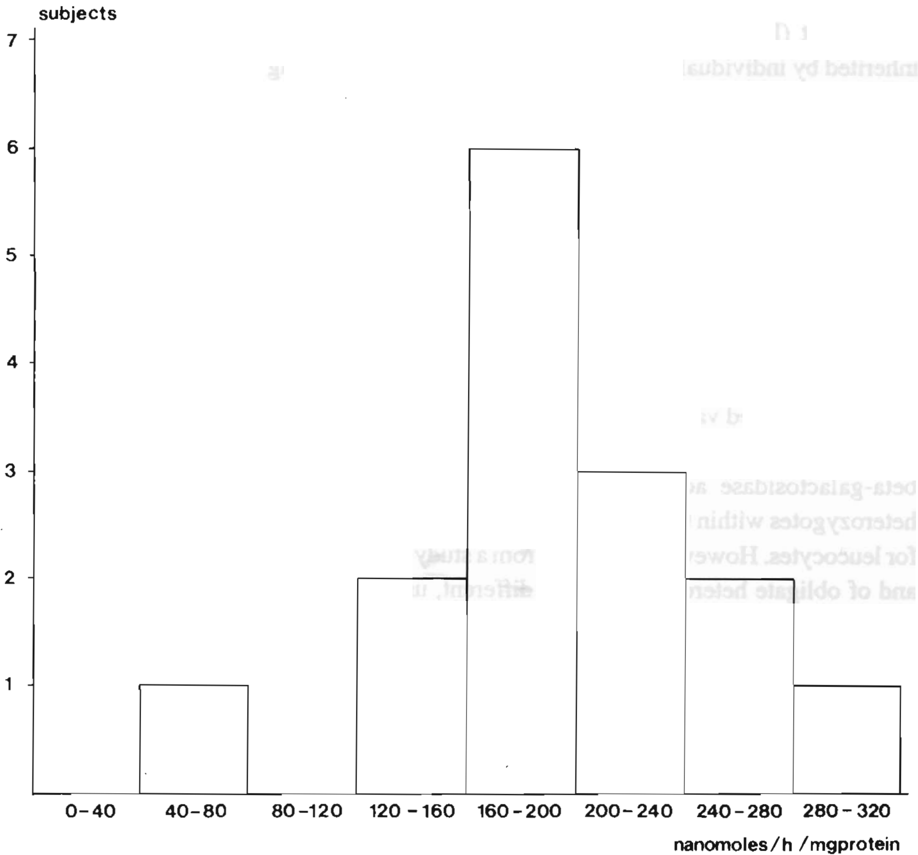

Figure 4 - Beta-galactosidase activity in fibroblasts divided into intervals of 40 nmol/h/mg protein.

could not be overlooked in the evaluation. Considering that this individual is within the same interval as two normal individuals (Figure 4), in which the position of the curve probably corresponds to an intercept with the curve of normal values, and since the curve could be shifted due to the fact that this is not a random sample (one family), we conclude that it would be more logical to classify him as normal.

Starting from the proposition of Suzuki (1977) that most sporadic affected individuals (those whose family has no consanguinity) present a double heterozygous genotype, with the parents carrying genetically different mutations, we return to our observation that individuals II.10 and II.11 have quite different enzyme activities. In parallel, individual II.10 is within the interval in which the beta-galactosidase activities

are highest (Figure 4). On the basis of these observations, we suggest that the allele inherited by individual II.10 and present as a segregant among his relatives is that whose mutation permits significant residual enzyme activity, since we determined that its real value is situated within an interval close to the normal curve. On the other hand, the enzyme activity of individual II.11 suggests that his recessive allele presents a more severe mutation deeply affecting the residual enzyme activity, a fact that places him at the left end of the heterozygote curve.

On the basis of the genotypes detected, we were able to calculate the genotypic frequencies of the progeny of I.1 and I.2. The result showed 18.2% (2/11) dominant homozygotes, 45.4% (5/11) heterozygotes and 36.4% (4/11) recessive homozygotes. According to the Mendelian proportion typical for monogenic inheritance, the deviation from the expected values may be explained by the reduced sample size.

The present study permitted us to conclude that the determination of beta-galactosidase activity in fibroblasts a viable method for the identification of heterozygotes within the appropriate family context, a fact that does not appear to be true for leucocytes. However, more data from a study of a larger number of normal individuals and of obligate heterozygotes from different, unrelated families are needed to confirm our conclusion.

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RESUMO

Através do estudo de uma família com diversos casos de Gangliosidose GM1, nos propusemos a identificar indivíduos heterozigotos através da medida da atividade da enzima beta-galactosidase em fibroblastos cultivados e a testar a possibilidade desta identificação também em leucócitos.

Pelos resultados obtidos em fibroblastos, e considerando os dados da história familiar, foi possível especular qual o provável genótipo de cada indivíduo da amostra. Foi também possível estimar o valor médio da atividade enzimática dos heterozigotos desta genealogia e a amplitude aproximada deste valor. Em leucócitos a atividade da beta-galactosidase esteve muito dispersa e não apresentou correlação com a observada em leucócitos.

Concluímos assim, que a determinação da atividade da beta-galactosidase em fibroblastos parece ser um meio viável para a identificação de heterozigotos dentro de um contexto familiar apropriado, o que não parece ser verdadeiro para leucócitos. No entanto, dados mais conclusivos dependem do estudo de um maior número de indivíduos normais e de heterozigotos obrigatórios, provenientes de diversas famílias não relacionadas.

REFERENCES

Beaudet, A.L., Scriver, C.R., Sly, W.S., Valle, D., Cooper, D.N., McKusick, V.A. and Schmidke, J. (1989). Genetics and biochemistry of variant human phenotypes. In: *The metabolic basis of inherited disease* (Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D., eds.) 6a. ed. McGraw Hill, New York, pp. 03-53.

Bruns, G.A.P., Leary, A.C., Regina, V.M. and Gerald, P.S. (1978). Lysosomal beta-D-galactosidase in man-hamster somatic cell hybrids. *Cytogenet. Cell Genet. 22*: 177-181.

De Wit, J., Hoeksema, A., Bootsma, D. and Westerveld, A. (1979). Assignment of structural beta-galactosidase loci to human chromosomes 3 and 22. *Hum. Genet. 51*: 259-267.

Dreyfus, J.C. and Poenaru, L. (1975). Le diagnostic enzymatique dans les maladies lysosomiales - et plus particulierement dans les Sphingolipidoses. *Arch. Franc. Ped. 32*: 503-514.

Galjaard, H., Willemsen, R., Hoogeveen, A.T., Mancini, G.M.S., Palmeri, S., Verheijen, F.W. and D'Azzo, A. (1987). Molecular heterogeneity in human beta-galactosidase and neuraminidase deficiency. *Enzyme 38*: 132-143.

Hoogeveen, A.T., Graham-Kawashima, H., D'Azzo, A. and Galjaard, H. (1984). Processing of human beta-galactosidase in GM1-gangliosidosis and Morquio B Syndrome. *J. Biol. Chem. 259*: 1974-1977.

Hoogeveen, A.T., Reuster, A.Y.Y., Kroos, M. and Galjaard, H. (1986). GM1-gangliosidosis. Defective recognition site on beta-galactosidase precursor. *J. Biol. Chem. 261*: 5702-5704.

Kolodny, E.H. (1977). General principles and techniques of case identification, carrier testing and prenatal diagnosis. In: *Practical Enzymology of the Sphingolipidoses.* (Glew, R.H. and Peters, S.P., eds.). Alan R. Liss Inc., New York, p. 1-38.

Lowry, O.H., Rosebrough, N.J., Lewis Farr, A. and Randall, R.J. (1951). Protein measurement with the folin reagent. *J. Biol. Chem. 193*: 265-277.

Mutoh, T., Sobue, T., Naoi, M., Matsuoka, Y., Kiuch, K. and Sugimura, K. (1986). A family with beta-galactosidase deficiency: three adults with atypical clinical patterns. *Neurology 36*: 54-59.

Nanba, E., Tsuji, A., Omura, K. and Suzuki, Y. (1988). GM1-gangliosidosis: abnormalities in biosynthesis and early processing of beta-galactosidase in fibroblasts. *Biochem. Biophys. Res. Comm. 152*: 794-800.

O'Brien, J.S. (1983). The gangliosidosis. In: *The metabolic basis of inherited diseases* (Stanbury, J.B., Wyngaarten, J.B., Fredrickson, D.S., Goldstein, J.L. and Brown, M.S., eds.). 5a. ed. McGraw Hill, New York, pp. 945-969.

O'Brien, J.S. (1989). Beta-galactosidase deficiency (GM1-gangliosidosis, Galactosialidosis and Morquio Syndrome Type B); Ganglioside sialidase deficiency (Mucolipidosis IV). In: *The metabolic basis of*

inherited diseases (Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D., eds.). 6a. ed. McGraw Hill, New York, pp. 1797-1806.

Pinsky, L., Miller, J., Shanfield, B., Watters, G. and Wolfe, L.S. (1974). GM1 gangliosidosis in skin fibroblasts culture: enzymatic differences between types 1 and 2 and observations on a third variant. *Am. J. Hum. Genet. 26*: 563-577.

Shows, T.B., Scrafford-Wolff, L.R., Brown, J.A. and Meisler, M.H. (1978). Assignment of a beta-galactosidase gene (beta GAL A) to chromosome 3 in man. *Cytogenet. Cell Genet. 5*: 219-222.

Singer, H.S. and Schafer, I.A. (1970). White-cell beta-galactosidase activity. *New Engl. J. Med. 282*: 571.

Singer, J.D., Cotlier, E. and Krimmer, R. (1973). Hexosaminidase A in tears and saliva for rapid identification of Tay-Sachs disease and its carriers. *Lancet 2*: 1116-1119.

Sips, H.J., De Wit-Verbeek, H.A., De Wit, J., Westerveld, A. and Galjaard, J. (1985). The chromosomal localization of human beta-galactosidase revisited: a locus for beta-galactosidase on human chromosome 3 and for its protective protein on human chromosome 22. *Hum. Genet. 69*: 340-344.

Skoog, W.A. and Beck, W.A. (1956). Studies on the fibrinogen, dextran and phytohemagglutinin methods of isolating leucocytes. *Blood 11*: 436-454.

Suzuki, K. (1977). Globoid cell leukodistrophy (Krabbe disease and GM1 Gangliosidosis). In: *Practical Enzymology of the Sphingolipidosis* (Glew, R.H. and Peters, S.P., eds.). Alan R. Liss Inc., New York, pp. 101-136.

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