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Authors

Devine, EA

Smith, M

Arredondo-Vega, FX

et al.

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Regional assignment of the structural gene for human acid β -glucosidase to q42 \rightarrow qter on chromosome 1

E.A. DEVINE, M. SMITH, F.X. ARREDONDO-VEGA, B. SHAFIT-ZAGARDO, and R.J. DESNICK
Division of Medical Genetics, Mount Sinai School of Medicine, New York, N.Y.

Abstract. The structural gene for human acid β -glucosidase (GBA) has been regionally assigned to a narrow region on chromosome 1 using somatic cell hybridization, specific immunoprecipitation, and assay with the natural substrate. A human fibroblast line, 46,XX,del(1)(pter \rightarrow q42:), was fused with mouse RAG fibroblasts and the heterokaryons were subcloned. All hybrid subclones containing a normal chromosome 1 were positive for GBA. In contrast, subclones with a single del(1) were negative for GBA by both immunoprecipitation and natural substrate assays. These results were consistent with the previous assignment of GBA to the region 1p11 \rightarrow qter and further localized the gene to the narrow region 1q42 \rightarrow qter.

Acid (E.C. 3.2.1.45) and neutral (E.C. 3.2.1.21) β -glucosidase isozymes have been distinguished in normal human tissues by their differential pH optima, subcellular localizations, substrate specificities, lectin binding affinities, activation by anionic detergents and acidic phospholipids, and electrophoretic mobilities. The acid isozyme (GBA) is a lysosomal hydrolase which normally hydrolyzes the β -glucosyl linkage of

the neutral glycosphingolipid, glucosyl ceramide. The deficiency of this isozyme is the basic enzymatic defect in the various subtypes of Gaucher disease (BRADY, 1978; SHAFIT-ZAGARDO et al., 1980).

Recently, we assigned the structural gene for human GBA to chromosome 1p11 \rightarrow qter using human \times mouse somatic cell hybrids and a sensitive immunoprecipitation assay (SHAFIT-ZAGARDO et al., 1981). We report here the regional localization of the locus for GBA near 1qter using an informative hybrid clone carrying a human chromosome 1 deletion. In addition to the immunoprecipitation assay, use of the specific natural substrate further supported our assignment of the structural gene for GBA to this region.

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Request reprints from: DR. R.J. DESNICK, Division of Medical Genetics, Mount Sinai School of Medicine, Fifth Avenue at 100th Street, New York, NY 10029 (USA).

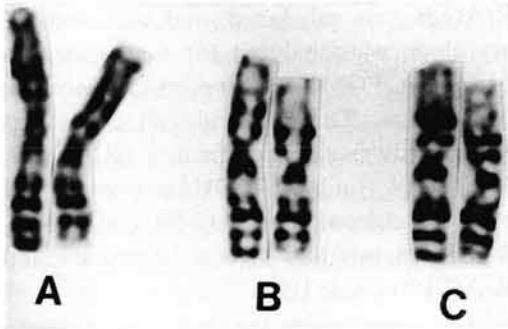


Fig. 1. Three pairs of chromosome 1 from cultured skin fibroblasts from patient AG, 46,XX, del(1)(pter \rightarrow q42:). Giemsa-trypsin banding. The normal chromosome is on the left; the deleted chromosome is on the right.

Parental and somatic cell hybrid lines. The parental lines used for hybridization were mouse RAG fibroblasts (KLEBE et al., 1970) and a human fibroblast line (AG) carrying a de novo deletion of the terminal region of the long arm of chromosome 1, 1q42 \rightarrow qter (fig. 1). A skin biopsy from the patient with this terminal deletion was provided by Dr. CARL B. MANKININ (Denton, Tex.).

The human and rodent lines were fused and heterokaryons were subcloned as previously described (SHAFIT-ZAGARDO et al., 1981). Parental and hybrid lines were grown in RPMI-1640 medium containing 12% fetal calf serum, 2 mM L-glutamine, penicillin (100 U/ml) and streptomycin (100 μ g/ml) (Gibco, Grand Island, N.Y.) using standard tissue culture techniques.

Determination of the human chromosomal 1 constitution of hybrid clones. The marker enzyme for the human 1, phosphoglucomutase 1 (PGM1), was determined electrophoretically (HARRIS and HOPKINSON, 1976). Metaphase spreads of the human \times mouse hybrid lines were prepared and the chromosomes were banded using the Giemsa 11 technique (BREG, 1972) and subsequently destained and banded with quinacrine hydrochloride (FRIEND et al., 1976). Individual hybrid clones were scored as positive for the normal or the deleted 1, when either or both were present in at least 30% of the metaphase spreads. Human GBA immunoprecipitation assays, electrophoretic studies

of marker enzymes, and cytogenetic analyses were performed on hybrid lines harvested from the same passage.

Artificial and natural substrate assays for GBA. Confluent fibroblast lines were harvested, the enzyme was solubilized, and GBA activity was assayed using the fluorogenic substrate, 4-methylumbelliferyl- β -D-glucopyranoside (4MU-Glc) (RPI, Inc., Elk Grove Village, Ill.) according to the procedure of SHAFIT-ZAGARDO et al. (1981). For assay with the natural substrate, the fluorescent glucosyl ceramide was prepared as previously described (GATT et al., 1981). The assay mixture contained 3.0 nmoles of 12-N-methyl-N-(7-nitrobenz-2-oxa-1,3-azol-4-yl)aminododecanyl glucosyl ceramide (NBD-GC) and 27.0 nmoles glucosyl ceramide in 200 μ l of 0.05 M sodium phosphate-citric acid buffer, pH 5.5, containing 0.5% taurocholate and 0.25% Triton X-100. The assay mixture was pipetted directly onto the cell pellets; the mixture was vortexed and incubated for 1 h at 37 $^{\circ}$ C. The reaction was stopped by the addition of 0.25 ml of distilled water, 1.5 ml of heptane, and 0.45 ml of isopropanol. The upper phase containing NBD-ceramide was removed and back-washed with 0.35 ml H₂O. The amount of substrate hydrolyzed was measured in a Perkin Elmer fluorometer (model 204-A, excitation 460 nm, emission 515 nm). One unit (U) of enzymatic activity represented 1 pmole of 4MU-Glc or 1 nmole of NBD-GC hydrolyzed/h at 37 $^{\circ}$ C.

Immunoprecipitation assay for human GBA. The immunoprecipitation assay was performed using mouse anti-human β -glucosidase antibody as previously described (SHAFIT-ZAGARDO et al., 1981). The antiserum was specific for human GBA; no GBA was precipitated from mouse fibroblasts. Assays of the antigen-antibody complex for neutral β -glucosidase, β -glucuronidase, β -hexosaminidase B, α -glucosidase at pH 4.0 and 6.0, β -galactosidase at pH 4.5 and 7.0, α -L-arabinosidase, and β -D-xylosidase activities were all negative. The assay was linear over a range of 0 to 2,000 U of human GBA activity. Hybrids were scored as positive when the percentage of GBA activity immunoprecipitated from them was greater than 10% of the GBA activity precipitated from normal human fibroblasts. This value was derived from the following calculations for gene dosage and chromosomal heterogeneity in the subclones: compared

to the amount of immunoprecipitable activity in human diploid fibroblasts, a hybrid clone containing one human and two mouse alleles for GBA would have one third of the immunoprecipitable activity. If the clone had at least 30% of metaphase spreads containing a single, intact 1, the minimal amount of activity for a clone to be scored as positive would be: one third of the activity precipitated from human diploid fibroblasts (for gene dosage) times 30% (for heterogeneity), i.e., $100\% \times 0.33 \times 0.3 \approx 10\%$.

From the fusion of the mouse RAG and human AG fibroblast lines, 14 hybrid clones were isolated. Of the three clones that expressed human PGM1, cytogenetic studies showed that one contained both the normal and deleted human 1. This hybrid line,

R/AG-B, was subcloned, and each secondary clone was analyzed for the expression of human PGM1 and for its chromosome constitution. Three hybrid cell lines contained only a single normal 1 (R/AG-B2, R/AG-B14, and R/AG-B11); two carried only the deleted 1 (R/AG-B9 and R/AG-B10), and two had neither (R/AG-B7 and R/AG-B8) (table I).

The results of the specific immunoprecipitation assays for human GBA in the seven secondary hybrid clones are shown in table I. Three subclones that were positive for PGM1 and contained only the normal 1 had 12% to 26% of the immunoprecipitated GBA activity of normal human fibroblasts.

Table I. Segregation of human GBA and intact human chromosome 1 in RAG \times AG fibroblast subclones

Cell line	Human PGM1	Chromosome 1		Human GBA activity ¹			Pos./Neg.
		Normal	del(1)	4MU-Glc	NBD-GC		
				Percentage of human control	nmol/h	Percentage of human control	
Normal human fibroblasts	+	+	-	100.0	7.30	100.0	+
AG	+	+	+	67.0	5.12	70.1	+
RAG	-	-	-	1.5	0.16	2.2	-
R/AG-B2	+	+	-	26.0	-	-	+
R/AG-B11	+	+	-	24.0	1.65	22.6	+
R/AG-B14	+	+	-	12.0	2.14	29.3	+
R/AG-B9	+	-	+	6.0	-	-	-
R/AG-B10	+	-	+	6.0	0.37	5.0	-
R/AG-B7	-	-	-	3.0	0.22	3.0	-
R/AG-B8	-	-	-	1.5	0.20	2.7	-

¹ The immunoprecipitate was resuspended and GBA activity was assayed with the artificial (4MU-Glc) and natural (NBD-GC) substrates. Hybrids scored as positive had more than 10% of the immunoprecipitated activity in normal human fibroblasts.

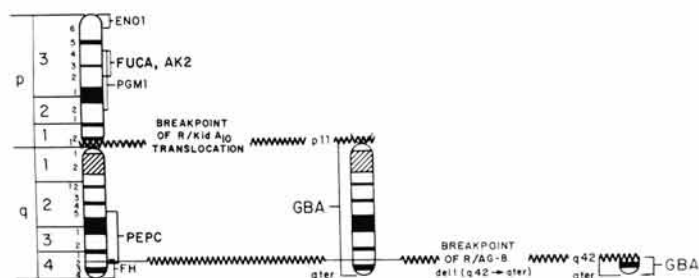
These subclones were scored as positive for the expression of human GBA. In contrast, the two subclones that were positive for PGM1 but carried only the deleted human 1 had 60% or less of the human GBA activity of normal fibroblasts. These subclones were scored as negative for human GBA. The two subclones that did not contain a human 1 had 30% or less of the mean immunoprecipitated GBA activity of normal fibroblasts.

To assure the specificity of the immunoprecipitation assay for human GBA, the natural substrate was used to assay the enzyme-antibody complex. As shown in table I, immunoprecipitated GBA activity was not detected in the mouse RAG cell line. The AG parental fibroblast line had 70.10% of the mean immunoprecipitable GBA activity in normal human fibroblasts. Five subclones of the hybrid line, R/AG-B, were examined for the presence of human GBA using the natural substrate. Cell lines R/AG-B7, R/AG-B8, and R/AG-B10 were cytogenetically shown to contain only the deleted 1 and were negative for enzyme activity with the natural substrate in the immunoprecipitation assay. In contrast, subclones R/AG-B11 and R/AG-B14, which

carried a normal 1, both had GBA immunoprecipitable activity toward the natural substrate.

The structural gene locus for human GBA recently has been assigned to the region 1p11 \rightarrow qter by somatic cell hybridization and specific double antibody immunoprecipitation (SHAFIT-ZAGARDO et al., 1981). Further localization of the GBA locus has been obtained by the analysis of somatic cell hybrids between murine RAG fibroblasts and a human cell line containing a de novo deletion of 1. Of the secondary clones derived from the primary hybrid, R/AG-B, which contained both the normal and deleted 1, seven were selected which contained only the normal, only the deleted, or neither human 1. These secondary hybrids were examined for immunoprecipitable human GBA activity using the specific immunoprecipitation assay. In clones positive for human PGM1, immunoprecipitated GBA activity segregated concordantly with the presence or absence of the deleted human 1. All of the hybrids carrying a normal 1 had immunoprecipitable GBA activity; those carrying only the deleted 1 contained no immunoprecipitable human GBA enzyme.

Fig. 2. Regional assignment of human GBA to chromosome 1. The human 1 and its enzymatic markers are shown diagrammatically on the left. Locations of the break point of the translocation carried in the hybrid line, R/KidA₁₀ (middle), and in the human line, AG (left), are designated by the broken lines. The structural gene locus for GBA has been mapped to the region 1q42 \rightarrow qter, as depicted on the right.



The specificity of the immunoprecipitation assay for human GBA was further demonstrated by the use of the fluorescent natural substrate, NBD-GC. This substrate is only hydrolyzed by acid β -glucosidase and is not degraded by any other glycosidase, including the neutral β -glucosidase (GATT et al., 1981). In the subclones examined (table I), only those hybrids carrying an intact 1 had immunoprecipitable human GBA activity using the natural substrate. These findings and the absence of detectable immunoprecipitable activity in the hybrids carrying only the deleted 1 are consistent with the regional assignment of the GBA gene to the region 1q42 \rightarrow qter (fig. 2).

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