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Genetics

Genetics of type II glycogenosis: Assignment of the human gene for acid α-glucosidase to chromosome 17

(cell hybrids/Pompe disease/hypoxanthine/aminopterin/thymidine selection/BrdUrd counterselection)

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ABSTRACT **We have studied somatic cell hybrids between thymidine kinase (EC 2.7.1.75) deficient mouse cells and human diploid fibroblasts for the expression of human acid α-glucosidase (EC 3.2.1.20). A deficiency in this enzyme is associated with the type II glycogenosis or Pompe disease. All 30 somatic cell hybrids selected in hypoxanthine/aminopterin/thymidine medium expressed human acid α-glucosidase and galactokinase (EC 2.7.1.6) and retained human chromosome 17; counterselection of the same hybrids in medium containing 5-bromodeoxyuridine resulted in the growth of hybrids that concordantly lost the expression of human acid α-glucosidase and galactokinase as well as human chromosome 17. Hybrids between thymidine kinase-deficient mouse cells and fibroblasts from a patient with Pompe disease that contained human chromosome 17 were found not to express human acid α-glucosidase. Because we have already shown that hybrids between mouse peritoneal macrophages and GM54VA simian virus 40-transformed human cells selectively retain human chromosome 17 and lose all other human chromosomes, we tested 13 independent mouse macrophage × GM54VA hybrid clones, including two that retained human chromosome 17 and no other human chromosomes, for the expression of human acid α-glucosidase and galactokinase. All 13 hybrid clones were found to express these human enzymes. Thus, we conclude that the gene coding for human acid α-glucosidase is located on human chromosome 17.**

The type II glycogen storage disease, or Pompe disease, which is characterized by a generalized glycogen deposit and by muscular hypotrophia, weakness, cardiomegaly, and cardiac decompensation, is associated with a deficiency in acid α-glucosidase (EC 3.2.1.20) (1–3). This enzyme catalyzes the cleavage of α-1,4 and α-1,6 glucosidic linkages (4, 5) and is localized in the lysosomes (6). The neutral α-1,4-glucosidase, which is not localized in the lysosome, is normally expressed in patients with Pompe disease (7).

Because we have found that mouse and human acid α-glucosidases have different electrophoretic mobilities in starch gel, probably due to different affinities of the mouse and the human enzymes for the glucosidic linkages present in this support medium, we analyzed mouse–human hybrids segregating human chromosomes for the expression of the human acid α-glucosidase to establish the chromosomal location of the gene coding for this enzyme in man. In addition, in order to determine whether the lack of expression of human acid α-glucosidase in patients with Pompe disease is due to a mutation in the structural gene for the enzyme or in another locus affecting its expression, we have also fused mouse cells and cells derived from patients with Pompe disease and analyzed the resulting hybrid clones for the expression of the mouse and the human enzyme.

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MATERIALS AND METHODS

Cells. Thymidine kinase-deficient LM-TK$^-$ (clone 1D) and Swiss mouse IT-22 cells (8) were fused in the presence of β-propiolactone-inactivated Sendai virus (9) to diploid human fibroblasts from different sources: human fibroblasts GM 0216 (10), GM 121 (11), GM 177 (12), and GM 1139 (13) were obtained from the Human Genetics Mutant Cell Repository (Institute for Medical Research, Camden, NJ). Human fibroblasts MP were derived from a patient carrying a translocation of the region p21 → pter of chromosome 6 to chromosome 17 and were obtained from E. Engel, Genetics Division, Department of Medicine, Vanderbilt University (Nashville, TN) (14). Clone 1D cells were also fused with human fibroblasts derived from a patient with Pompe disease (Repository No. GM 248, Human Genetics Mutant Cell Repository. Hybrids were selected in hypoxanthine/aminopterin/thymidine (HAT) medium (15) containing 10 mM ouabain (16). Sixteen independent hybrid clones, originally selected in HAT medium, were counterselected by growth in medium containing 100 μg of BrdUrd per ml.

In addition, GM54VA simian virus 40-transformed human cells that contain the genome of simian virus 40 integrated in human chromosome 17 (17) were fused with BALB/c and C57BL mouse peritoneal macrophages, and the hybrids were selected in medium containing 10 mM ouabain (16).

Karyologic Analysis. Chromosomes present in hybrid cells were identified after trypsin–Giemsa banding staining according to a modification (18) of the method described by Seabright (19). At least 15 metaphases from each hybrid clone were photographed and karyotyped.

Neuraminidase Treatment. The method described by Swallow *et al.* (7) was followed: samples were incubated for 3 hr at 7°C with neuraminidase and centrifuged at 10,000 × *g*. The supernatants were electrophoresed immediately after the enzyme treatment.

Detection of the Expression of Human Acid α-Glucosidase in Hybrids. Horizontal starch gel electrophoresis was carried out with 0.2 M Na phosphate buffer at pH 7.3 in the electrode chambers and 0.02 M Na phosphate buffer at pH 7.3 in the gel buffer. The concentration of hydrolyzed starch was 12%. Alternatively, a 4-mm-thick flat bed of Sephadex G-75 superfine (Pharmacia) was used as support according to the method described by Radola (20), except that Ampholines and electrode solutions were substituted by the buffer system described above. For the enzyme assay of acid α-glucosidase on starch gel, 100 V per 15 cm was applied for 22 hr at 4°C. For the enzyme assay on the Sephadex support, the electrophoresis was carried out

Abbreviations: HAT, hypoxanthine/aminopterin/thymidine; MPM, mouse peritoneal macrophages.

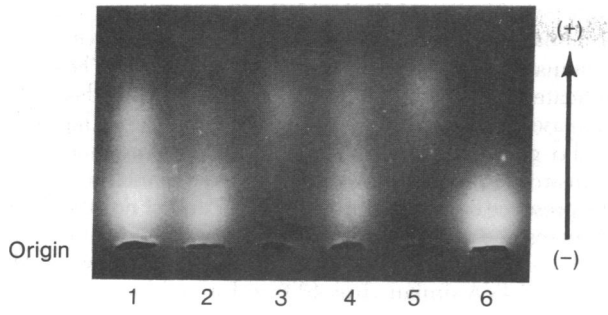

FIG. 1. Starch gel electrophoresis of acid α-glucosidase. Lanes 1 and 2, two hybrid clones between mouse peritoneal macrophages (MPM) and GM54VA cells. The only human chromosome these two hybrids contain is human chromosome 17. Lane 3, extract of LM-TK⁻ (clone 1D) thymidine kinase-deficient mouse cells. Lane 4, extract of hybrid clone 11-14-77, obtained from a fusion between clone 1D cells and human fibroblasts MP (13) and selected in HAT medium. Lane 5, extract of the same hybrid, which was counterselected in medium containing 100 μg of BrdUrd per ml. Lane 6, extract of human fibroblasts.

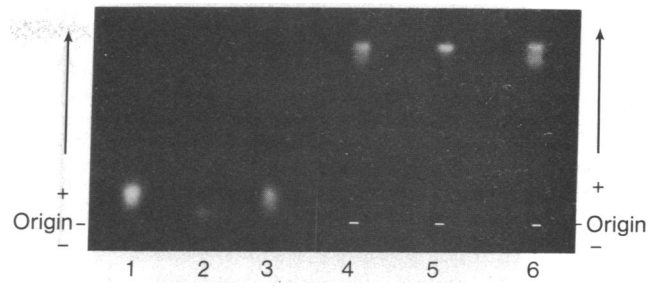

FIG. 2. Zymogram of acid α-glucosidase (lanes 1, 2, and 3) and neutral α-glucosidase (lanes 4, 5, and 6). The left and right parts of the figure represent the top and bottom slices of the same gels stained at optimal pH for the acid and neutral forms, respectively. In this electrophoresis, the voltage was not sufficient to separate the mouse and human acid α-glucosidase isozymes; however, low voltage is necessary if one wants to maintain the bands of neutral α-glucosidase in the gel (see *Materials and Methods*). Lanes: 1 and 6, extract from clone 1D cells; 2 and 5, extract from GM54VA cells; 3 and 4, extract from hybrid BALB/c MPM × GM54VA clone 9.

at 50 V per 20 cm for 15 hr. The electrophoresis of the neutral α-glucosidase was carried out on starch gel at 80 V per 15 cm for 15 hr and on Sephadex at 30 V per cm for 15 hr. Acid and neutral α-glucosidase activity was visualized by following the methods described by Swallow *et al.* (7): after starch gel electrophoresis at 50 V per 20 cm for 15 hr, the gel was cut into two slices. A no. 1 Whatman filter paper sheet was embedded in a solution of 0.3 mg of 4-methyl-umbelliferyl-2-D-glucoside (Sigma) in 0.2 M Na acetate buffer, pH 4.0, and then put in contact with the internal surface of the bottom slice for detection of acid α-glucosidase activity. The top slice was stained for neutral α-glucosidase with the same procedure, except that the buffer was 0.2 M Na phosphate, pH 7.3. After 30 min both slices were photographed under UV light.

Galactokinase (GK) Assay. The basic method of Orkwiszewski *et al.* (21) was used to test galactokinase as a marker for human chromosome 17. A shorter time of electrophoresis (15 hr) was sufficient to achieve good resolution of the mouse and human bands.

Other Isoenzyme Markers. Twenty-two additional isoenzymes were assayed after electrophoresis, as markers for the other 22 human chromosomes: peptidase-C (EC 3.4.11.1), soluble isocitric dehydrogenase-1 (EC 1.1.1.42), β-galactosidase (EC 3.2.1.23), phosphoglucomutase-2 (EC 2.7.5.1), hexosaminidase B (EC 3.2.1.30), glyoxalase (EC 4.4.1.5), β-glucuronidase (EC 3.2.1.31), glutathione reductase (EC 1.6.4.2), soluble aconitase (EC 4.2.1.3), soluble glutamic oxalactic transaminase (EC 2.6.1.1), lactic dehydrogenase A and B (EC 1.1.1.27), esterase D (EC 3.1.1.1), nucleoside phosphorylase (EC 2.4.2.1), mannose phosphate isomerase (EC 5.3.1.8), adenine phosphoribosyl transferase (EC 2.4.2.7), peptidase A (EC 3.4.11.1), glucose phosphate isomerase (EC 5.3.1.9), adenosine deaminase (EC 3.5.4.4), superoxide dismutase-1 (EC 1.15.1.1.), arylsulphatase-A (EC 3.1.6.1), glucose 6-phosphate dehydrogenase (EC 1.1.1.49). Methods followed, enzyme symbols, and current chromosomal assignments are summarized in the report of the Third Annual International Workshop on Human Gene Mapping (22). Methods for electrophoretic analysis of β-galactosidase and arylsulphatase have been reported elsewhere (23, 24).

RESULTS AND DISCUSSION

As shown in Fig. 1, it is possible to separate human and mouse acid α-glucosidase by starch gel electrophoresis. This is probably due to different affinities of the human and mouse enzymes for the starch (7). No change was noted after neuraminidase treatment (data not shown). Analysis of a panel of 14 independent hybrid clones between thymidine kinase-deficient LM-TK⁻ (clone 1D) mouse cells and different human diploid fibroblasts indicated that all hybrid clones expressed human acid α-glucosidase and galactokinase activity and retained human chromosome 17. Human chromosome 17 was the only human chromosome consistently present in all hybrid clones. Under the conditions described, neutral α-glucosidase migrated more anodally, but it was not possible to resolve the human from

Table 1. Concordant segregation of the expression of human galactokinase (GK) and acid α-glucosidase (α-GLU) and presence of human chromosome 17 in mouse–human hybrids

Hybrid clones	Independent hybrid clones tested	Hybrids expressing human GK	Hybrids expressing human acid α-GLU	Hybrids containing human chromosome 17
LM-TK⁻ or IT-22 × HF in HAT	30	30	30	30*
LM-TK⁻ or IT-22 × HF in BrdUrd†	16	0	0	0
MPM × GM54VA	13	13	13	13‡

* In each of these clones, human chromosome 17 was identifiable in more than 90% of the metaphases analyzed.

† The 16 clones originally selected in HAT medium were counterselected in medium containing 100 μg of BrdUrd per ml.

‡ In each of these clones, human chromosome 17 was retained by 100% of the hybrid cells.

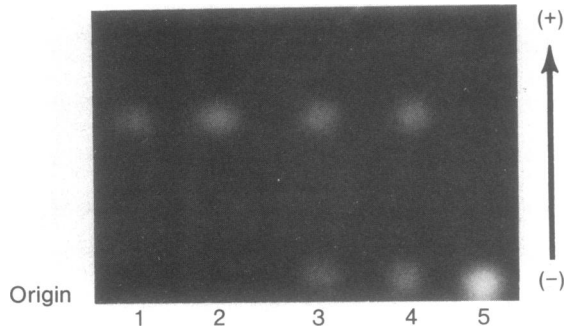

FIG. 3. Starch gel electrophoresis of galactokinase. Lanes: 1, extract of hybrid clone 11-14-77 (see legend of Fig. 1) counterselected in BrdUrd; 2, extract of LM-TK$^-$ (clone 1D) mouse cells; 3, extract of an MPM × GM54VA hybrid clone that retains human chromosome 17 and no other human chromosomes; 4, extract of hybrid clone 11-14-77 (see legend of Fig. 1) that was selected in HAT medium; 5, extract of human fibroblasts.

the mouse form (Fig. 2). In order to establish conclusively whether the human gene for acid α-glucosidase is located on human chromosome 17, we chose 16 additional independent hybrid clones between thymidine kinase-deficient mouse cells (LM-TK$^-$ and IT-22) and human diploid fibroblasts (GM0216, GM 121, GM 177, GM 1139, and MP) that had been selected in HAT medium. These hybrids were also counterselected in medium containing 100 μg of BrdUrd per ml. As shown in Table 1, all the additional 16 HAT-selected clones expressed human galactokinase and acid α-glucosidase activity and retained human chromosome 17. In contrast, the same hybrid clones that had been counterselected in medium containing a high concentration of BrdUrd lost concordantly the expression of human galactokinase and acid α-glucosidase activity, as well as human chromosome 17 (Figs. 1 and 3, Table 1). These results indicate that the human genes for acid α-glucosidase, galactokinase, and thymidine kinase are syntenic and that human acid α-glucosidase is located on human chromosome 17.

In order to confirm this assignment and to establish whether the presence of human chromosome 17 and of no other human chromosome is required for the expression of human acid α-glucosidase, we studied somatic cell hybrids between MPM and GM54VA simian virus 40-transformed human cells (17). We have already assigned the site of simian virus 40 integration to human chromosome 17 in these transformed cells and have shown that hybrids between MPM and GM54VA cells selectively retain human chromosome 17 in all their cells (17). Two of these MPM × GM54VA hybrids were found to contain human chromosome 17 as the only human chromosome (Fig. 4) (17, 25). As shown in Table 1, all these clones expressed human acid α-glucosidase and galactokinase activities.

We have also hybridized clone 1D mouse cells with fibroblasts (GM 248) derived from a child with Pompe disease. As shown in Fig. 5, the fibroblasts derived from the patient do not express any acid α-glucosidase activity. Three independent clone 1D × GM 248 hybrid clones containing human chromosome 17 were found to express only the mouse form of acid α-glucosidase (Fig. 5). These observations are compatible with the hypothesis that cells of patients with Pompe disease contain mutations in the structural gene for acid α-glucosidase. In addition, the results presented in this paper clearly indicate that the gene for human acid α-glucosidase is syntenic with the genes for galactokinase and thymidine kinase and is located on human chromosome 17. Because it is possible to select for the

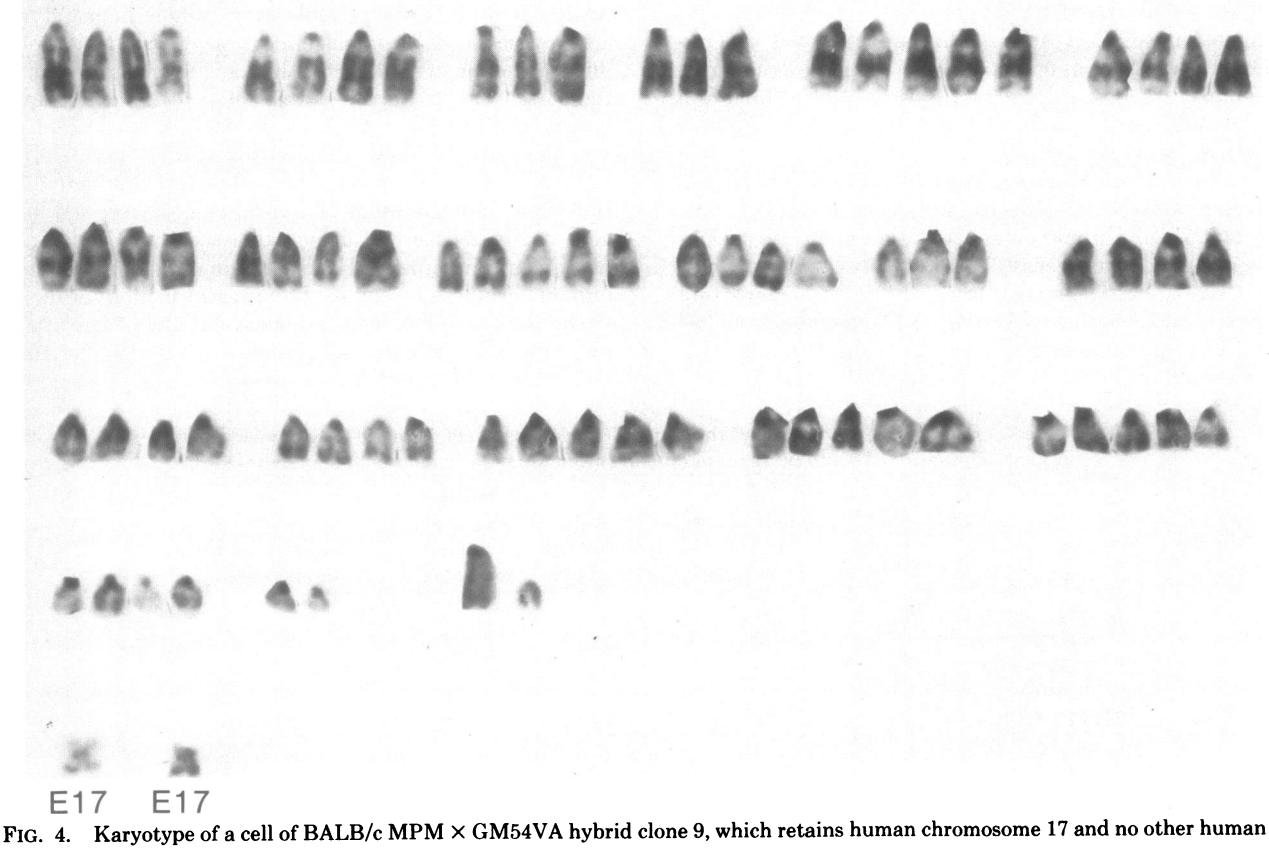

FIG. 4. Karyotype of a cell of BALB/c MPM × GM54VA hybrid clone 9, which retains human chromosome 17 and no other human chromosomes.

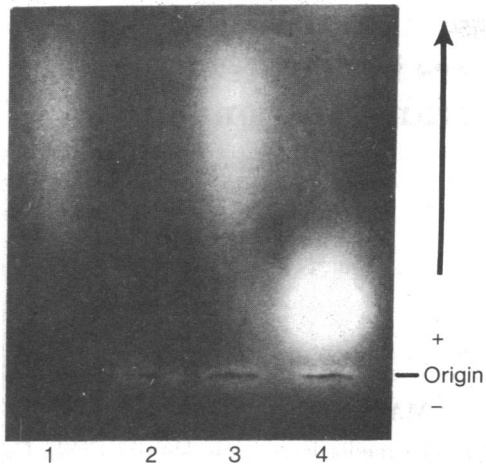

FIG. 5. Starch gel electrophoresis of acid α-glucosidase. Lane 1, extract of a hybrid clone between clone 1D mouse cells and GM 248, human fibroblasts from a patient with Pompe disease; only mouse acid α-glucosidase is expressed. Lane 2, extract from GM 248 cells; the human enzyme is not expressed. Lane 3, extract from clone 1D cells. Lane 4, extract from GM54VA cells.

expression of human thymidine kinase after chromosome-mediated gene transfer (26), the availability of an additional gene marker on this chromosome should allow the establishment of the order of these genes on human chromosome 17 and provide an additional marker for studies of chromosome-mediated gene transfer.

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