UC Irvine
UC Irvine Previously Published Works

Title
Assignment of the gene locus for human α-l-fucosidase to chromosome 1 by analysis of somatic cell hybrids

Permalink
https://escholarship.org/uc/item/0cj8z4sr

Journal
Somatic Cell Genetics, 4(1)

ISSN
0098-0366

Authors
Turner, BM
Smith, M
Turner, VS
et al.

Publication Date
1978

DOI
10.1007/bf01546492

Copyright Information
This work is made available under the terms of a Creative Commons Attribution License, availalbe at https://creativecommons.org/licenses/by/4.0/

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Somatic Cell Genetics, Vol. 4, No. 1, 1978, pp. 45–54

Assignment of the Gene Locus for Human α-L-Fucosidase to Chromosome 1 by Analysis of Somatic Cell Hybrids

B.M. Turner,[1] M. Smith,[1] V.S. Turner,[1] R.S. Kucherlapati,[2,3] F.H. Ruddle,[2] and K. Hirschhorn[1]

[1]*Division of Medical Genetics, Department of Pediatrics, Mount Sinai School of Medicine of the City University of New York, Fifth Avenue and 100th Street, New York, New York, 10029 and* [2]*Department of Biology, Kline Biology Tower, Yale University, New Haven, Connecticut.*

Received 16 June 1977—Final 2 September 1977

Abstract—*The α-L-fucosidases (EC 3.2.1.51) from human and mouse cells could be separated by isoelectric focusing of neuraminidase-treated cell extracts in acrylamide slab gels. Fourteen hybrid clones derived from the fusion of mouse and human cultured fibroblasts and 37 hybrid clones derived from the fusion of human long-term lymphoid lines with mouse RAG cells were tested for expression of human α-L-fucosidase. A strong correlation between the expression of the human enzyme and the presence or absence of human chromosome 1 was found. The presence of human α-L-fucosidase in clones scored as positive by isoelectric focusing was confirmed by Ouchterlony double immunodiffusion against IgG from rabbits immunized with purified human α-L-fucosidase. It is concluded that the structural gene locus for human α-L-fucosidase is located on chromosome 1.*

INTRODUCTION

Human α-L-fucosidase (EC 3.2.1.51) is a lysosomal hydrolase essential for the catabolism of fucose-containing glycolipids and glycoproteins. The enzyme can be resolved into a series of 6 or more isozymes by starch–gel electrophoresis (1, 2) or isoelectric focusing in acrylamide gel (3, 4) followed by staining for enzyme activity with the fluorogenic

[3]Dr. Kucherlapati's present address is the Department of Biochemical Sciences, Princeton University, Princeton, New Jersey.

0098-0366/78/0100-0045$05.00/0 © 1978 Plenum Publishing Corporation

substrate 4-methylumbelliferyl-α-L-fucoside. Treatment of cell or tissue extracts with purified bacterial neuraminidase results in the disappearance of the most acidic isozymes and a parallel increase in staining intensity of the 2 or 3 least acidic forms (1, 4). In human populations there are three common phenotypes of the enzyme distinguishable by differences in isozyme patterns after electrophoresis or isoelectric focusing (4). They have been designated αFUC 1, 2-1, and 2 and are attributable to two common alleles αFUC^1 and αFUC^2 at an autosomal locus (4). The polymorphism was shown to be expressed in a variety of different tissues and cell types, suggesting that the same gene locus codes for the enzyme in most, if not all, human cells. A third, rare allele αFUC^0 causes severe deficiency of the enzyme in homozygotes and gives rise to the clinical manifestations of the lysosomal storage disease fucosidosis (5).

In this report we describe methods for the separation of human and mouse α-L-fucosidases by isoelectric focusing and by the use of antiserum specific for the human enzyme. Using these techniques the expression of the human enzyme in human × mouse somatic cell hybrids has been examined. Evidence is presented for the assignment of the gene locus for human α-L-fucosidase to chromosome 1.

MATERIALS AND METHODS

Isoelectric Focusing. Slab gel isoelectric focusing was carried out using the LKB 2117 Multiphor apparatus (LKB Instruments, Stockholm, Sweden). Ampholyte-containing acrylamide gels were made up as follows: 10 ml of 29.1% acrylamide, 10 ml of 0.9% *NN'*-methylenebisacrylamide, 37.1 ml of water containing 7.5 g of sucrose, 0.4 ml of riboflavin, and the following ampholyte solutions (Ampholine carrier ampholytes, LKB) 0.5 ml of pH 4–6, 1.5 ml of pH 5–8, and 0.5 ml pH 7–9. All chemicals apart from ampholytes were from British Drug Houses Ltd., Poole, England. Preparation of gel slabs, neuraminidase treatment of cell extracts, isoelectric focusing, and staining for α-L-fucosidase activity were all exactly as described previously (4), with the exception that the running time for isoelectric focusing was extended to 5 h. The pH gradient was measured on completion of the run by cutting a strip 1 cm wide from the gel and immersing 0.5 cm sections of this strip in 0.5 ml of distilled water in glass tubes. The tubes were vortexed, kept overnight at 4°C, and then vortexed again. The pH of the solution was then measured with a glass electrode at 4°C.

Cell extracts for enzyme analysis were prepared in distilled water (0.2–0.3 ml for cells from one 75 cm² flask) either by 5 cycles of freezing and thawing or by sonication.

Enzyme and Chromosome Analysis. Enzymes were analyzed by horizontal starch–gel electrophoresis using the methods described by Harris

and Hopkinson (6). Metaphase spreads for chromosome analysis were prepared as previously described (7). Chromosomes were banded using quinacrine hydrochloride fluorescence and trypsin–Giemsa treatment.

Cell Lines and Hybridization Procedures. Fourteen clones derived from fusions between mouse cells (A9 or RAG) and a variety of human fibroblast cell lines were tested. These were AIM3a, AIM8a, AIM11a, AIM23a, WA1a, WA11a, J10H12, JFA14b, AIM4a, AIM8b, AIM10a, YA26a, YA52a, and YA65a. The first 8 clones constitute the "clone panel" described previously (8). All these clones were isolated and characterized at Yale University. Thirty-seven clones (17 primary and 20 secondary) derived from 5 separate hybridizations between the mouse hypoxanthineguanine phosphoribosyltransferase-deficient cell line RAG and human long-term lymphoid lines, were isolated and characterized at Mount Sinai School of Medicine. Five human parent lines were used. These were ODY (13 clones) and EB_4 (2 clones), obtained from Dr. Michael Steel, Edinburgh; Cali (13 clones), obtained from Dr. J. Fogh, Sloan Kettering Human Tumor Bank; NB60E (5 clones), established by Dr. N.G. Beratis, Mount Sinai School of Medicine and RPMI 1788 (4 clones) from Dr. N. Tanigaki, Roswell Park Memorial Institute. All cell lines except EB_4 had a normal karyotype. Cells were fused with inactivated Sendai virus as described previously (7, 9). Cells were grown in HAT-selective medium (10) to eliminate RAG cells while the human parental cells, which grow in suspension, were eliminated by successive medium changes. Hybrid cells were cloned by the technique of Ham and Puck (11).

Purification of Human α-L-Fucosidase and Preparation of Antibodies. α-L-Fucosidase was purified approximately 12,000 fold from human placenta by ammonium sulfate precipitation and affinity chromatography. The affinity gel was prepared by binding L-fucosylamine to *N*-hydroxysuccinimide activated Sepharose 4B, prepared as described by Cuatrecasas and Parikh (12). Details of the purification procedure will be presented elsewhere (manuscript in preparation). The purified enzyme preparation was at least 95% pure on the basis of densitometric analysis of acrylamide gels stained with Coomassie Brilliant Blue. New Zealand White Rabbits were immunized with several subcutaneous injections of 50 or 100 μg of enzyme protein in Freund's complete adjuvant. IgG was purified from immune serum by ion-exchange chromatography (13) and gelfiltration on Sephadex G-100.

RESULTS

The isozymes of α-L-fucosidase in mouse A9 cells before and after neuraminidase treatment are shown in Fig. 1. In untreated extracts 5 major isozymes, evenly distributed over the pH range 7.5–5.7, were seen

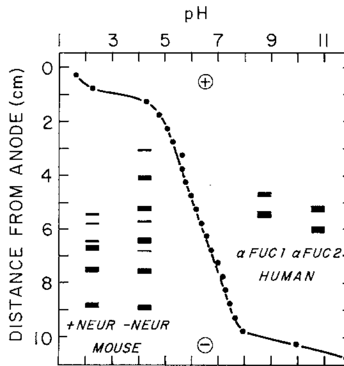

Fig. 1. Diagram of the isozymes of human and mouse α-L-fucosidase after isoelectric focusing in thin-layer acrylamide gel and staining for enzyme activity. The two homozygous phenotypes (αFUC1, αFUC2) of the human enzyme are shown. The human isozymes are those remaining after overnight incubation of lymphoid line extracts with purified bacterial neuraminidase (4). The isozymes present in mouse RAG cell extracts are shown with (+NEUR) and without (−NEUR) neuraminidase treatment. The isoelectric points of the different isozymes can be read from the pH gradient (●——●), measured across the gel as described in the text.

together with 4 isozymes of lower staining intensity. After treatment with neuraminidase, 3 major isozymes and 3 minor components were detected. Two of the major isozymes had isoelectric points of pH 7.5 and 7.0 and corresponded to the 2 least acidic isozymes seen in untreated extracts. The third major isozyme had an isoelectric point of pH 6.7 and corresponded to a minor component present in the cell extract prior to neuraminidase treatment. As shown in Fig. 1, the three major forms of α-L-fucosidase in mouse cells, following neuraminidase treatment, were clearly separated from the human isozymes by the gel isoelectric focusing method.

The α-L-fucosidase isozymes in several human × mouse hybrid clones are shown in Fig. 2. In those clones which were positive for the human enzyme the isozyme pattern was a simple mixture of the patterns observed in human and mouse cells, with no evidence for heteromeric enzyme forms. As noted above, α-L-fucosidase is polymorphic in human populations (4). Thus, depending on the phenotype of the human parent cells, hybrid clones may retain either the αFUC^1 or αFUC^2 allele. In the positive clones shown in Fig. 2, channels 2, 3, and 5, the human α-L-fucosidase is of the αFUC1 phenotype. The human enzyme in channel 6 seems to be of the αFUC2 phenotype, the major isozymes of which have higher isoelectric points than their αFUC1 counterparts (see Fig. 1). Unfortunately, it was not possible to test the parental cell line from which this clone was derived. The presence of the human enzyme in this clone was confirmed by immunodiffusion as described below.

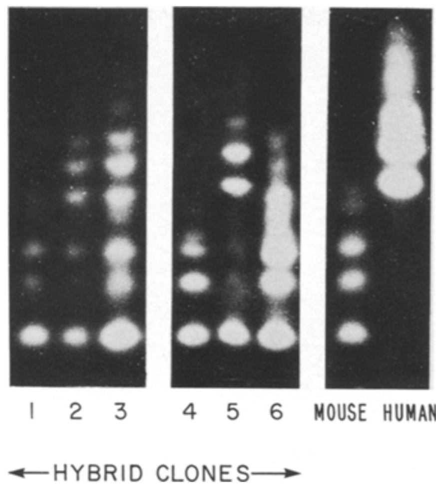

Fig. 2. A photograph of the isozymes of α-L-fucosidase in human and mouse cultured cells and human × mouse hybrids. All cell extracts were treated with purified bacterial neuraminidase prior to separation of the isozymes by gel isoelectric focusing, as described in the text. Lanes 2, 3, 5, and 6 show hybrid clones positive for human α-L-fucosidase. Lanes 1 and 4 are negative for the human enzyme. The human control is the purified placental enzyme used for preparation of rabbit antiserum.

Fourteen human × mouse fibroblast hybrid clones were tested for human α-L-fucosidase. Eight of these comprise the "clone panel" described previously (8). The human chromosome complements of the hybrid cell lines in this panel are such that each human chromosome has a unique distribution among the 8 clones. We have shown previously that the expression of human α-L-fucosidase in these clones correlates exactly with the presence or absence of chromosome 1 (14). Four clones were chromosome 1+,αFUC+ and four were chromosome 1−,αFUC−. Six additional human × mouse fibroblast hybrids have since been tested for α-L-fucosidase and for the chromosome 1 marker enzymes phosphoglucomutase 1 (PGM_1) and peptidase C (PEPC). Two clones (AIM10a and YA52a) were positive for human α-L-fucosidase and four were negative. The latter were also negative for both PGM_1 and PEPC. The two positive clones were both positive for PGM_1. However, only YA52a was also positive for PEPC and chromosome 1. AIM10a was negative for PEPC and had no intact human chromosome 1.

In 37 clones and subclones derived from five separate hybridizations between human lymphoid lines and mouse RAG cells, we observed concordant expression of human α-L-fucosidase, PGM_1, and PEPC. The results are shown in Table 1. Twenty-seven clones were tested for all three markers and 23 showed concordant expression of these markers

Table 1. Expression of αFUC, PGM_1 and PEPC in 37 human (lymphoid line) × mouse (RAG) hybrid clones

αFUC	PGM_1	PEPC	Number of clones
+	+	+	12
+	+	−	1
+	−	−	1
−	−	+	2
−	−	−	11
+	+	NT[a]	6
+	NT	+	1
−	NT	−	3

[a]NT indicates "not tested."

(Table 1, lines 1 and 5). Only a single clone was discordant for αFUC and PGM_1, (Table 1, line 3). This clone became negative for αFUC when later passages were tested. Three additional clones were discordant for αFUC and PEPC (Table 1, lines 2 and 4). It was noted that two of these clones were αFUC−,PGM_1−,PEPC+ and one was αFUC+,PGM_1+,PEPC−. Thus, as in the case of AIM-10a described above, the expression of human α-L-fucosidase in these discordant clones correlated with PGM_1 but not with PEPC. Six clones were tested for αFUC and PGM_1 alone and all were concordant (Table 1, line 6). Four clones were tested for αFUC and PEPC alone and were also concordant (Table 1, lines 7 and 8).

The segregation of human α-L-fucosidase and different human chromosomes is shown in Table 2. The presence or absence of a particular chromosome was determined by karyotyping alone (chromosomes 3, 5, 7, 8, 16, 17, and 22) or by a combination of karyotyping and analysis of marker enzymes. Complete karyotype data was available for 22 clones. Thirteen of these clones contained an apparently intact chromosome 1 and also expressed human α-L-fucosidase, while 9 were negative for both chromosome 1 and α-L-fucosidase. Thus, there was a perfect correlation between the presence of an intact chromosome 1 and expression of human α-L-fucosidase. It can be seen from the results shown in Table 2 that the expression of human α-L-fucosidase did not correspond to the presence or absence of any human chromosome other than chromosome 1.

Antibodies raised in rabbits against purified human placental α-L-fucosidase cross-reacted with the human lymphoid-line enzyme but not with the enzyme from mouse RAG cells (Fig. 3). The enzyme–antibody complex remained catalytically active and the precipitin lines on Ouchterlony double diffusion plates could therefore be visualized by staining with the fluorogenic substrate. The human lymphoid-line enzyme formed a line

Table 2. Segregation of human α-L-fucosidase (αFUC) with human chromosomes and enzyme markers in human (lymphoid-line) × mouse (RAG) hybrids[a]

Chromosome	Enzyme markers[b]	αFUC segregation	
		Concordant	Discordant
1	PGM_1, PEPC	22	0
2	ICD_s, MDH_s	15	14
3		9	13
4	PGM_2	11	15
5		7	15
6	ME_s, SOD_m	15	14
7		9	13
8		9	13
9	AK_1, AK_3	7	19
10	GOT_s	19	6
11	LDHA	11	14
12	LDHB, PEPB	11	16
13	ESD	10	16
14	NP	10	18
15	MPI	8	19
16		6	16
17		9	13
18	PEPA	12	14
19	PHI	18	10
20	ADA	6	19
21	SOD_s	14	16
22		9	13
X	G6PD	15	12

[a] Complete karyotype data was available for 22 clones and the figures given for chromosomes 1,3,5,7,8,16,17, and 22 are derived from this set of clones alone. Chromosomes 3,8,17, and 22 were absent from all 22 clones. Additional clones were tested for various marker enzymes and thus the figures for chromosomes other than those listed above are derived from both karyotyping and analysis of enzyme markers.

[b] Nomenclature is that recommended by the nomenclature committee of the Baltimore Conference (1975); see reference 16, pp. 65–74.

of complete identity with the placental enzyme on immunodiffusion. A single precipitin line was detectable by staining with the fluorogenic substrate and a line corresponding to this could be visualized by staining for protein with Coomassie Brilliant Blue. An additional faint precipitin line closer to the antibody well was occasionally detected by protein staining in both purified enzyme preparations and cell extracts. This line had no catalytic activity and its significance is unclear. Four of the hybrid clones which were shown to be positive for human α-L-fucosidase by isoelectric focusing were subsequently tested by immunodiffusion and a distinct precipitin line was obtained. An example is shown in Fig. 3, well 5. Ten clones which were negative by isoelectric focusing were also tested by immunodiffusion and seven formed no detectable precipitin line. However, three of these (all subclones from the same primary clone) gave

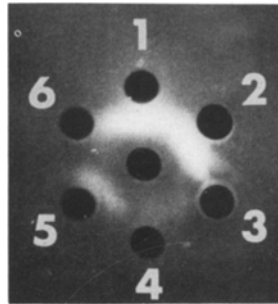

Fig. 3. Ouchterlony double diffusion in agarose: rabbit antibodies against human α-L-fucosidase are in the center well. The outer wells contain (1) human lymphoid-line extract (2) purified human placental α-L-fucosidase (3) and (4) human × mouse hybrid cell extract negative for human α-L-fucosidase (5) human × mouse hybrid cell extract positive for human α-L-fucosidase (6) mouse RAG cell extract. Precipitin lines were stained for enzyme activity with the fluorogenic substrate.

a weak positive reaction in 2 separate tests. This may simply be a consequence of the greater sensitivity of the immunodiffusion method (compared to the isoelectric focusing technique) which detects small populations of positive cells present in some cell lines. However, more complex explanations are possible, as discussed below.

DISCUSSION

The slab gel isoelectric focusing method provides a sensitive and reliable means of detecting human α-L-fucosidase in human × mouse somatic cell hybrids. The data presented show that the expression of the human enzyme is correlated with the presence or absence of chromosome 1. This is consistent with our earlier results obtained by analysis of a "clone panel" (14) and with recent family studies (15) which have demonstrated close linkage of α-L-fucosidase and the Rh blood group system. The *Rh* locus has been assigned to the lpter→1p32 region, an area of the chromosome which also includes the PGM_1 locus (reviewed in 16). In view of this, it is not surprising that in 4 clones that we found to be discordant for PGM_1 and PEPC, the α-L-fucosidase was concordant with PGM_1 rather than PEPC, which is located at the opposite end of chromosome 1 in the region lqter→1q41.

Human α-L-fucosidase is a heterogeneous enzyme. In addition to the isozymes separable by electrophoresis or isoelectric focusing, several forms that presumably differ in molecular size can be resolved by gel filtration (17, 18). Recent studies on the purified placental enzyme have shown that the largest form of the enzyme has a molecular weight,

measured by gel filtration, of approximately 300,000 and that the smallest catalytically active form has a molecular weight of about 50,000. This latter value corresponds to the size of an enzyme subunit, as measured by acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. This form of the enzyme is therefore presumably a monomer. The enzyme forms resolved by isoelectric focusing are also probably monomers, in view of the absence of heteromeric, "hybrid" isozymes with isoelectric points intermediate between those of the human and mouse enzymes. Thus, the gene locus which we have assigned to chromosome 1 probably codes for the catalytic, 50,000 molecular weight subunit of human α-L-fucosidase. It seems likely that two alleles at this locus give rise to the common polymorphism described previously (4). This conclusion is consistent with the expression of both the αFUC1 and αFUC2 phenotypes in hybrid cells retaining chromosome 1 and with the assignment of the polymorphic *α-L-fucosidase* gene locus to chromosome 1 by analysis of family data (15). Finally, on the basis of previous studies (5), it can be concluded that the inborn error of metabolism fucosidosis is a result of homozygosity for a rare "silent" allele at this same gene locus.

Studies on purified human liver α-L-fucosidase have shown that the enzyme has a single subunit, of molecular weight about 50,000 and exists as a tetramer (19). However, recent work on the placental enzyme has raised the possibility that the enzyme contains a second subunit that associates, under appropriate conditions, with the catalytic subunit to generate higher molecular weight enzyme forms (manuscript in preparation). These high molecular weight forms can be dissociated under relatively mild conditions to yield free, enzymatically active, catalytic subunits. It remains to be established whether or not the second subunit is synthesized in cultured cells and to what extent it can react with antiserum raised against the purified placental enzyme. The possibility exists that the occasional positive results obtained by double immunodiffusion analysis of hybrid clones shown to be negative for human α-L-fucosidase by isoelectric focusing are due to the existence in these clones of hybrid enzymes containing a mouse catalytic subunit and the human second subunit. More detailed analysis of hybrid clones by immunodiffusion and immunoprecipitation is now underway in order to clarify this possibility.

ACKNOWLEDGMENTS

The authors thank Bridget Shafit-Zagardo and Constantin Zamfirescu for skilled technical assistance. This work was supported in part by U.S. Public Health Service Grant HD-02552, Clinical Genetics Center Grant GM-19443 and a grant from the National Foundation–March of Dimes.

LITERATURE CITED

1. Turner, B. M., Beratis, N. G., Turner, V. S., and Hirschhorn, K. (1974). *Clin. Chim. Acta* **57**:29–35.
2. Alhadeff, J. A., Miller, A. L., Wenger, D. A., and O'Brien, J. S. (1974). *Clin. Chim. Acta* **57**:307–313.
3. Thorpe, R., and Robinson, D. (1975). *FEBS Lett.* **54**:87–92.
4. Turner, B. M., Turner, V. S., Beratis, N. G., and Hirschhorn, K. (1975). *Am. J. Hum. Genet.* **27**:651–661.
5. Turner, B. M., Beratis, N. G., Turner, V. S., and Hirschhorn, K. (1975). *Nature (London)* **257**:391–392.
6. Harris, H., and Hopkinson, D. A. (1976). *Handbook of Enzyme Electrophoresis in Human Genetics*. North Holland Publishing Co., Amsterdam.
7. Smith, M., Gold, P., Freedman, S. O., and Shuster, J. (1975). *Ann. Hum. Genet.* **39**:21–31.
8. Satlin, A., Kucherlapati, R., and Ruddle, F. H. (1975). *Cytogenet. Cell Genet.* **15**:146–152.
9. Harris, H., and Watkins, J. F. (1965). *Nature (London)* **205**:640–646.
10. Littlefield, J. W. (1964). *Science* **145**:709–710.
11. Ham, R. G., and Puck, T. T. (1962). *Proc. Soc. Exp. Biol. Med.* **111**:67–77.
12. Cuatrecasas, P., and Parikh, I. (1972) *Biochemistry* **11**:2291–2299.
13. Weir, D. M. (1967). *Handbook of Experimental Immunology*. F. A. Davis Co., Philadelphia.
14. Turner, V. S., Turner B. M., Kucherlapati, R., Ruddle, F. H., and Hirschhorn, K. (1976). *Baltimore Conference (1975): Third International Workshop on Human Gene Mapping. Birth Defects: Original Article Series, XII, 7. The National Foundation–*March of Dimes, New York, pp. 238–240.
15. Corney, G., Fisher, R. A., Cook, P. J. L., Noades, J., and Robson, E. B. (1977). *Ann. Hum. Genet.* **40**:403–405.
16. Hamerton, J. L. (1976). *Baltimore Conference (1975): Third International Workshop on Human Gene Mapping. Birth Defects: Original Article Series XII, 7.* The National Foundation–March of Dimes, New York, pp. 7–23.
17. Ya Wiederschain, G., Kolibaba, L. G., and Rosenfeld, E. L. (1973). *Clin. Chim. Acta* **46**:305–310.
18. Robinson, D., and Thorpe, R. (1973). *Clin. Chim. Acta* **47**:403–407.
19. Alhadeff, J. A., Miller, A. L., Wenaas, H., Vedvick, T., and O'Brien, J. S. (1975). *J. Biol. Chem.* **250**:7106–7113.