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The human lactase-phlorizin hydrolase gene is located on chromosome 2

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The lactase-phlorizin hydrolase gene was assigned to chromosome 2 by analysis of Southern blots of DNA from a panel of human-rodent cell hybrids containing characteristic sets of human chromosomes. The hybridization probe used was a recently isolated cDNA clone of the human lactase-phlorizin hydrolase gene.

Lactase-phlorizin hydrolase; Gene mapping; Chromosome 2; (Human)

1. INTRODUCTION

Lactase (EC 3.2.1.23) of the small-intestinal brush border membrane is the enzyme involved in the most frequent genetic disturbance in man, adult-type hypolactasia [1,2] (reviews [3–5]). This condition affects one-third to one-half of mankind – mostly in the third world – and severely limits the possibility of ingesting fresh milk: in these adults intestinal lactase (and the accompanying, but distinct, phlorizin hydrolase activity, EC 3.2.1.62 [6]) declines to levels 5–10% of those at birth. The mode of inheritance of adult-type hypolactasia is monofactorial, autosomal and recessive.

The genetic anomaly underlying this condition is still unknown, although it is generally assumed that adult-type hypolactasia is the human equivalent of the decline in intestinal lactase [7,8] and phlorizin hydrolase [9] activities which take place in the vast majority of mammals at the time of weaning. But again, the mechanism leading to this decline is also little understood – it may be related to a shorter lifespan of the enterocyte [10], or to a decreased rate of synthesis [11] (processing, or homing) of lactase, or to a combination thereof [12].

Recently, the complete primary structures of human and rabbit lactase-phlorizin hydrolase have been established via cDNA cloning and sequencing [13]: the deduced primary translation product (pre-pro-lactase-phlorizin hydrolase, of 1927 or 1926 amino acid residues) is subjected to major proteolytic processing (unique in its magnitude among brush border proteins), the result of which is that only approx. 60% of the pre-pro-form appears eventually as the 'final' lactase-phlorizin hydrolase in the brush border membrane. It is quite possible that the decline in intestinal lactase which takes place in most mammals at the time of weaning is related to deficient processing and/or homing of

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an enzymatically inactive precursor from the site of synthesis to the final target membrane [14]. This would be in keeping with the observation that in the adult rabbit the level of lactase mRNA is as high as in baby rabbits (Sebastio, G. et al., in preparation), suggesting that it is not the mRNA which is regulated.

As to human adult-type hypolactasia, the residual lactase-phlorizin hydrolase is, at least in some pedigrees, indistinguishable from the 'normal' counterpart in its immunological reactivity, enzymatic activity or apparent molecular size [15–17].

As a first step towards an understanding of the genetics of human lactase-phlorizin hydrolase, we report here the localization of its gene to chromosome 2.

2. MATERIALS AND METHODS

2.1. *Parental cells*

The parental HPRT-deficient rodent cell lines were mouse RAG and A9 cells and Chinese hamster Wg3-h. The parental human cells were female fibroblasts with different balanced X-autosome translocations, except one case where 46,Y, der(X) (Xqter-p22.3::Yq11-qter) cells were used to produce the hybrid 445 × 392.K1.

2.2. *Hybrid cells*

The cell hybrids were produced by polyethylene glycol-mediated fusion of rodent cell lines and human fibroblasts and human chromosomes in hybrid cell metaphases were analysed as described in [18,19] and table 1. About 12 metaphases were analysed for each cell hybrid.

2.3. *DNA extraction, Southern transfer, and filter hybridization*

DNA was extracted from somatic cell hybrids according to

Table 1

Analysis of human chromosomes and human lactase-phlorizin hydrolase gene in 15 rodent-human cell hybrids

Human-rodent hybrids	Human chromosomes																						Human lactase
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
445 × 393 K1	+	+	+	+	+	+	+	−	−	−	+	−	−	/	−	−	−	−	+	−	/	−	+
697 × 175 K36	−	/	−	+	−	+	−	−	−	−	−	−	−	+	/	/	−	−	−	−	/	/	+
617 × 347 K6	−	−	−	−	−	/	−	−	−	−	/	+	−	−	/	/	/	/	−	/	+	−	−
422 K2	−	−	+	−	+	+	−	+	+	−	+	−	+	/	−	/	−	−	−	+	+	+	−
749	+	−	+	+	+	+	+	−	−	−	/	+	−	+	−	/	−	+	−	−	+	/	−
494 × 393 K6	−	+	−	−	−	−	−	−	−	+	−	−	/	−	−	−	−	−	−	−	−	−	+
790 × 175 K6	−	−	−	−	−	−	−	−	−	−	−	−	+	−	/	−	−	−	−	−	+	−	−
750	/	−	−	/	/	−	−	−	−	−	+	−	−	+	/	−	+	−	−	/	−	/	−
PI-RAG-72	+	−	+	−	+	+	+	+	+	−	−	+	−	+	+[d]	−	−	+	−	−	−	+	−
GO-RAG-4	−	−	+	/	−	−	/	+	−	−	−	−	−	+	+	−	−	+	−	−	−	+	−
GM194-RAG-7	−	−	+[b]	+	+	−	−	−	−	/	−	−	−	−	−	−	−	/	+	−	+	−	−
GM194-RAG-5-5	−	−	+[b]	+	+	+	+	−	−	+	−	+	+	+	−	+	−	−	−	+	+	+	−
GM97-RAG-8-13	+[a]	−	−	+	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	+	−
GM89-A9-9c-7	−	−	+	+	−	−	+	−	−	−	+	−	−	+	+	/	−	+	/	+	+	+	−
MS58-A9-26	−	−	−	−	−	−	−	−	−	−	+	−	+	+[c]	+	−	−	−	−	−	−	−	−
+/+	1	2	1	2	1	2	1	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	
−/−	8	12	5	5	6	7	7	9	9	10	6	8	8	4	5	7	10	6	10	7	5	4	
+/−	2	0	5	5	5	4	4	3	3	1	4	4	4	6	3	1	1	4	1	3	7	6	
−/+	2	0	2	1	2	1	2	3	3	2	2	3	2	1	2	2	3	3	2	3	1	2	
Concordant	9	14	6	7	7	9	8	9	9	10	7	8	8	5	5	7	10	6	11	7	5	4	
Discordant	4	0	7	6	7	5	6	6	6	3	6	7	6	7	5	3	4	7	3	6	8	8	

[a] GM97-RAG-8-13 only contains the region pter-q12 of chromosome 1

[b] GM194-RAG-7 and GM194-RAG-5-5 only contain the regions q21-qter and pter-q21 of chromosome 3, respectively

[c] MS58-A9-26 only contains the region pter-q32 of chromosome 14

[d] PI-RAG-72 only contains the region q13-qter of chromosome 15

+, chromosome detected in at least 30% of hybrid cells; /, chromosome detected in less than 30% of hybrid cells; −, chromosomes not detected

Aldridge et al. [20]. DNA samples were digested with *Hin*dIII, separated electrophoretically on 0.7% agarose gels at 50 V for 20 h and transferred onto Hybond-N membranes (Amersham, England) as described [21]. The probe used was a 6.0 kb *Eco*RI fragment of a human intestinal lactase cDNA clone [13]. The DNA probe was labelled with ^{32}P by nick-translation to a specific activity of above 10^8 cpm per μg. Hybridization, stringent washes and autoradiography were performed using established methods [20].

3. RESULTS AND DISCUSSION

DNA from 15 different human rodent cell hybrids was analysed by Southern blotting after digestion with the restriction enzyme *Hin*dIII (fig.1). Using a 6.0 kb *Eco*RI fragment of human lactase-phlorizin hydrolase cDNA as the probe 9 hybridizing bands are revealed in human genomic DNA whereas 7 cross-hybridizing bands are seen in mouse and 6 bands in hamster. The two largest human fragments of approx. 18 and 14 kb are significantly more intense than the other human fragments and since in the hybrid cells the fainter human fragments are not always clearly detectable, the presence (+) or absence (−) of the lactase-phlorizin hydrolase sequence in the hybrids is based on the distribution of the two intense bands. The results in table 1 show 100% concordant segregation of chromosome 2 and the lactase-phlorizin hydrolase gene in the cell hybrids, whereas other chromosomes show at least 21% discordant segregation.

The brush border of the small intestine contains a characteristic set of enzymes like the peptidases and the glucosidases [22,23]. Of these, sucrase-isomaltase has been localized to chromosome 3 [24], γ-glutamyl transpeptidase to chromosome 22 [25,26], aminopeptidase N to chromosome 15 [27] and now lactase-phlorizin hydrolase to chromosome 2. It is clear that the genes of enzymes belonging to the same organelle (brush border) are not located on the same chromosome, which is in line with data from other systems [28]. The genes of the brush border enzymes are then far from each other and not under a common regulatory DNA element. In fact, this is reflected by differences in appearance during development [29].

The molecular anomaly underlying adult-type hypolactasia is still unknown. If the phenotypic variation is caused by a variation at the lactase-phlorizin hydrolase locus, genetic markers at or close to the gene locus should show genetic linkage to adult-type alactasia. Now that the lactase-phlorizin hydrolase gene has been cloned and its chromosomal location has been determined it is possible to investigate this question. Work in this direction is in progress.

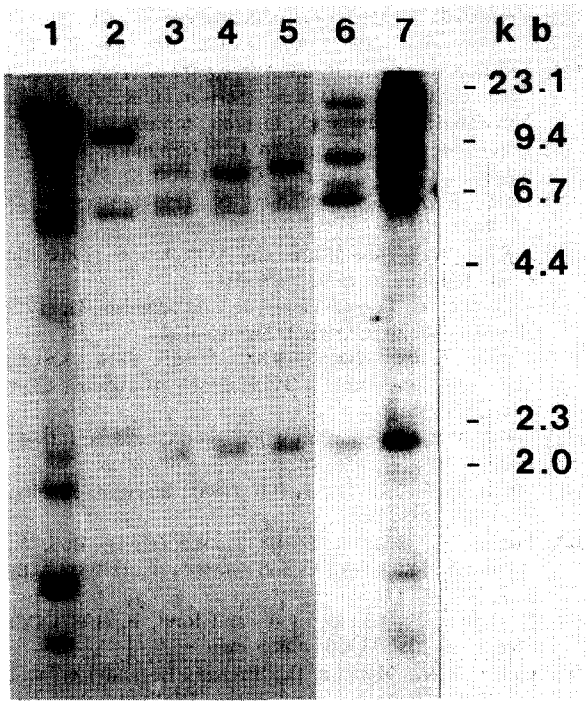

Fig.1. Detection of human lactase-phlorizin hydrolase gene sequences by Southern blot analysis. Lanes: 1, human DNA; 2, Wg3-h (hamster); 3, RAG (mouse); 4, A9 (mouse); 5, hybrid 750; 6, hybrid 697 × 175 K36; 7, hybrid 445 × 393 K1.

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