Amer J Hum Genet 25:200–207, 1973

Assignment of the Genes for Malate Oxidoreductase Decarboxylating to Chromosome 6 and Peptidase B and Lactate Dehydrogenase B to Chromosome 12 in Man

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INTRODUCTION

Somatic cell hybrids can be used to make gene assignments in man [1, 2]. Mouse/human hybrids are useful for this purpose for the following reasons: human chromosomes are preferentially lost, mouse and human homologous enzyme phenotypes can be discriminated, and human chromosomes can be identified in the hybrid cells. It is, therefore, possible to make correlations between the individual human chromosomes and specific human enzyme phenotypes using hybrid clones which possess different human chromosome constitutions.

In this report, we present evidence for the assignment of the gene specifying the soluble form of malate oxidoreductase decarboxylating (MOD; EC 1.1.1.40) to human chromosome 6, and the genes for peptidase B (Pep B) and lactate dehydrogenase B (LDH B; EC 1.1.1.27) to chromosome 12.

MATERIALS AND METHODS

Hybrid cell populations were produced by mixing mouse and human cells together in ratios ranging from 1:1 to 1:10 human-to-mouse cells at a total cell density of between 2×10^6 and 4×10^6 [3]. Cells were stimulated to form heterokaryons by treatment with β-propiolactone–inactivated Sendai virus at concentrations of 1,000 hemagglutinating units (HAU) per milliliter. In instances where both parent cell populations were monolayer cultures, fusion was performed with the cells attached. In those hybrids which were formed from a leukocyte parental cell population, fusion was performed with the cells in suspension [4].

The following six hybrids were analyzed in this study. *J hybrids* were formed between mouse RAG cells which are deficient in hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and normal human leukocytes [4]. *WA hybrids* were formed between mouse L cells deficient in adenine phosphoribosyltransferase (APRT) and the normal human female fibroblastic cell strain WI-38 [5]. *KOP hybrids* (coded RK) were formed between RAG and the KOP human fibroblastic cell strain which carries an X/14 reciprocal

Received September 22, 1972; revised November 3, 1972.

This work was supported by grant 5-R01-GM-09966 from the U.S. Public Health Service.

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translocation [6]. *JBA hybrids* were formed between APRT-deficient mouse L cells and normal human leukocytes which carry a 14/22 centric fusion [5]. *IL hybrids* were formed between the human neuroblastoma IMR-32 and the mouse cell line LM (TK$^-$) which is deficient in the enzyme thymidine kinase [7]. *NM hybrids* were formed between the C-1300 mouse neuroblastoma cell clone NA (HGPRT$^-$) and the human primary fibroblastic cell strain MRC-5 [7]. The RK, NM, and IL hybrids were isolated in the HAT selection medium in Dulbecco-Vogt modified Eagle's minimal medium (DVME) with 10% fetal calf serum [8]. Human HGPRT was selectively retained in hybrids J, RK, and NM, complementing the mouse enzyme deficiency. The IL hybrids preferentially retained human thymidine kinase activity [9]. The WA and JBA hybrids were isolated in AA selection medium which enriches for cells which possess APRT activity [5]. In these clones, the human form of APRT was retained. The AA selection medium was made from DVME plus alanosine (7 μg/ml; the gift of Dr. Piero Sensi) and adenine (5×10^{-5}M).

Hybrid clones were examined for the human forms of the following 22 enzymes by starch gel and acrylamide gel electrophoresis [10, 11]: adenosine deaminase (EC 3.5.4.4); adenine phosphoribosyltransferase (EC 2.4.2.7); glutamate oxaloacetate transaminase (EC 2.6.1.1); glucose-6-phosphate dehydrogenase (EC 1.1.1.49); glucose phosphate isomerase (EC 5.3.1.9); hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8); isocitrate dehydrogenase (EC 1.1.1.42); indolephenol oxidase A—tetrameric form; indolephenol oxidase B—dimeric form; lactate dehydrogenase A (EC 1.1.1.27); lactate dehydrogenase B (EC 1.1.1.27); malate oxidoreductase decarboxylating (EC 1.1.1.40); malate oxidoreductase (EC 1.1.1.37); mannose phosphate isomerase (EC 5.3.1.8); nucleoside phosphorylase (EC 2.4.2.1); peptidases A, B, C, and D; phosphoglycerate kinase (EC 2.7.2.3.); phosphoglucomutase 1 (EC 2.7.5.1); and thymidine kinase (EC 2.7.1.21).

The human chromosomes were identified in metaphase cells prepared to reveal quinacrine banding [12], Giemsa banding [13], and constitutive heterochromatin [14]. A total of 1,135 metaphase cells were analyzed. In some instances the same cell was stained both for quinacrine banding and for Giemsa banding. The majority of the metaphase spreads were prepared as karyotypes.

RESULTS

Twenty-two primary clones of independent origin and 21 secondary clones derived from primary clones were analyzed (table 1). Six different hybrid combinations were used, and within a particular hybrid combination secondary clones were frequently descended from different primary clones. Therefore, many of the secondary clones in this study are unrelated to many of the primary clones and other secondary clones. To a very considerable degree the clones listed in table 1 are independent of each other. The importance of clone independence for linkage analysis has been discussed elsewhere [15].

The combined results (table 2) show a strong correlation between chromosome 6 and the MOD phenotype and chromosome 12 and the Pep B phenotype (fig. 1). Two clones in a total of 51 (6%) possessed C-6 chromosomes but *lacked* detectable MOD activity. All of these discrepant clones had low frequencies of C-6 chromosomes in the sampled cells. Clone J-3-G had two C-6 positive cells in a total of 15 (13%); and clone WA IIa had one positive in 32 (3%). The average frequency of C-6 chromosomes in the two discrepant clones was 8%. In the 13 clones which were positive both for chromosome 6 and MOD, the average frequency of cells having a C-6 chromosome was 63% with the lowest frequency being 15%. Thus

TABLE 1

EXPRESSION OF ENZYME PHENOTYPES AND CHROMOSOMES IN 43 HYBRID CLONES

Clone	Class	No. Cells Analyzed	6+ Cells	MOD	12+ Cells	Pep B
J-3-G	S	15	2*	—	15	+
J-3-G-(AG)G	S	26	0	—	25	+
J-3-G-(AG)S	S	13	0	—	12	+
J-3-S	S	10	0	—	10	+
J-10-H-5	S	31	0	—	14	+
J-10-H-7	S	28	25	+	0	—
J-10-H-9	S	27	0	—	0	—
J-10-H-12	S	25	24	+	23	+
J-10-J	S	42	39	+	35	+
J-10-J-(AG)C	S	6	3	+	5	+
J-10-J-(AG)Q	S	9	7	+	6	+
J-10-J-(AG)S	S	14	11	+	13	+
J-10-P	S	13	12	+	12	+
WA Ia	P	23	0	—	22	+
WA IIa	P	32	1*	—	17	+
WA III	P	50	0	—	0	—
WA IVa	P	29	0	—	10	+
WA V	P	59	0	—	0	—
WA VIa	P	40	0	—	0	—
WA VIIa	P	25	0	—	19	+
WA VIIIa	P	31	0	—	0	—
WA IXa	P	27	8	+	24	+
WA IVa(1-21)†	S	52	0	—	0	—
RK 2-6A	P	9	6	+	4	+
RK 2-6B	P	14	0	—	0	—
RK 2-7	P	6	0	—	0	—
RK 2-14	P	7	0	—	6	+
RK 3-2B	P	15	9	+	6	+
RK 3-4	P	32	0	—	0	—
RK 3-5	P	18	7	+	0	—
RK 3-5zj	S	17	0	—	0	—
RK 3-5zh'	S	8	0	—	0	—
RK 3-5za	S	24	0	—	0	—
RK 3-51	S	19	3	+	0	—
RK 2-7i	S	33	0	—	0	—
RK 2-7zd	S	14	0	—	0	—
RK 2-7zr	S	44	0	—	0	—
JBA-1	P	55	0	—	0	—
IL-I-20A	P	45	0	—	0	—
IL-I-20B	P	37	0	—	0	—
IL-II-5	P	70	0	—	50	+
IL-II-16	P	31	0	—	0	—
NM-VII-10	P	9	4	+	6	+

NOTE.—Clones are classified as primary (P) and secondary (S). Secondary clones are derived from primary or secondary clones. Total number of cells subjected to chromosome analysis is indicated and presence of chromosomes 6 or 12 is tabulated. Presence or absence of MOD and Pep B activity is signified by + or — symbols. RK = code for KOP hybrids.

* From clones which are believed to give false negative test for MOD activity (see text).

† Subclones of WA IVa, all of which lack Pep B phenotype.

TABLE 2

CORRELATIONS BETWEEN MOD PHENOTYPE AND CHROMOSOME 6 AND PEP B PHENOTYPE AND CHROMOSOME 12 IN 43 HYBRID CLONES

ENZYME/CHROMOSOME	CLONAL TYPE				TOTAL
	+/+	−/−	+/−	−/+	
MOD/6	13	28	0	2*	43
Pep B/12	21	22	0	0	43

* Discrepant clones described in text.

all of the discrepant clones had significantly lower frequencies of C-6 chromosomes. This property of the discrepant clones most probably explains their apparent discordancy. The MOD enzyme activity is weak relative to other enzymes such as Pep B. If only a small subpopulation of cells possess the C-6 chromosome, then the MOD activity in the overall population may fall below the level of detection. Reconstruction experiments using LDH A have shown that dilution of the cell extracts to 10% of the standard concentration resulted in failure to detect the enzyme phenotype in all instances [16]. The MOD activity is considerably less than LDH A activity in cultured fibroblasts. After weighing all these points, it is reasonable to consider the discrepant clones as false negatives and to reassign them as clones which are chromosome 6–positive and MOD–positive. The data, therefore, provide very strong support for the assignment of the structural gene for MOD to chromosome 6 and the structural gene for Pep B to chromosome 12.

It is also possible to strengthen the proposed assignments by the demonstration of negative correlations between the MOD and Pep B phenotypes and chromosomes other than 6 and 12. It has been possible to demonstrate such negative correlations in the J series of clones where the chromosome data have been completely analyzed and compiled (tables 3 and 4). It can be seen that negative correlations exist between each of the phenotypes and all the chromosomes save one. The combination of negative and positive correlations strongly support the proposed chromosome assignments.

DISCUSSION

Previous reports have provided evidence that Pep B and LDH B are syntenic (linked to the same chromosome) [3, 17]. The human and mouse forms of LDH B possess identical electrophoretic mobilities and cannot be resolved qualitatively. In some hybrid combinations the human form of LDH B is expressed more strongly, and thus the human phenotype can be scored quantitatively with a fair degree of reliability. In the J series of hybrids the human parent is heterozygous for an electrophoretic variant for LDH B, and thus the human variant form can be distinguished reliably. In this study, the expected positive correlation between Pep B and LDH B was observed, providing additional support for the syntenic association.

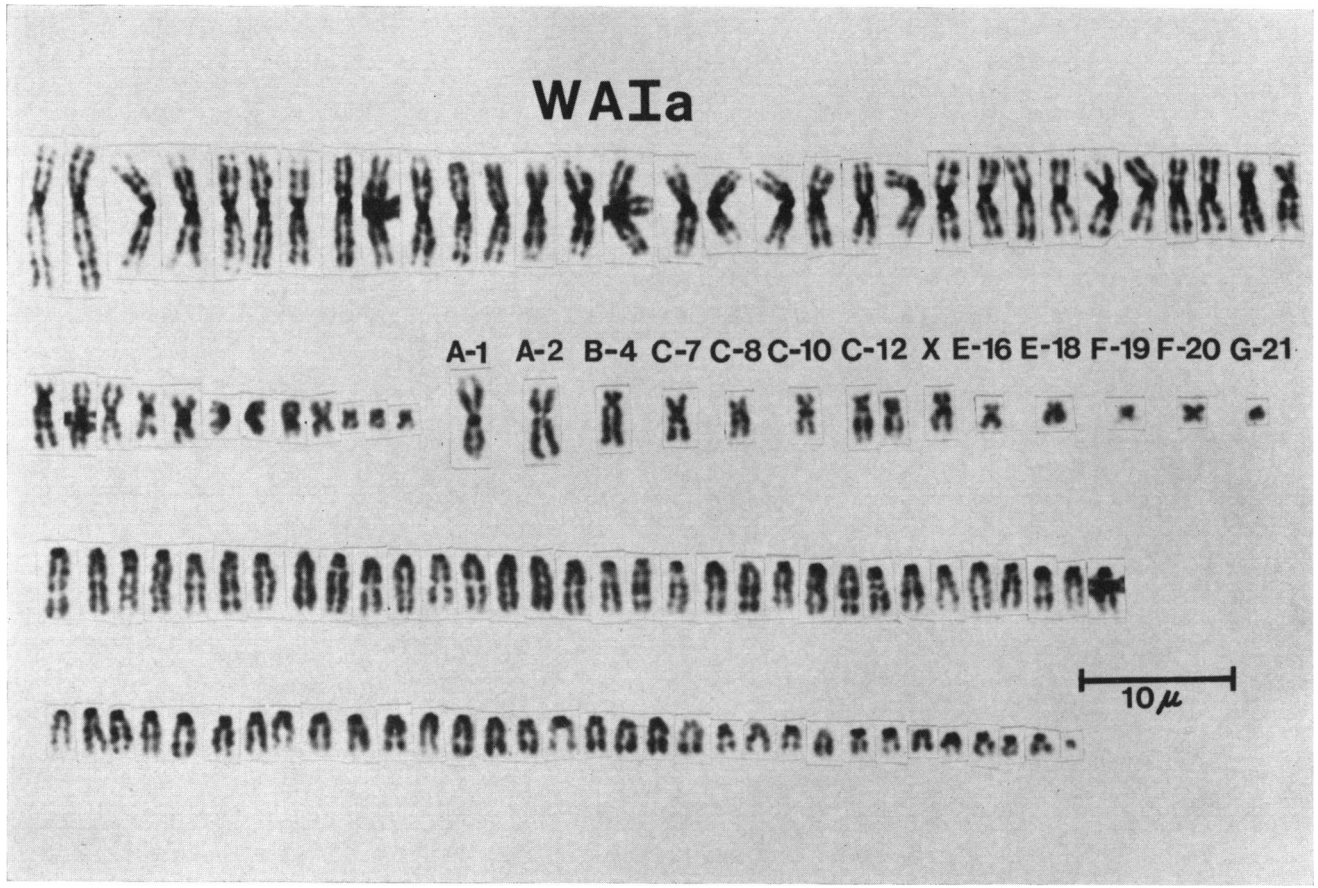

FIG. 1—Karyotype of a hybrid cell which is both LDH B- and Pep B- positive and which carries human chromosome 12. Human chromosomes are identified. Clone WA Ia results from the hybridization of mouse cell line A9 and a human diploid cell, WI-38.

TABLE 3

CORRELATIONS BETWEEN MOD PHENOTYPE AND ALL HUMAN CHROMOSOMES IN J CLONES

CHROMOSOME	CLONAL TYPE (MOD/CHROMOSOME)			
	+/+	−/−	+/−	−/+
1	7	2	0	4
2	0	6	7	0
3	7	4	0	2
4	0	4	7	2
5	0	6	7	0
6	7	5	0	(1)*
7	7	1	0	5
8	4	4	3	2
9	0	6	7	0
10	7	2	0	4
11	5	4	2	2
12	6	2	1	4
13	0	6	7	0
14	0	6	7	0
15	0	5	7	1
16	6	5	1	1
17	7	1	0	5
18	7	2	0	4
19	2	5	5	1
20	0	6	7	0
21	6	3	1	3
22	4	6	3	0
X	4	2	3	4
Y	0	0	0	0

* Single discrepant J clone; probably +/+ type (see text).

Recently, Puck and his associates (personal communications), using human–Chinese hamster hybrids, have reported a syntenic association between the human complement to the Chinese hamster glycine-A auxotrophic marker and human LDH B. Evidence exists which relates the glycine-A auxotroph to a deficiency in the enzyme serine hydroxymethylase [18], and thus it can be postulated that this enzyme is syntenic with Pep B and LDH B in man. The human–Chinese hamster hybrid system does not provide information on Pep B because the human and Chinese hamster enzymes are electrophoretically identical. The data support the assignment of the structural genes for peptidase B, lactate dehydrogenase B, and serine hydroxymethylase to chromosome 12. The map distances between these genes and their order along the chromosomes are currently unknown.

It is of some interest that LDH A is located on chromosome 11, and LDH B is on chromosome 12. Chromosomes 11 and 12 are similar in terms of their overall length, arm ratios, and quinacrine banding patterns. The LDH A and B subunits are similar in their molecular weights and amino-acid constitutions. This observation is therefore compatible with hypotheses [19] which advocate a polyploid event in the evolution of the primate karyotype, but does not of itself provide strong evidence in support of such hypotheses. It will be of interest to search for additional

TABLE 4

CORRELATIONS BETWEEN PEP B PHENOTYPE AND ALL HUMAN CHROMOSOMES IN J CLONES

CHROMOSOME	CLONAL TYPE (PEP B/CHROMOSOME)			
	+/+	−/−	+/−	−/+
1	10	1	1	2
2	0	3	11	0
3	7	1	4	2
4	3	3	8	0
5	0	3	11	0
6	7	2	4	1
7	11	1	0	2
8	7	3	4	0
9	0	3	11	0
10	10	1	1	2
11	5	1	6	2
12	11	3	0	0
13	0	3	11	0
14	0	3	11	0
15	0	2	11	1
16	6	2	5	1
17	9	0	2	3
18	9	1	2	2
19	3	3	8	0
20	0	3	11	0
21	8	2	3	1
22	4	3	7	0
X	6	1	5	2
Y	0	0	0	0

relationships of this type. If such can be found, then a comparison of the amino-acid compositions of the subunits coded by the duplicated genes may provide an estimate of occurrence during evolution of the theorized polyploid event. Moreover, it will be of interest to establish the linkage relationships of genes in representative species of the order Primates by means of somatic cell genetics. Such information should provide additional useful information on the evolution of the human genome.

SUMMARY

Evidence is presented for the assignment in man of the gene coding for malate oxidoreductase decarboxylating to chromosome 6 and the genes coding for peptidase B and lactate dehydrogenase B to chromosome 12. These findings are based on chromosome segregation in six different somatic cell hybrid combinations between human and mouse cell populations. Twenty-two primary and 21 secondary hybrid clones and 1,135 cells were examined.

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