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The PKU Locus in Man Is on Chromosome 12

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SUMMARY

Classical phenylketonuria (PKU) is a typical example of inborn errors in metabolism and is characterized by a complete lack of the hepatic enzyme phenylalanine hydroxylase, which normally converts phenylalanine to tyrosine. The genetic disorder causes impairment of postnatal brain development, resulting in severe mental retardation in untreated children. The disease is transmitted as an autosomal recessive trait and has a collective prevalence of about one in 10,000 among Caucasians, so that 2% of the population are carriers of the PKU trait. We have recently reported the cloning of human phenylalanine hydroxylase cDNA and that the human chromosomal phenylalanine hydroxylase gene is encoded by a unique DNA sequence. Using the human phenylalanine hydroxylase cDNA clone to analyze a clonal human/mouse hybrid cell panel by Southern hybridization, the phenylalanine hydroxylase gene has been assigned to human chromosome 12. Since the hypothesis that classical PKU is caused by structural mutations in the phenylalanine hydroxylase gene itself rather than through some transregulatory mechanisms has recently been confirmed by gene mapping, the PKU locus in man is determined to be on chromosome 12.

INTRODUCTION

The PKU locus in man has been studied by linkage analysis with other polymorphic gene loci in PKU families with inconclusive results [1–5]. The possible assignment

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of the PKU locus on chromosome 1 was suggested by moderate linkage data with the phosphoglucomutase locus PGM-1 (1p3) [3] and the amylase loci Amy-1 and Amy-2 (1p1) [4]. More recently, linkage studies between the PKU locus and 15 chromosome markers including PGM-1, Amy-1, and Amy-2, using improved methods for obligate heterozygote determination among siblings in the PKU families, have been performed. These experiments, however, failed to establish genetic linkage between the PKU locus and any of the markers and are in disagreement with the previous assignment [5].

Chromosomal assignments for genetic loci can be made using cloned genes as probes in molecular hybridization to genomic DNAs isolated from human/rodent cell hybrids that contain different assortments of human chromosomes. We have recently reported the cloning of human phenylalanine hydroxylase cDNA and its use in studying the chromosomal phenylalanine hydroxylase gene in PKU families [6]. The cDNA clone (phPH72) contains 1.2 kilobase (kb) of human phenylalanine hydroxylase cDNA inserted into the *Pst* I site of pBR322 and was used to detect the presence of the human phenylalanine hydroxylase gene in a clonal human/mouse hybrid cell panel by molecular hybridization. Comparison of these data with the presence of various human chromosomes in each of the hybrid cell lines has shown that the human phenylalanine hydroxylase gene is on chromosome 12.

MATERIALS AND METHODS

Hybrid Cell Lines and DNA Isolation

Hybrid parental cell lines were made by fusing mouse A9 cells with human GM144 cells (AHA), human GM589 cells (BDA), and human WI-38 cells (WA); or between mouse L-cells and human GM126 cells (41pT2A and FRY1). Subclones of the original hybrid cell lines were isolated by dilution plating. Mouse-human hybrid cells were cultivated without antibiotics in α-minimal essential medium, 10% heat-inactivated fetal bovine serum, in roller bottles. Cytogenetic and isozyme samples were taken concurrently with DNA isolation. Monolayers were twice washed with saline and once with TNE (50 mM Tris-HCl, pH 8, 10 mM NaCl, 10 mM EDTA) at 4°C. TNE supplemented with 0.5% w/v SDS (BRL) and proteinase K (100 μ/ml) was added followed by 37°C overnight incubation on a roller mill. DNA was extracted twice with redistilled phenol, twice with chloroform-isoamyl alcohol (24:1), concentrated in dialysis bags against PEG 8000 (Sigma, St. Louis, Mo.), and exhaustively dialyzed against TE (10 mM Tris-HCl, pH 8, 1 mM EDTA) at 4°C.

Karyotype and Isozyme Analysis of Hybrid Cell Line

Genetic analysis of cell hybrids included G-banding of 25–50 metaphases and isozyme analysis. The isozyme constitution of each hybrid cell line was determined by starch gel or cellulose acetate electrophoresis and histochemical staining for the following loci: chromosome 1 (enolase-1, E.C.4.2.1.11; phosphoglucomutase-1, E.C.2.7.5.1; and peptidase-C, E.C.3.4.11*); chromosome 2 (malate dehydrogenase-1, E.C.1.1.137; and isocitrate dehydrogenase-1, E.C.1.1.1.42); chromosome 3 (acylase-1, E.C.3.5.1.14); chromosome 4 (peptidase-S, E.C.3.4.11*; and phosphoglucomutase-2, E.C.2.7.5.1); chromosome 5 (hexosaminidase B, E.C.3.2.1.30); chromosome 6 (glyoxylase-1, E.C.4.4.1.5; and malic enzyme-1, E.C.1.1.1.40); chromosome 7 (β-glucuronidase, E.C.3.2.1.31; and uridine phosphorylase, E.C.2.4.2.3); chromosome 8 (glutathione reductase, E.C.1.6.4.2); chromosome 9 (adenylate kinase-1, E.C.2.7.4.3; and aconitase-

S, E.C.4.2.1.3); chromosome 10 (adenosine kinase-1, E.C.2.7.1.20; and glutamate oxaloacetate transaminase, E.C.2.6.1.1); chromosome 11 (lactate dehydrogenase-A, E.C.1.1.1.27); chromosome 12 (triose phosphate isomerase, E.C.5.3.1.1; peptidase-B, E.C.3.4.11*; and lactate dehydrogenase-B, E.C.1.1.1.27); chromosome 13 (esterase-10, E.C.3.1.1.1); chromosome 14 (nucleoside phosphorylase, E.C.2.4.2.1); chromosome 15 (mannose phosphate isomerase, E.C.5.3.1.8; pyruvate kinase-3, E.C.2.7.1.40; and hexosaminidase-A, E.C.3.2.1.30); chromosome 16 (adenosine phosphoribosyl transferase, E.C.2.4.2.7); chromosome 17 (galactokinase, E.C.2.7.1.6); chromosome 18 (peptidase-A, E.C.3.4.1*); chromosome 19 (glucose phosphate isomerase, E.C.5.3.1.9); chromosome 20 (adenosine deaminase, E.C.3.5.4.4); chromosome 21 (superoxide dismutase-1, E.C.1.15.1.1); chromosome 22 (aconitase-M, E.C.3.1.6.1); and the X chromosome (phosphoglycerate kinase, E.C.2.7.2.3; hypoxanthine-guanine phosphoribosyl transferase, E.C.2.4.2.8; and glucose-6-phosphate dehydrogenase, E.C.1.1.1.49).

Southern Blotting Analysis

About 10 μg of DNA were digested to completion with *Bam* HI at 2 U/μg DNA followed by electrophoresis in 1% agarose gels. DNA was transferred to a nitrocellulose filter according to the method of Southern [7] and allowed to hybridize with the inserted *Pst* I fragment of phPH72 DNA after labeling with ^{32}P by nick-translation [8]. Conditions of hybridization and washing were as those reported by Wahl et al. [9]. Hybridization bands were detected by autoradiography using Fuji Medical X-ray films with intensifying screens at −70° for up to 3 days.

RESULTS

Digestion of human genomic DNA with *Bam* HI followed by Southern hybridization analysis revealed an intense hybridization band at 20.5 kb (fig. 1, lanes 1–3). Analysis of total mouse DNA under the same conditions yields an intense band at 10.4 kb and a weaker signal at 5.7 kb (fig. 1, lane 4), suggesting a rather

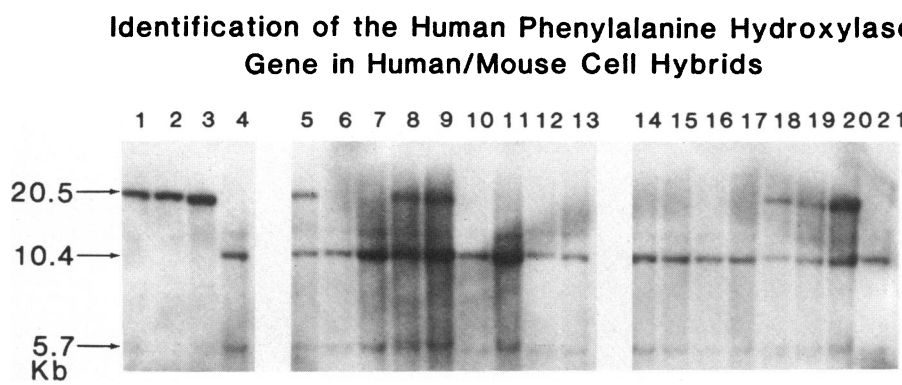

FIG. 1.—Southern hybridization analysis of *Bam* HI-digested DNA from a series of human/mouse cell hybrids. Using as a probe the nick-translated phenylalanine hydroxylase cDNA clone phPH72: *lane 1*, human parental line GM144; *lane 2*, human parental line GM589; *lane 3*, human parental line GM126; *lane 4*, mouse parental line A9; *lane 5*, hybrid BDA-10a3; *lane 6*, hybrid BDA-14b25; *lane 7*, hybrid BDA-14b25-2; *lane 8*, hybrid AHA-16e; *lane 9*, hybrid AHA-3d2; *lane 10*, hybrid WAV R4dA19; *lane 11*, hybrid AHA 11a; *lane 12*, hybrid Fry-1; *lane 13*, hybrid 41pT2A; *lane 14*, hybrid BDA-17b17; *lane 15*, hybrid BDA-17b17-1; *lane 16*, hybrid BDA-17b17-3; *lane 17*, hybrid AHA-3d2-1; *lane 18*, hybrid AHA-3d2-3; *lane 19*, AHA 16e-1; *lane 20*, hybrid AHA-16e-3; *lane 21*, hybrid BDA-14b25-3.

TABLE 1

ANALYSIS OF THE HUMAN PHENYLALANINE HYDROXYLASE GENE IN MOUSE/HUMAN HYBRID CELLS

MOUSE/HUMAN CELL HYBRIDS	HUMAN CHROMOSOMES																							PHENYLALANINE HYDROXYLASE GENE
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
BDA-10a3	−	+	+	−	−	+	−	+	−	+	+	+	0	+	+	+	+	−	+	+	+	+	+	+
BDA-10a3-1	−	+	+	−	−	+	−	+	−	+	−	+	−	−	−	−	−	−	−	+	−	+	+	+
BDA-10a3-4	−	+	+	+*	−	+	−	0	−	+	−	+	0	−	−	0	0	−	−	+	−	0	+	+
BDA-10a3-6	−	+	+	+*	−	+	−	0	−	+	−	+	0	−	−	0	0	−	−	+	−	0	+	+
BDA-14b25	+	−	+	+	−	−	0	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−
BDA-14b25-2	+	−	+	+	−	−	−	0	−	−	−	−	0	−	−	−	0	−	−	−	−	0	+	−
WAV R4dA19	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−
AHA-16e	−	+	+	−	0	−	0	0	−	+	+	+	+	+	−	−	0	+	+	+	+	0	+	+
AHA-16e-1	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	+	+	+	−	+	+
AHA-3d2	−	−	+	+	0	−	0	0	−	+	+	+	−	−	−	−	−	+	+	−	−	0	+	+
AHA-11a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Fry-1	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Concordance frequency (%)	23	77	69	36	45	69	44	88	46	92	69	100	64	62	46	54	56	54	69	85	53	62	54	...
AHA-3d2-1	−	−	0	−	0	−	−	−	−	−	−	−	−	−	−	0	0	+	+	−	−	0	−	−
AHA-3d2-3	−	−	+	+	0	−	−	−	−	−	+	+	−	−	−	−	−	+	+	−	−	−	−	+
AHA-16e-3	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	+	−	+	−	−	+
BDA 17b17-3	−	−	−	+	0	−	−	+	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−
BDA 14b25-3	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−
Concordance frequency (%)	...	...	...	...	...	...	...	40	...	60	...	100	...	...	...	...	...	...	...	60	...	...	...	...

NOTE: + = karyotype > 20% of cells with chromosome or isozyme expression or both. − = karyotype < 5% of cells with chromosome or no isozyme expression or both. +* = karyotype > 5%, but < 20% of cells with chromosome; inconsistent or negative isozyme expression. 0 = data insufficient to score clear positive or negative. Concordance frequency = no. of concordant hybrids/total no. of hybrids × 100 (+* and 0 phenotypes not included in calculation). Phenylalanine hydroxylase gene: +/− = presence/absence of 20.5-kb band in Southern blot analysis.

high level of sequence homology between the phenylalanine hydroxylase genes in the two species. Since the phenylalanine hydroxylase gene is a unique DNA sequence in the human genome with no apparent pseudogenes [6], the presence of the human phenylalanine hydroxylase gene in human/mouse hybrid cells can be detected because of the distinct fragment sizes of the human and mouse genes.

A clonal panel of 12 human/mouse hybrid cells was used to localize the phenylalanine hydroxylase gene on a particular human chromosome. High molecular weight DNA was isolated from each hybrid cell line, digested with *Bam* HI, and subjected to Southern hybridization analysis using ^{32}P-labeled phPH72 DNA. Every hybrid cell contained the 10.4-kb and 5.7-kb mouse DNA bands, and the presence of the 20.5-kb human DNA fragments was found in some of the hybrid lines (fig. 1, lanes 5–13). A comparison of these data with the human chromosome contents in each of the hybrid lines as determined by karyotype and isozyme data has demonstrated complete segregation concordance between the phenylalanine hydroxylase gene and the presence of chromosome 12 (table 1, upper panel). The only other human chromosomes with relatively high segregation concordance with the phenylalanine hydroxylase gene were 8 (88%), 10 (92%), and 20 (85%).

To further substantiate the assignment of the phenylalanine hydroxylase gene to chromosome 12, an additional panel of five hybrids was analyzed. The lower panel of table 1 shows the isozyme phenotype and karyotype data for each of these hybrids. DNA samples from two hybrid lines (AHA 3d2-3 and AHA 16e-3) containing chromosome 12 showed the presence of the 20.5-kb human DNA fragment after digestion with *Bam* HI and Southern blot analysis. Complete segregation concordance is again observed only with chromosome 12, and the potential association with the other human chromosomes can be ruled out on the basis of their reduced levels of segregation concordance with the phenylalanine hydroxylase gene. These results confirmed the previous assignment of the phenylalanine hydroxylase gene to chromosome 12.

The hybrids 41pT2A, BDA 17b17, and BDA 17b17-1 contain the cellular oncogene Ki-ras DNA sequence that has been assigned to chromosome 12 [10], and are each positive for the TPI-1 isozyme marker that is located at 12p13 (data not shown). However, they are negative for the PEP-B marker that is located at 12q21, indicating the presence of only a part of chromosome 12 in these cells as a result of translocation. All three hybrids gave no hybridization signal corresponding to the human phenylalanine hydroxylase gene (table 2). These results

TABLE 2

ANALYSIS OF HUMAN/MOUSE HYBRIDS CONTAINING TRANSLOCATED CHROMOSOME 12 AS DETERMINED BY KARYOTYPE AND ISOZYME ANALYSIS

Human/Mouse cell hybrid	Chromosome 12 karyotype	PEP-B (12q21)	TPI (12p13)	Phenylalanine hydroxylase gene
BDA-17b17	–	–	+	–
BDA-17b17-1	–	–	+	–
41pT2A	–	–	+	–

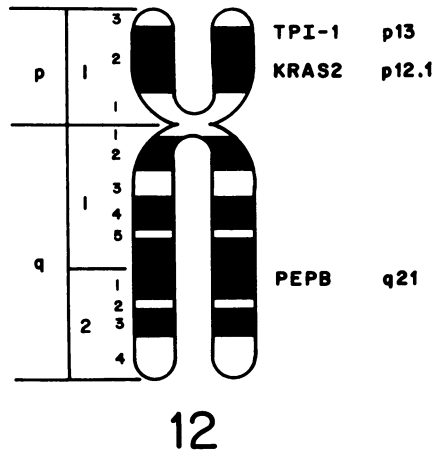

FIG. 2.—Idiogram of human chromosome 12 shows the relationship between the three loci (KRAS2, TPI-1, and PEP-B) screened in characterization of the hybrids BDA-17b17, BDA-17b17-1, and 41pT2A. The mapping results indicate that the phenylalanine hydroxylase locus may be located on the long arm of human chromosome 12.

suggest that the phenylalanine hydroxylase gene is located on the long arm of chromosome 12 (fig. 2). This possibility should be tested directly by subchromosomal localization of the phenylalanine hydroxylase gene by in situ hybridization.

DISCUSSION

The use of recombinant DNA probes in conjunction with human/rodent cell hybrid panels to assign specific genes in the human genome is a powerful tool in the construction of the human gene map. Here we have added the assignment of the gene coding for phenylalanine hydroxylase to human chromosome 12 using a previously cloned human cDNA probe in Southern analysis of genomic DNA isolated from a human/mouse clone panel. The human phenylalanine hydroxylase cDNA clone has also been used as a hybridization probe to detect the existence of restriction fragment length polymorphisms in the phenylalanine hydroxylase locus [6]. These restriction enzymes have subsequently been used to analyze families with one or more PKU children plus unaffected siblings. In all families studied, complete segregation concordance between the mutant phenylalanine hydroxylase gene and the disease phenotype has been observed. Furthermore, the allelic segregation between the probands and the unaffected siblings was totally discordant. These results strongly suggest that PKU is the result of mutational events in the phenylalanine hydroxylase gene itself and is not caused by some other transregulatory mechanisms [6]. Since the phenylalanine hydroxylase gene has been assigned to human chromosome 12, the PKU locus in man is also on chromosome 12. Finally, the existence of multiple restriction fragment length polymorphisms in the phenylalanine hydroxylase locus would also mean that the human cDNA clone can serve as a polymorphic marker for chromosome 12,

which will be very useful in linkage analysis between the PKU and other human gene loci.

REFERENCES

1. PENROSE LS: Data for the study of linkage in man: phenylketonuria and the ABO and MN loci. *Ann Eugen (Lond)* 16:241–248, 1951
2. HSIA DYY, STEINBERG AG: Studies on linkage between phenylketonuria and the blood groups. *Am J Hum Genet* 12:277–286, 1960
3. BERG K, SAUGSTAD LF: A linkage study of phenylketonuria. *Clin Genet* 6:147–152, 1974
4. KAMARYT J, MRSKOS A, PODHRADSKA O, ET AL.: PKU locus: genetic linkage with human amylase (Amy) loci and assignment to linkage group 1. *Hum Genet* 43:205–210, 1978
5. PAUL TD, BRANDT IK, ELSAS LJ, JACKSON CE, NANCE CS, NANCE WE: Linkage analysis using heterozygote detection in phenylketonuria. *Clin Genet* 16:217–232, 1979
6. WOO SLC, LIDSKY AS, GUTTLER F, CHANDRA T, ROBSON KJH: Cloned human phenylalanine hydroxylase gene permits prenatal diagnosis and carrier detection of classical phenylketonuria. *Nature* 306:151–155, 1983
7. SOUTHERN EM: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98:503–517, 1975
8. MANIATIS T, JEFFREY A, KLEID DG: Nucleotide sequence of the rightward operator of phage λ. *Proc Natl Acad Sci USA* 72:1184–1188, 1975
9. WAHL GM, STERN M, STARK GR: Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethol-paper and rapid hybridization by using dextran sulfate. *Proc Natl Acad Sci USA* 76:3683–3687, 1979
10. RYAN J, BARKER PE, SHIMIZV K, WIGLER M, RUDDLE FH: Chromosomal assignment of a family of human oncogenes. *Proc Natl Acad Sci USA* 80:4460–4463, 1983