SHORT COMMUNICATION

Human *PCK1* Encoding Phosphoenolpyruvate Carboxykinase Is Located on Chromosome 20q13.2

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Cytoplasmic liver phosphoenolpyruvate carboxykinase (GTP) (PEPCK) catalyzes a rate-limiting step in gluconeogenesis. Primers derived from the rat liver PEPCK sequence were used to amplify a portion of the human liver cDNA and to screen a YAC library of human genomic DNA. The sequences of human and rat PEPCK cDNA differed at 16% of the nucleotides compared (45/291). Analysis of a human/rodent hybrid mapping panel demonstrated concordant segregation of *PCK1* with chromosome 20. Fluorescence *in situ* hybridization with YAC DNA further localized *PCK1* to subband 20q13.2.

A major target for regulation of gluconeogenesis is the enzyme phosphoenolpyruvate carboxykinase (PEPCK; EC 4.1.1.32). Transcription of PEPCK is regulated by insulin, glucocorticoids, cAMP, and diet, in order to adjust glucose production to physiological requirements (11, 13). Elevated gluconeogenesis in the liver may be a contributing factor in diabetic hyperglycemia (5). The mouse *Pck-1* locus encoding PEPCK is located on Chr 2 at a position 7 ± 3 cM from *Ada* (14). A human gene responsible for maturity-onset diabetes of the young (MODY) has recently been localized to the 20-cM region distal to the *ADA* locus on chromosome 20 (2, 3). If the murine linkage were conserved, human *PCK1* would be located close to the MODY locus. In view of the relationship between PEPCK function and glucose homeostasis, the present study was undertaken to determine the chromosomal location of the human PEPCK gene.

To generate a hybridization probe, a 400-bp fragment of human PEPCK cDNA corresponding to exons 4 and 5 of the rat gene (1) was amplified from 1 ng of human liver cDNA (Clontech, No. 7121-1) using a coding strand primer from exon 4 (primer 1: CGC ACC ATG TAT GTC ATC CC) and a noncoding primer from exon 5 (primer 2: ATG CCC AGG ATC AGC ATG TG). The amplified fragment was electroeluted and both strands were sequenced. Comparison with the rat cDNA revealed 85% identity of nucleotide sequence in the region compared (246/291), with 6/97 amino acid substitutions. The similarity between the human and the rat sequences demonstrates that the amplified product is an authentic PEPCK cDNA.

Genomic DNA from NIGMS mapping panel 1 was digested with *Bgl*II and analyzed by Southern blotting with the human cDNA fragment described above. The probe hybridized with a 6.7-kb *Bgl*II fragment from human DNA, which migrated more slowly than the hybridizing fragments from hamster (4.5 kb) and mouse (6.0 kb). Comparison of the segregation of human *PCK1* with the chromosome content of the hybrid cells demonstrated at least one discordance with every chromosome except chromosome 20 (Table 1).

To localize the gene further by *in situ* hybridization, YAC clones were isolated by screening the total human genomic YAC library constructed at the Center for Genetics for Medicine, Washington University (4). The screening procedure, including the identification of the final colony, was carried out by a PCR-based method (7, 9) using primer 1 and a noncoding primer from the 3′ end of human exon 4 (primer 3: GAG GCA TTT GAT AAA CTC CC). Two YAC clones, PCKA (A111D5) and PCKB (B160B6), were obtained by this procedure. Pulsed-field gel electrophoresis and Southern blotting with a vector probe indicated that PCKA is 220 kb in length and PCKB is 170 kb in length. To confirm the identity of the clones, DNA was amplified with primers 1 and 2 and with a second set of primers amplifying exon 5. The products obtained from the YAC clones were identical in length to those obtained from human genomic DNA, indicating that both clones contain the *PCK1* gene. The *Bgl*II and *Hind*III fragments observed on Southern blots of YAC DNA were also identical to those of human genomic DNA.

Normal human chromosome spreads were G-banded and photographed, and the slides were hybridized with biotinylated DNA from YAC clones PCKA and PCKB. Thirty mitoses were examined with each probe, and all

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TABLE 1

Mapping of Human *PCK1* in NIGMS Human/Rodent Somatic Cell Hybrid Mapping Panel 1

		Human chromosome																						
Hybrid clone	*PCK1*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
GM/NA09925	+	+	+	−	+	+	+	+	+	−	−	/	+	/	+	+	+	+	+	+	+	−	+	−	−
GM/NA09926	+	+	+	+	+	/	+	+	+	−	+	−	/	+	+	+	/	+	+	+	+	/	/	/	−
GM/NA09927	+	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−	+	+	+	+	−	−	−	−
GM/NA09929	+	−	−	+	+	−	+	/	+	−	−	+	+	−	+	/	−	+	−	/	+	−	−	/	−
GM/NA09930A	+	−	+	+	/	+	−	+	/	−	−	/	+	+	+	+	−	+	+	−	+	+	+	/	/
GM/NA09931	+	−	−	−	−	+	−	+	−	−	+	−	+	−	+	−	−	+	−	−	+	+	−	−	+
GM/NA09933	+	+	−	+	+	+	+	+	+	−	/	−	+	+	+	+	−	+	+	+	+	+	+	−	+
GM/NA09934	+	−	+	−	−	+	+	/	+	−	−	+	+	−	/	+	−	+	+	−	+	+	/	−	−
GM/NA09938	+	−	−	/	+	+	+	+	/	−	−	+	+	−	+	/	−	+	−	/	+	+	+	−	/
GM/NA09928	−	−	+	+	−	+	+	−	+	−	/	−	−	/	+	+	−	+	−	+	−	+	+	−	+
GM/NA09932	−	−	−	−	+	+	+	−	+	−	/	+	+	−	−	−	−	+	−	/	−	+	−	−	−
GM/NA09937	−	−	−	+	+	−	+	+	+	−	/	−	+	−	+	+	−	+	+	−	−	−	−	−	−
No. of discordant hybrids		5	5	5	4	4	5	1	4	9	5	5	3	4	2	3	7	3	4	4	0	5	4	6	6

Note. Cell lines containing the 6.7-kb *Bgl* II fragment are designated + for *PCK1*. The human chromosomes are designated + if present in >12% of the >25 metaphases analyzed at first passage and at final harvest, − if absent, and / if present in 2–8% of cells (data not included in calculation of discordance).

of the cells showed specific hybridization signals. Twenty-eight of the mitoses probed with PCKA and 25 of those probed with PCKB contained double signals on both homologous pairs of chromosome 20, with four specific signals per mitosis. Representative examples of signals on chromosome 20 are shown in Fig. 1. Comparison of the G-banded chromosomes and the FISH localized PCKA and PCKB to 20q13.2. No other hybridization signals were observed, suggesting that neither clone is chimeric.

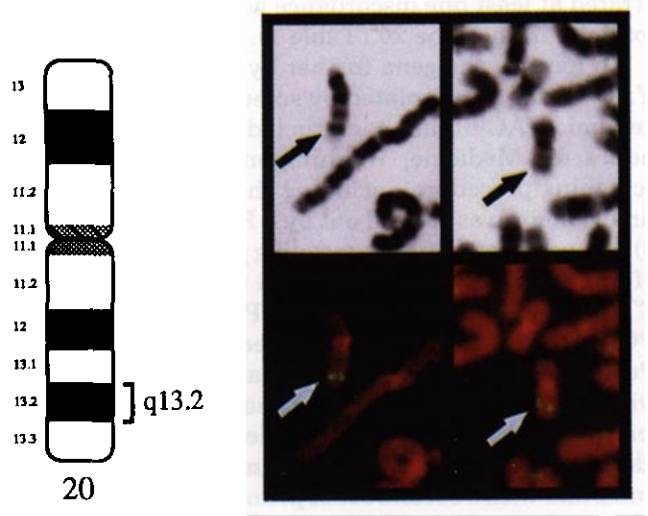

FIG. 1. Localization of *PCK1* to 20q13.2 by FISH. DNA from YAC PCKA was biotinylated using the BRL nick-translation kit and denatured in hybridization solution containing 50% formamide and 2× SSC. The labeled YAC was prehybridized to repetitive sequence enriched total human DNA (Cot-1 DNA, BRL). Following hybridization of the probe to denatured chromosomal DNA of normal human chromosome spreads, the probe was detected by repeated cycles of FITC-conjugated avidin and anti-avidin antibody as previously described (12). Chromosomes were counterstained with propidium iodide and signals were visualized under a fluorescence microscope. **(Left)** Idiogram of chromosome 20 indicating the localization of *PCK1*. **(Right)** Examples of FISH with the biotinylated PCK1 probe. The top panels represent two partial metaphases illustrating G-banded chromosome 20 (black arrows). The bottom panels document the same cells after FISH, demonstrating double fluorescent signals over the distal long arm of chromosome 20 (white arrows).

The predictive value of the mouse genetic map for locating human genes is increasing with the number of genes mapped in both species. In the mouse, *Pck-1* is located 7 ± 3 cM from *Ada* (14). Our results demonstrate that human *PCK1* is part of a conserved linkage group on human chromosome 20 that includes *ADA*. Other loci in this conserved linkage group are *HCK1, SRC, RPN2,* and *GNAS1* (6, 8). Human *ADA* has been mapped by *in situ* hybridization to 20q12–q13.1 (8), proximal to our localization of *PCK1* at 20q13.2. The data indicate that gene order is also conserved within this linkage group in the two species.

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REFERENCES

1. Beale, E. G., Chrapkiewicz, N. B., Scoble, H. A., Metz, R. J., Quick, D. P., Noble, R. L., Donelson, J. E., Biemann, K., and Granner, D. K. (1985). Rat hepatic cytosolic phosphoenolpyruvate carboxykinase (GTP): Structures of the protein, mRNA and gene. *J. Biol. Chem.* **260:** 10748–10760.
2. Bell, G. I., Xiang, K-S., Newman, M. V., Wu, S-H., Wright, L. G., Fajans, S. S., Spielman, R. S., and Cox, N. J. (1991). Gene for non-insulin-dependent diabetes mellitus (maturity-onset diabetes of the young subtype) is linked to DNA polymorphism

on human chromosome 20q. *Proc. Natl. Acad. Sci. USA* **88:** 1484–1488.

3. Bowden, D. W., Akots, G., Rothschild, C. B., Falls, K. F., Sheehy, M. J., Hayward, C., Mackie, A., Baird, J., Brock, D., Antonarakis, S. E., and Fajans, S. S. (1992). Linkage analysis of maturity-onset diabetes of the young (MODY): Genetic heterogeneity and nonpenetrance. *Am. J. Hum. Genet.* **50:** 607–618.
4. Brownstein, B. H., Silverman, G. A., Little, R. D., Burke, D. T., Korsmeyer, S. J., Schlessigner, D., and Olson, M. V. (1989). Isolation of single-copy human genes from a library of yeast artificial chromosome clones. *Science* **244:** 1348–1351.
5. Consoli, A., Nurjhan, N., Reilly, J. J., Bier, D. M., and Gerich, J. E. (1990). Mechanism of increased gluconeogenesis in non-insulin-dependent diabetes mellitus. *J. Clin. Invest.* **86:** 2038–2045.
6. GBASE: The genomic database for the mouse maintained at The Jackson Laboratory by D. P. Doolittle, A. L. Hillyard, L. J. Maltais, J. N. Guidi, M. T. Davisson, and T. H. Roderick (1992).
7. Green, E. D., and Olson, M. V. (1990). Systematic screening of yeast artificial-chromosome libraries by use of the polymerase chain reaction. *Proc. Natl. Acad. Sci. USA* **87:** 1213–1217.
8. Grzeschik, K. H., and Skolnick, M. H. (1991). Report of the committee on the genetic constitution of chromosome 20. *Cytogenet. Cell Genet.* **58:** 785–799.
9. Kwiatkowski, T. J., Zoghbi, H. Y., Ledbetter, S. A., Ellison, K. A., and Chinault, A. C. (1990). Rapid identification of yeast artificial chromosome clones by matrix pooling and crude lysate PCR. *Nucleic Acids Res.* **18:** 7191–7192.
10. Lugo, T. G., Handelin, B., Killary, A. M., Housman, D. E., and Fournier, R. E. (1987). Isolation of microcell hybrid clones containing retroviral vector insertions into specific human chromosomes. *Mol. Cell. Biol.* **7:** 2814–2820.
11. McGrane, M. M., de Vente, J., Yun, J., Bloom, J., Park, E., Wynshaw-Boris, A., Wagner, T., Rottman, F. M., and Hanson R. W. (1988). Tissue-specific expression and dietary regulation of a chimeric phosphoenolpyruvate carboxykinase/bovine growth hormone gene in transgenic mice. *J. Biol. Chem.* **263:** 11443–11451.
12. Meltzer, P. S., Guan, X. Y., Burgess, A., and Trent, J. M. (1992). Micro-FISH: A novel strategy to identify cryptic chromosomal rearrangements. *Nature Genet.* **1:** 24–28.
13. O'Brien, R. M., and Granner, D. K. (1991). Regulation of gene expression by insulin. *Biochem. J.* **278:** 609–619.
14. Siracusa, L. D., Alvord, W. G., Bickmore, W. A., Copeland, N. G., and Jenkins, N. A. (1991). Interspecific backcross mice show sex-specific differences in allelic inheritance. *Genetics* **128:** 813–821.