Title

Human dopa decarboxylase: Localization to human chromosome 7p11 and characterization of hepatic cDNAs

Journal

Genomics, 13(2)

ISSN

0888-7543

Authors

Scherer, Lisa J
McPherson, John D
Wasmuth, John J
et al.

Publication Date

1992-06-01

DOI

10.1016/0888-7543(92)90275-w

SHORT COMMUNICATION

Human Dopa Decarboxylase: Localization to Human Chromosome 7p11 and Characterization of Hepatic cDNAs

LISA J. SCHERER,[1] JOHN D. MCPHERSON,* JOHN J. WASMUTH,* AND J. LAWRENCE MARSH[2]

*Developmental Biology Center and Department of Developmental and Cell Biology, *Department of Biological Chemistry, University of California, Irvine, California 92717*

Received September 30, 1991; revised December 20, 1991

We have cloned full-length DDC cDNAs from a human hepatoma cDNA library [DDC; dopa decarboxylase; aromatic-L-amino acid decarboxylase, EC 4.1.1.28]. The protein encoded by hepatoma cells is the same as that encoded by adrenal chromaffin derived pheochromocytoma cells, despite reported differences in biochemical properties. We have confirmed the location of the *DDC* gene to chromosome 7 using a new panel of somatic cell hybrids, and we have localized the gene to band p11 on chromosome 7 by fluorescent *in situ* hybridization. The human gene retains 65% amino acid identity with Drosophila DDC (Accession No. X04426) and considerable structural similarity with other enzymes (F. R. Jackson, 1990, *J. Mol. Evol.* 31: 325–329, and references therein).

Dopa decarboxylase is required for the synthesis of both catecholamines and indolalkylamines. DDC is found in the central and peripheral nervous systems including neural crest-derived pheochromocytoma cells, as well as in peripheral tissues that do not produce these neurotransmitters. Differences in biochemical properties have raised the question of whether the same enzyme is present in both neuronal and nonneuronal tissues (e.g., (2)).

Two degenerate oligonucleotide primers (5′-AA(T/C)TT(T/C)AA(T/C)CCNCA(C/T)AA(A/G)TTG-3′ and 5′-C(G/T)(A/G)A(A/G)NAC(A/G)AACCACAT-(C/T)TT-3′) were used to amplify *DDC* DNA from a λgt11 HepG2 (Hepatoma) cDNA library (5). A single 200-bp fragment was amplified, cloned, and sequenced, revealing strong homology to Drosophila DDC (4). The 200-bp amplification product was used to screen the HepG2 library, and seven near full-length clones had inserts of ~1.8 kb. Two of the longest cDNAs (cHsDDC1 and 10) were sequenced, revealing a protein identical to that obtained from human pheochromocytoma cells (6). Thus, the same coding region is expressed in liver and adrenal cells. Some differences were noted in the 5′ and 3′ untranslated regions, and these are incorporated in the GenBank file (HumDDC, Accession No. M88700).

Genomic DNA clones were isolated from a λ phage library (described in Ref. (10)) by screening with the cDNA. A 4.1-kb *Hin*dIII fragment from genomic clone g5 was identified as repeat-free by probing a Southern blot of genomic DNA. This fragment was used to isolate a clone (cosDDC1) from a human placental cosmid library (Stratagene).

Chromosomal assignment of the *DDC* gene was carried out by probing a panel of 24 Chinese hamster/human cell hybrids (described in fig. 1) with the 4.1-kb fragment. Hybridization occurs only in lanes containing total human DNA (HeLa) or the hybrid cell line, HHW1126, which is the only hybrid containing an intact human chromosome 7. No hybridization is detected in DNA from HHW1107, which has a derivative chromosome 5 t(5;7)(q35.2;q22)] containing 7q22–7qter. These results confirm those of Bruneau *et al.* (1), localizing the *DDC* gene to chromosome 7, and extend the localization of *DDC* to 7pter–7q22. Further confirmation of the chromosome 7 assignment was accomplished by analyzing four other cell hybrids (Fig. 1B) that contain: chromosome 5 exclusively (HHW105); chromosome 5 plus 7 (HHW1126); chromosomes 6 plus 12 (HHW484); or chromosome 7 plus 12 (HHW151). Only the two hybrids with a chromosome 7 show hybridization signals (Fig. 1B).

The *DDC* gene was further localized by fluorescent *in situ* hybridization. Biotinylated cosDDC1 DNA was hybridized to metaphase chromosomes prepared from lymphoblastoid cultures. Hybridization signals were observed only near the centromere on the short arm of chromosome 7 (Fig. 2). These results confirm the assignment of DDC to chromosome 7 and localize it to 7p11.

In Drosophila, naturally occurring allelic variation in enzyme activity is very common (e.g., (8)) and allelic differences in DDC activity of greater than twofold are seen even among common laboratory strains (9). The possibility of scoring allelic states at loci involved in synthesis and reception of catecholamine neurotransmitters may help in assessing the genetic contributions to complex genetic traits such as hypertension and other disorders. We are continuing to develop informative polymorphisms within or near the *DDC* gene, which will be useful for linkage studies.

[1] Present address: Department of Biology, California Institute of Technology, Pasadena, CA.

[2] To whom correspondence should be addressed.

GENOMICS 13, 469–471 (1992)
0888-7543/92 $5.00

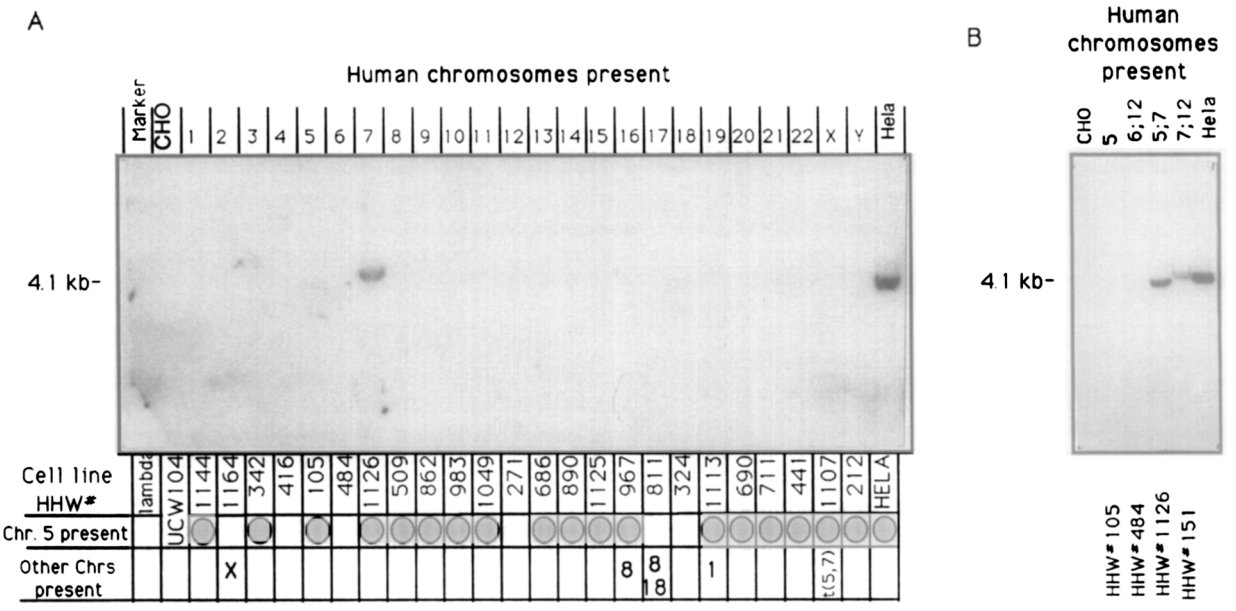

FIG. 1. (**A**) Southern blot analysis of DNA from a human chromosome mapping panel. A unique 4.1-kb DNA fragment was hybridized to a blot of human/hamster hybrid cell line DNA. The mapping panel was made up of hybrid cell lines, each of which contain a derivative chromosome 5 and also stably maintain 1 or in a few cases 2 additional human chromosomes. The parental CHO cell line used to construct most of the hybrids was UCW56, which harbors a LARS gene mutation encoding a temperature-sensitive leucyl-tRNA synthetase (3). The temperature-sensitive phenotype is complimented by the human LARS gene product, thus allowing selection at 39°C of hybrids retaining human chromosome 5. Four additional hybrids that used other modes of selection specific for the retention of chromosome X, 12, or 18 were included. Cell line HHW1164 is a subclone of UV24HL5, provided by Dr. Larry Thompson of the Lawrence Livermore National Laboratory. Each hybrid was characterized by trypsin-Giemsa banding and G-11 staining. Together, the 24 hybrids allow for the assignment of probes to any one of the 24 distinct human chromosomes. Cell lines were chosen so that each line contains 1 relevant human chromosome, which is listed above the lanes. At the bottom, the HHW numerical designation of the cell line is shown. Presence of chromosome 5, which was used to select hybrids initially, is shown by a dot. Presence of additional chromosomes is shown at the bottom. Cell line HHW 1107 contains a translocation of the distal half of chromosome 7q to 5 [t(5;7)(q35.2;q22)] in addition to the X chromosome. (**B**) Confirming Southern blot analysis of selected human/rodent hybrid cells. Chromosome composition of hamster/human hybrid cell lines is shown above. The HeLa cell line provides a positive control. CHO is Chinese hamster ovary DNA, which provides a negative control. Hybridization is seen only in the HeLa lane and in hybrids containing chromosome 7.

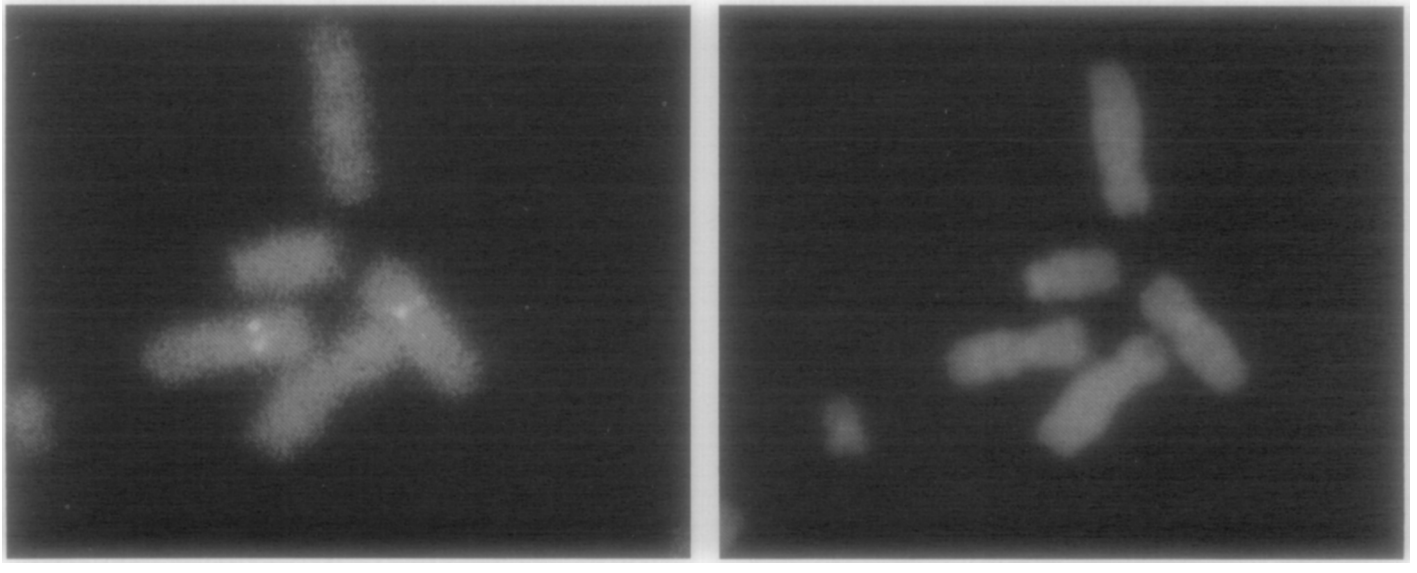

FIG. 2. *In situ* hybridization of cosDDC1 cosmid DNA to metaphase chromosomes. Biotinylated DNA was hybridized to lymphoblastoid metaphase chromosomes prepared by standard procedures. Hybridization is consistently detected only near the centromere of the short arm of chromosome 7. (**Left**) The fluorescent signal; (**right**) the DAPI stained chromosomes for reference.

ACKNOWLEDGMENTS

This work was supported by NIH Grant GM28972 to J.L.M. L.J.S. is supported by PHS Training Grant T32 CA09054 and J.D.M. by a California Biotechnology Grant. J.J.W. is supported by NIH Grant HG00320. We are especially indebted to Dr. M. Smith for advice, encouragement, and materials and to Ulla Bengtsson for performing the *in situ* hybridizations.

REFERENCES

1. Bruneau, G., Gross, M., Krieger, M., Bernheim, A., Thibault, J., and Nguyen, V. C. (1990). Preparation of a human dopa decarboxylase cDNA probe by PCR and its assignment to chromosome 7. *Ann. Genet.* **33:** 208–213.
2. Cooper, J. R., Bloom, F. E., and Roth, R. H. (1986). "The Biochemical Basis of Neuropharmacology," pp. 309–313, Oxford Univ. Press, London/New York.
3. Dana, S., and Wasmuth, J. J. (1982). Linkage of the *leuS, emtB* and *chr* genes on chromosome 5 in humans and expression of human genes encoding protein synthesis components in human–Chinese hamster hybrids. *Somat. Cell Genet.* **8:** 245.
4. Eveleth, D. D., Gietz, R. D., Spencer, C. A., Nargang, F., Hodgetts, R. B., and Marsh, J. L. (1986). Sequence and structure of the *Dopa Decarboxylase* gene of Drosophila: Evidence for novel RNA splicing variants. *EMBO J.* **5:** 2663–2672.
5. Hagen, F. S., Gray, C. L., and Kuijper, J. L. (1988). Assaying the quality of cDNA libraries. *Biotechniques* **6:** 340–345.
6. Ichinose, H., Kurosawa, Y., Titani, K., Keisuke, F., and Nagatsu, T. (1989). Isolation and characterization of a cDNA clone encoding human aromatic L-amino acid decarboxylase. *Biochem. Biophys. Res. Comm.* **164:** 1024–1030.
7. Jackson, F. R. (1990). Prokaryotic and eukaryotic pyridoxal dependent decarboxylases are homologous. *J. Mol. Evol.* **31:** 325–329.
8. Laurie-Ahlberg, C. C., Wilton, A. N., J. W. Cutsinger, and Emigh, T. H. (1982). Naturally occurring enzyme activity variation in *Drosophila melanogaster.* I. Source of variation for 23 enzymes. *Genetics* **102:** 191–206.
9. Marsh, J. L., and Wright, T. R. F. (1986). Evidence for regulatory variants of the *dopa decarboxylase* and *alpha methyldopa hypersensitive* loci in Drosophila. *Genetics* **112:** 249–265.
10. Wyman, A. R., Wertman, K. F., Barker, D., Helms, C., and Petri, W. H. (1986). Factors which equalize the representation of genome segments in recombinant DNA libraries. *Gene* **49:** 263–271.