Cytogenet Cell Genet 56:176–177 (1991)

0301–0171/91/0564–0176

Localization of the human dihydrolipoamide dehydrogenase gene (DLD) to 7q31→q32

S.W. Scherer,[1,4] G. Otulakowski,[3] B.H. Robinson,[2–4] and L.-C. Tsui[1,4]

Departments of [1] Medical Genetics, [2] Biochemistry, and [3] Pediatrics, University of Toronto, and [4] Department of Genetics, Research Institute, The Hospital for Sick Children, Toronto, Ont. (Canada)

Abstract. The gene for human dihydrolipoamide dehydrogenase (DLD) has been localized to the long arm of chromosome 7, within bands q31→q32, by gel-blot hybridization analysis with DNA from a panel of somatic cell hybrids containing various portions of human chromosome 7.

Dihydrolipoamide dehydrogenase (commonly known as lipoamide dehydrogenase, or DLD [E.C.1.8.1.4.]) is a 50-kDa subunit protein comprised of at least three distinct mitochondrial multienzyme complexes: the pyruvate dehydrogenase complex, the 2-oxoglutarate dehydrogenase complex, and the branched chain α-keto and dehydrogenase complex (Sakurai et al., 1970). Functionally, this enzyme acts to transfer a pair of electrons from reduced lipoyl groups to NAD within each of these complexes, thus releasing oxidized lipoate to participate in reductive acylation. It may also participate in another multienzyme complex that is responsible for glycine cleavage (Kochi et al., 1986; Carothers et al., 1987). A rare inborn error of metabolism in man characterized by reduced lipoamide dehydrogenase activity and concomitant reduction of all α-keto acid dehydrogenase complex activities has been described in a small number of patients (Robinson, 1989). Isolation and sequencing have been reported for cDNAs encoding the entire protein sequence of human (Otulakowski and Robinson, 1987; Pons et al., 1988) and porcine (Otulakowski and Robinson, 1987) lipoamide dehydrogenase and the human gene assigned to chromosome 7 (Otulakowski et al., 1988; Olson et al., 1990).

Using a panel of rodent × human somatic cell hybrids containing various portions of chromosome 7, we have refined the chromosome localization of the human DLD gene to 7q31→q32.

Supported by grants from the Medical Research Council of Canada (MRC). L.-C.T. is an MRC Scientist and Sellers Chair of Cystic Fibrosis Research.

Received 29 August 1990; accepted 29 October 1990.

Request reprints from Dr. Lap-Chee Tsui, Department of Genetics, The Hospital for Sick Children, 555 University Avenue, Toronto, Ont. M5G 1X8 (Canada).

The somatic cell hybrids used in the hybridization analysis have be described previously (Zengerling et al., 1987; Rommens et al., 1988). Th amount of human chromosome 7 present in each of these hybrids is shown Fig. 1. High-molecular-weight DNA (5–10 μg) was isolated from hybrid c lines, digested with *Eco*RI, separated by electrophoresis on a 0.8% agaro gel, and transferred to nylon membranes (Zeta-probe, Bio-Rad). A 1.1-k fragment from the cDNA clone LD4a (Otulakowski and Robinson, 198 labeled with [α-^{32}P]dCTP by the random priming method (Feinberg a Vogelstein, 1983), was used as the probe in the hybridization analysis. Filte were prehybridized and hybridized in CGH solution (0.5 M sodium pho phate buffer [pH 7.2], 1 mM EDTA, 7% SDS) at 65 °C (Church and Gilbe 1984). Following hybridization, the blots were washed three times consec tively in 2 × SSC, 1 × SSC, and 0.1 × SSC (all with 0.1% SDS) for 20 m each at 65 °C (SSC = 150 mM NaCl, 15 mM sodium citrate). The filters we exposed to Kodak XAR-5 film with intensifying screens for 1–6 days a –70 °C.

An example of the hybidization results is shown in Fig. 2 and the data are summarized in Fig. 1. As shown in Fig. 2, th DLD cDNA probe detected a single 4.0-kb band in all of th hybrids containing human DNA from the 7q31→q32 regio but not in hybrids that did not contain this region. The 4.0-k human-specific band was easily distinguished from cross-hy bridizing sequences (presumably the corresponding genes) i the mouse and hamster DNAs. The regional localization infor mation for DLD was derived mainly from the hybridization result with the human × mouse hybrid GM1059Rag5, which contained a single human chromosome 7 with an interstitial deletion (7pter→q22::q32→qter), and with 2068Rag22-2 which contained a single translocated chromosome 7 (7qter→q22:) (Rommens et al., 1988). The result clearly showed that the DLD sequence was present in 2068Rag22-2 but absent in GM1059Rag5, indicating that the DLD gene maps within 7q31→q32.

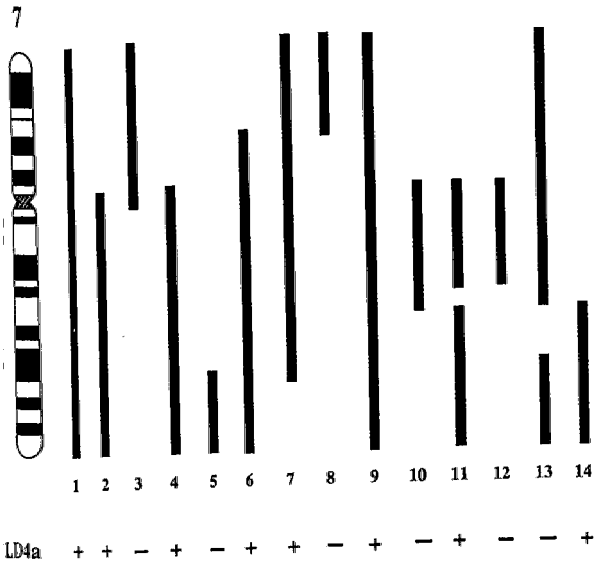

Fig. 1. Somatic cell hybrid lines and summary of hybridization data. The top panel shows the human × rodent somatic cell hybrid lines used in this study and the portion of human chromosome 7 contained in each of them. The presence (+) or absence (−) of the DLD gene in each of these cell lines was determined by gel-blot hybridization analysis with the cDNA probe. Examples of the hybridization results are presented in Fig. 2, but not all the results are shown.

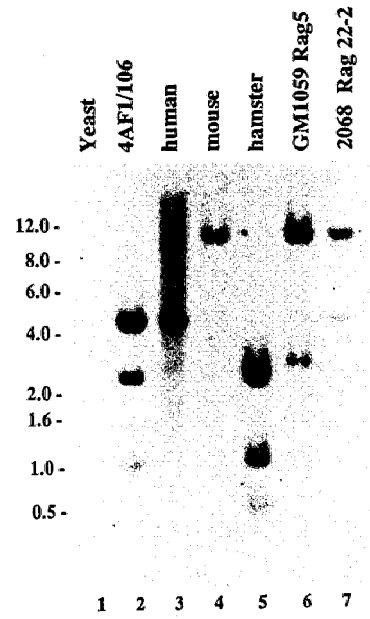

Fig. 2. Examples of gel-blot hybridization. The autoradiograph shows the result of hybridization of the LD4a cDNA to two of the somatic cell hybrids, GM1059Rag5 and 2068Rag22-2. Total human, mouse, and hamster DNAs are included as controls. The arrow indicates the 4.0-kb human-specific sequence present in lanes 2, 3, and 7.

A detailed long-range restriction map has been constructed for a 4.5×10^6 bp region encompassing the gene for cystic fibrosis (CF) (Drumm et al., 1988; Poustka et al., 1988; Rommens et al., 1989). Preliminary data (not shown) suggest that DLD is not in close proximity of the met protooncogene (MET), CF, and D7S8, which, together, span approximately 1.5×10^6 bp. Over 60 unique chromosome 7–specific DNA fragments have been localized to the 7q31→q32 interval with use of the somatic cell hybrids described here (Rommens et al., 1988). The localization of the DLD gene to this interval, which is estimated to be approximately 30×10^6 bp in size, adds yet another DNA segment for use in the construction of a long-range physical map of 7q31→q32, now underway.

The authors wish to thank Prof. Karl-Heinz Grzeschik for providing most of the somatic hybrid cell lines for this study.

Carothers DJ, Raefsky-Estrin C, Pons G, Patel MS: Rat liver mitochondria contain two immunologically distinct dihydrolipoamide dehydrogenases. Archs Biochem Biophys 256:597–605 (1987).
Church GM, Gilbert W: Genomic sequencing. Proc natl Acad Sci, USA 81:1991–1995 (1984).
Drumm ML, Smith CL, Dean M, Cole JL, Iannuzzi M, Collins FS: Physical mapping of the cystic fibrosis region by pulsed-field gel electrophoresis. Genomics 2:346–354 (1988).
Feinberg AP, Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Analyt Biochem 132:6–13 (1983).
Kochi H, Seino H, Ono K: Inhibition of glycine oxidation by pyruvate, α-ketoglutarate and branched-chain α-keto acids in rat liver mitochondria: interaction between the glycine cleavage system and the α-keto acid dehydrogenase complexes. Archs Biochem Biophys 249:263–272 (1986).
Olson S, Song BJ, Huh T-L, Chi Y-T, Veach RL, McBride OW: Three genes for enzymes of the pyruvate dehydrogenase complex map to human chromosomes 3, 7 and X. Am J hum Genet 46:340–349 (1990).
Otulakowski G, Robinson BH: Isolation and sequence determination of cDNA clones of porcine and human lipoamide dehydrogenase. J biol Chem 262:17313–17318 (1987).
Otulakowski G, Robinson BH, Willard HF: Gene for lipoamide dehydrogenase maps to human chromosome 7. Somat Cell molec Genet 14:411–414 (1988).
Pons G, Raefsky-Estrin C, Carothers D, Pepin R, Javid AA, Jesse BW, Ganapathi MK, Samols D, Patel MS: Cloning and cDNA sequence of the dihydrolipoamide dehydrogenase component of human α-ketoacid dehydrogenase complexes. Proc natl Acad Sci, USA 85:1422–1426 (1988).
Poustka A, Lehrach H, Williamson R, Bates G: A long-range restriction map encompassing the cystic fibrosis locus and its closely linked genetic markers. Genomics 2:337–345 (1988).
Robinson BH: Lactic acidemia, in Scriver CR, Beaudet AL, Sly WS, Valle D (eds): The Metabolic Basis of Inherited Disease, pp 869–888 (McGraw-Hill, New York/Toronto 1989).
Rommens JM, Zengerling S, Burns J, Melmer G, Kerem B, Plasvic N, Zsiga M, Kennedy D, Markiewicz D, Rozmahel R, Riordan JR, Buchwald M, Tsui L-C: Identification and regional localization of DNA markers on chromosome 7 for the cloning of the cystic fibrosis gene. Am J hum Genet 43:645–663 (1988).
Rommens JM, Zengerling-Lentes S, Kerem B, Melmer G, Buchwald M, Tsui L-C: Physical localization of two DNA markers closely linked to the cystic fibrosis locus by pulsed-field gel electrophoresis. Am J hum Genet 45:932–941 (1989).
Sakurai Y, Fukuyoshi Y, Hameda M, Hazakawa T, Koike M: Multiple forms of lipoamide dehydrogenase in pig heart α-keto acid dehydrogenase complexes. J biol Chem 245:4453–4458 (1970).
Zengerling S, Olek K, Tsui L-C, Grzeschik K-H, Riordan JR, Buchwald M: Mapping of DNA markers linked to the cystic fibrosis locus on the long arm of chromosome 7. Am J hum Genet 40:228–236 (1987).