Vol. 168, No. 2, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 30, 1990 Pages 677-682

MOLECULAR CHARACTERIZATION OF GENETIC MUTATION IN HUMAN LACTATE DEHYDROGENASE-A (M) DEFICIENCY

Masato Maekawa[1,2], Kayoko Sudo[1], Takashi Kanno[2] and Steven S.-L. Li[1,3]

[1]Laboratory of Genetics
National Institute of Environmental Health Sciences
National Institutes of Health
Research Triangle Park, North Carolina 27709

[2]Department of Laboratory Medicine
Hamamatsu University School of Medicine
Handa-cho 3600, Hamamatsu City 431-31
Japan

Received March 6, 1990

Human lactate dehydrogenase-A mutant gene was isolated from the genomic DNA library of a patient deficient in LDH-A (Muscle) subunit. The nucleotide sequences of seven protein-coding exons were determined and a deletion of 20 base-pairs in exon 6 was found. This mutation results in a frameshift translation and premature termination. The predicted incomplete LDH-A (M) subunit containing only 259 instead of 331 amino acids appears to be degraded rapidly, since no protein was detected immunologically (Maekawa et al., Am J Hum Genet 39:232-238, 1986). In addition, three synonymous (silent) substitutions, A to C, T to C, and G to A, were observed at codons 115, 160 and 172, respectively, in this LDH-A mutant gene. © 1990 Academic Press, Inc.

In human somatic tissues, five isozymes of tetrameric lactate dehydrogenase (LDH; E.C.1.1.1.27) are formed *in vivo* by combination of the LDH-A (muscle) and LDH-B (heart) subunits. An additional homotetrameric LDH-C4 isozyme is present in mature testis and sperm. The expression of mammalian LDH-A, LDH-B and LDH-C genes is developmentally regulated and tissue-specific (1,2). Human LDH-A and LDH-C genes are closely linked on chromosome 11, while LDH-B gene is located on chromosome 12 (3-7). The genomic organizations of human LDH-A, LDH-B and LDH-C genes have been elucidated, and their protein-coding sequences are interrupted by six introns at homologous positions (8-10). In humans, enzyme deficiencies and variant subunits of either LDH-A (M) or LDH-B (H) have been reported (11-15). The frequencies of either LDH-A or LDH-B subunit deficiency were estimated by screening a Japanese population (16). In that survey, a female was found

[3]To whom correspondence should be addressed.

Abbreviation: LDH, Lactate dehydrogenase.

0006-291X/90 $1.50
Copyright © 1990 by Academic Press, Inc.
All rights of reproduction in any form reserved.

to possess only one LDH-B4 isozyme, and enzymatically inactive LDH-A protein and heterotetramers with LDH-B subunit were not detectable immunologically (17). Here we describe the molecular nature of the genetic lesion in this human LDH-A mutant gene.

Materials and Methods

The DNA from white blood cells was isolated from the female with the deficiency of the LDH-A subunit, and a genomic library was constructed from the MboI partially-restricted DNA fragments inserted at BamHI site of the lambda FIX II vector (Stratagene). The full-length human LDH-A cDNA (18), excluding poly(A) tail, and the unique-flanking sequences of the LDH-A functional gene (8) were labelled with (α-^{32}P) dCTP (Amersham) with the use of random primers (Boehringer-Mannheim). These were used as probes to isolate genomic clones containing LDH-A mutant gene by plaque hybridization as described previously (19). The plaque DNAs bound to the plaque-screen-hybridization membrane (DuPont) were hybridized at 65°C overnight and the filters were washed as recommended by the supplier. Autoradiography of the filters was done with Kodak XAR-5 film and DuPont Cronex Lighting-plus intensifying screens at -70°C. The positive plaques were subsequently purified to homogeneity through repeated rounds of screening.

The DNA purified from the positive genomic clone was analyzed by restriction endonuclease mapping and Southern blotting, as described previously (19). The isolated DNA fragments were further cleaved and subcloned into M13 mp18 or mp19 bacteriophages (20). The M13 bacteriophages exhibiting positive hybridization to the human LDH-A cDNA probe (18) were isolated and the nucleotide sequences of the inserted DNA were determined by the dideoxy chain termination method with the sequencing protocol modified to use deoxyadenosine 5'-(α-^{35}S thio)triphosphate (21).

Results

Several genomic clones were found to exhibit strong hybridization signals to probes of the human LDH-A cDNA and unique flanking-sequences of the LDH-A functional gene. A positive clone, A02, containing an insert of approximately 20Kb, was tentatively identified as possessing LDH-A mutant gene. The DNA isolated from the clone A02 was further characterized and compared with that of clone H448, containing LDH-A functional gene, by restriction endonuclease cleavage and Southern blot analyses (Fig. 1). The sizes of genomic DNA fragments cleaved by EcoRI and/or HindIII from clones A02 and H448 appear to be very similar, indicating either a small deletion or a nucleotide substitution.

The isolated DNA fragments of EcoRI and/or HindIII cleavage from clone A02 were further cleaved with Sau3A, AluI, BamHI or XbaI, and subcloned into M13 bacteriophages. The nucleotide sequences of the inserted DNA from M13 bacteriophages exhibiting positive hybridization to the LDH-A cDNA probe were determined, and the protein-coding exons were identified by comparison with the nucleotide sequence of the LDH-A cDNA (18). As the previously reported LDH-A functional gene (8), the protein-coding sequence is interrupted by six introns at codons 41-42, 81, 139, 197, 236, and 277-

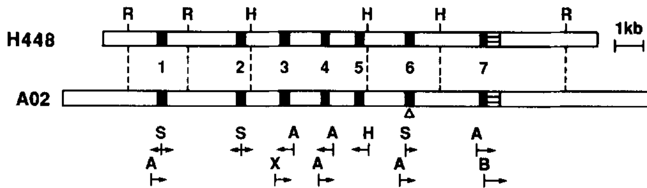

Figure 1. Comparison of genomic structure of human LDH-A functional and mutant genes along with restriction endonuclease map and nucleotide sequencing strategy of mutant DNA.

The seven protein-coding exons are numbered in Arabic and shown by solid blocks. The 3' non-coding region is stippled. The sizes of six introns are not known precisely. Human genomic clone A02 was isolated from lambda FIX II library (Stratagene). The restriction endonuclease map was deduced from cleavage with EcoRI (R), HindIII (H), and/or SalI (located at both ends of genomic DNA inserts) and Southern blot analysis probed with human LDH-A cDNA and flanking sequences (EcoRI-SalI DNA fragments) of LDH-A functional gene. The isolated EcoRI-EcoRI, HindIII-HindIII, and EcoRI-HindIII DNA fragments were further cleaved with Sau3A (S), AluI (A), BamHI (B), or XbaI (X) and subcloned into mp18 or mp19 bacteriophages. The nucleotide sequences of the inserted DNA from the M13 bacteriophages exhibiting positive hybridization to human LDH-A cDNA probe were determined by the dideoxy chain termination method. A deletion in exon 6 of mutant clone A02 is denoted by open triangle.

278. The 5' non-coding region of the human LDH-A gene is also interrupted by an intron 24 nucleotides 5' to the ATG translation initiation site, while there is no intron in the 3' non-coding sequence. A comparison between the nucleotide sequence of seven exons determined from clone A02 with that of the LDH-A functional gene (H448), reveals a deletion of 20 nucleotides at codons 252 to 259 in exon 6 (Fig. 2), as well as three synonymous substitutions, A to C, T to C, and G to A at codons 115, 160 and 172, respectively. This deletion results in a frame-shift translation of seven amino acids and premature termination, producing a predicted LDH-A mutant protein of 259 amino acids.

Discussion

As noted previously (16), the female patient deficient in LDH-A subunit is presumably homozygous for the mutant allele, since her parents are first cousins. In this study, the genetic mutation causing the LDH-A deficiency was shown to be a deletion of 20 base-pairs in exon 6 of the human LDH-A gene. This deletion occurred between two direct repeats of tetranucleotides TTGG, one copy of which is retained in the mutant gene (26). Thus, this mutation results in a frame-shift translation at codon 252 and premature termination, producing an incomplete LDH-A subunit of 259 amino acids. In the serum and erythrocyte samples of this patient, proteins which could react with anti-LDH-A antibodies and heterotetramers with LDH-B subunits which could react with anti-LDH-B antibodies were not detectable

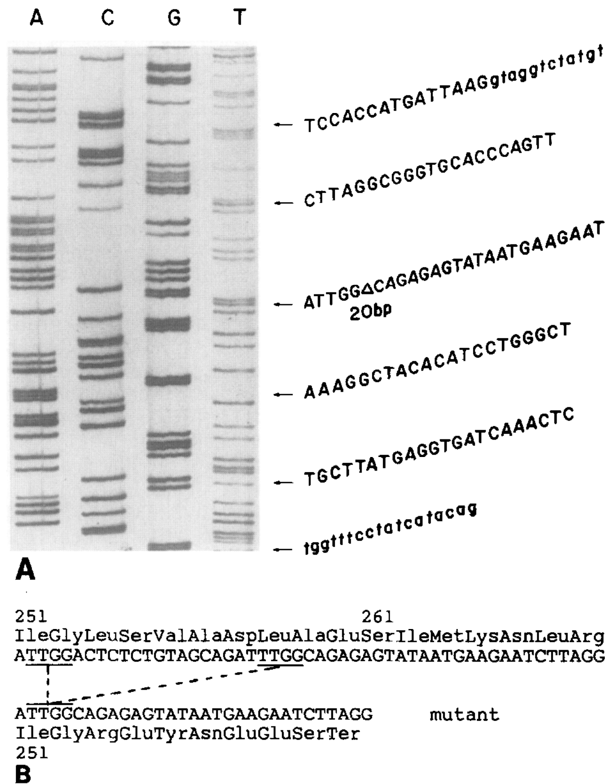

Figure 2. Nucleotide sequences of exon 6 and its flanking regions from human LDH-A mutant gene.

A. The nucleotide sequences of exon 6 are shown in higher case, and the intervening sequences are given in lower case. A deletion of 20 nucleotides is denoted by open triangle.

B. The deletion of 20 nucleotides occurred between two direct repeats of tetranucleotides TTGG (underlined), one copy of which is retained in the mutant gene. This deletion results in a frameshift translation and premature termination (Ter) as indicated.

immunologically (17). Thus, the incomplete LDH-A subunit may be degraded rapidly *in vivo* by cellular protease(s) if this mutant gene is transcribed and its mRNA is translated. It should be noted that the interaction between the COOH-tail αβββα subdomain (amino acids 251 and 331) of one subunit and the NH2-arm (first 20 amino acids) of another subunit is a major factor responsible for forming stable tetrameric LDH isozymes (22).

In addition to the deletion of 20 base-pairs, three synonymous substitutions, A to C, T to C, and G to A, were found at codons 115, 160 and 172, respectively, in this LDH-A mutant gene. It may also be noted that the 3' noncoding sequence of 565 nucleotides determined from the LDH-A mutant gene was found identical to that of the LDH-A cDNA and functional gene (8,18).

This female patient with the LDH-A deficiency frequently complained of uterine stiffness during her pregnancy. Uterine stiffness was a problem in the early stage of delivery and, thus, she required a Caesarean section (15). It is of interest that synthesis of the LDH-A subunit in human breast cancer MCF-7 cells and mouse uterus was shown to be induced significantly by estrogen (23,24) and that the promoter region of human and mouse LDH-A genes contains an estrogen-responsive element (25). The sequence and function of the promoter region from this human LDH-A mutant gene remain to be investigated.

Acknowledgments

We thank Dr. B. H. Judd for his interest in and support of this project, Drs. M. Eisenberg and F. Johnson for reading the manuscript, and Ms. N. Gore for preparing it. This work was supported in part by Japan Clinical Pathology Foundation for International Exchange.

References

1. Markert, C.L., Shakelee, J.B., and Whitt, G.S. (1975) Science 189, 102-114.
2. Li, S. S.-L. (1989) Biochem. Soc. Transact. 17, 304-307.
3. Boone, C., Chen, T.R., and Ruddle, F.H. (1972) Proc. Natl. Acad. Sci. USA 69, 510-514.
4. Mayeda, K., Weiss, L., Linahl, R., and Dully, M. (1974) Am. J. Hum. Genet. 26, 59-64.
5. Franke, U., and Busby, N. (1975) Cytogenet. Cell Genet. 14, 313-319.
6. Kao, R.T., Wu, K.C., Law, M.L., Hartz, J.A., and Lau, Y.-F. (1988) Somatic Mol. Cell Genet. 14, 515-518.
7. Li, S.S.-L., Luedemann, M., Sharief, F.S., Takano, T. and Deaven, L.L. (1988) Cytogenet Cell Genet. 48, 16-18.
8. Chung, F.Z., Tsujibo, H., Bhattacharyya, U., Sharief, F.S., and Li, S.S.-L. (1985) Biochem. J. 231, 537-541.
9. Takano, T., and Li, S.S.-L. (1989) Biochem. J. 257, 921-924
10. Takano, T., and Li, S.S.-L. (1989) Biochem. Biophys. Res. Commun. 159, 579-583.
11. Kitamura, M., Iijima, N., Hashimoto, F. and Hiratsuka, A. (1971) Clin. Chim. Acta. 34, 419-423.
12. Mohrenweisser, H.W., and Novotny, J.E. (1982) Biochem. Biophys. Acta. 702, 90-98.
13. Houki, N., Matsushima, Y., Kitamura, M., Tukada, T. Nishina, T., and Nakayama, T. (1986) Jap. J. Clin. Chem. 15, 85-90.
14. Joukyuu, R., Mizuno, S., Amakawa, T., Tsukada, T., Nishina, T., and Kitamura, M. (1989) Clin. Chem. 35, 687-690.
15. Kanno, T., Sudo, K., Maekawa, M., Nishimura, Y., Ukita, M., and Fukutake, K. (1988) Clin. Chim. Acta. 173, 89-98.
16. Maekawa, M., Kanda, S., Sudo, K. and Kanno, T. (1984) Am. J. Hum. Genet. 36, 1204-1214.
17. Maekawa, M., Sudo, K. and Kanno, T. (1986) Am. J. Hum Genet. 39, 232-238.
18. Tsujibo, H., Tiano, H.F., and Li, S.S.-L. (1985) Eur. J. Biochem. 147, 9-15.
19. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular Cloning, a laboratory manual. Cold Spring Harbor Laboratory, New York.

20. Messing, J., Crea, R., and Seeberg, P.H. (1981) Nucleic Acids Res. 9, 309-321.
21. Biggin, M.D., Gibson, T.J., and Hong, G.F. (1983) Proc. Natl. Acad. Sci. USA 80, 3963-3965.
22. Eventoff, W., Rossmann, M.G., Taylor, S.S., Torff, H.J., Meyer, H., Keil, W., and Kiltz, H.H. (1977) Proc. Natl. Acad. Sci. USA 74, 2677-2681.
23. Burke, R.E., Harris, S.C., and McGurie, W.L. (1978) Cancer Res. 38, 2773-2776.
24. Li, S. S.-L., and Hou, E.W. (1989) Cell Biol. Int. Reports 13, 619-624.
25. Fukasawa, K.M., and Li, S.S.-L. (1987) Genetics 116, 99-105.
26. Nalbantoglu, J., Phear, G., and Meuth, M. (1987) Mol. Cell. Biol. 7, 1445-1449.