Genet Res., Camb. (1989), **53**, *pp.* 101–104 *Printed in Great Britain*

Eight independent *Ldh-1* mutations of the mouse recovered in mutagenicity experiments: biochemical characteristics and chromosomal localization

WALTER PRETSCH

Institut für Säugetiergenetik, Gesellschaft für Strahlen- und Umweltforschung, D-8042 Neuherberg, Federal Republic of Germany

(*Received 3 October 1988*)

Summary

Eight mouse mutants with altered charge or activity of lactate dehydrogenase-1 have been detected in offspring derived from mutagen-treated spermatogonia. Using two chromosome-7 marker genes pooled recombination frequencies are estimated as *c*–14·4 ± 0·8–*p*–6·9 ± 0·6–*Ldh-1*.

1. Introduction

The locus for the A subunit of lactate dehydrogenase (LDH) (EC 1.1.1.27), *Ldh-1*, in mice was first shown to be on chromosome 7 by use of mouse × human and mouse × Chinese hamster somatic cell hybrids (O'Brien, Linnenbach & Croce, 1978; Lalley, Minna & Francke, 1978), has been mapped between *Gpi-1* and *Hbb* by Soares (1978). Peters & Andrews (1985) used an electrophoretically detectable variant to locate the structural gene for the B subunit, *Ldh-2*, on chromosome 6.

In various mutagenicity experiments to recover genetically inherited enzyme-charge and enzyme-activity alterations in erythrocytes a total of eight mutants with altered charge and/or activity of LDH compared to the wild type were detected (Pretsch & Charles, 1980, 1984; Charles & Pretsch, 1987). These mutations have been suspected of the *Ldh-1* locus as in blood of mice mainly the isozyme containing LDH-A polypeptides is expressed. In this paper a short genetical characterization of these mutants and their assignment to the structural locus *Ldh-1* on chromosome 7 is given.

2. Materials and methods

(i) *Mice*

The original LDH mutants were recovered in different mutagenicity experiments in which males were treated with 1-ethyl-1-nitrosourea or procarbazine hydrochloride. The F_1 offspring were screened for protein charge alterations with polyacrylamide gel isoelectric focusing (PAGIF) or for enzyme activity alterations (Charles & Pretsch, 1981, 1987; Pretsch & Charles, 1980, 1984). Variants were genetically confirmed and congenic lines established by backcrossing the heterozygous mutants to C3H/El inbred mice.

For linkage studies, the chromosome-7 markers c^{ch}, chinchilla, and *p*, pink-eyed dilution, were used.

(ii) *Lactate dehydrogenase assays*

For the determination of LDH charge alterations lysed blood samples or crude liver extracts were isofocused on ultrathin-layer polyacrylamide gels with a final pH range of 3·5–9·5 prepared according to the procedure of Radola (1980). The staining for LDH was done in the dark at 37 °C (Charles & Pretsch, 1981).

The determination of LDH specific activity in blood was performed at 334 nm with an Eppendorf ACP 5040 analyser (Eppendorf, Hamburg, FRG) (Charles & Pretsch, 1987). Wild-type and heterozygous animals could be clearly discerned based on their different enzyme activities.

(iii) *Linkage studies*

Heterozygous LDH mutants were mated with animals homozygous for c^{ch} and *p*. These two loci are linked on chromosome 7 with a recombination frequency (RF ± S.E.) of 14·7 ± 0·3 % (Davisson & Roderick, 1981). Recombination frequencies were calculated in offspring of backcrosses of the triple heterozygous mutants with $c^{ch}p/c^{ch}p$ animals.

3. Results and discussion

A total of eight independent mutants with altered charge and/or activity of LDH were detected in various mutagenicity experiments (Table 1). LDH charge alteration was accompanied with LDH activity

Table 1. *Characteristics of eight independent* Ldh-1 *mutations*

LDH mutant no.	Experiment[a]	Blood LDH activity (%)[b]		LDH-PAGIF banding pattern	References
		Heterozygotes	Homozygotes		
1049	250 mg ENU	55	—	Shifted[c]	Pretsch & Charles (1984)
1592	600 mg PHCl	60	10	Shifted	Pretsch & Charles (1980) Charles & Pretsch (1981)
1623	160 mg ENU	95	85	Shifted	—
1962	160 mg ENU	65	25	Shifted	Charles & Pretsch (1987)
2014	250 mg ENU	65	30	Normal	Charles & Pretsch (1987)
9546	250 mg ENU	50	2	Normal	Charles & Pretsch (1987)
10866	160 mg ENU	100	100	Shifted	Pretsch & Charles (1984)
29804	80 mg ENU	60	25	Normal	Charles & Pretsch (1987)

[a] Paternal treatment expressed as dose of the mutagen/kg body weight. ENU, 1-ethyl-1-nitrosourea; PHCl, procarbazine hydrochloride.
[b] Specific activity in homozygous wild types set at 100%.
[c] Several weak LDH banding deficiencies can be seen after PAGIF of crude liver extracts.

deficiency in three mutants (nos. 1049, 1592, 1962) whereas two mutants (1623, 10866) showed normal activity. Finally, three mutants (2014, 9546, 29804) had reduced LDH activity without changed LDH banding pattern after isoelectric focusing.

The results of the genetic studies are given in Table 2. In backcrosses, the transmission was calculated as the percent of mutant offspring observed to that expected for segregation of an autosomal codominant gene. Transmission was normal for all mutants and ranged between 94 and 106%. By crossing heterozygous mutants *inter se* homozygous wild types, heterozygous mutants as well as homozygous mutants with a third phenotype were recovered for four mutant lines (1592, 1623, 10866, 29804) in the approximate Mendelian ratio, 1:2:1. Homozygotes of mutant line no. 1049 are lethal. Of the three remaining mutant lines (1962, 2014, 9546) there is a significant reduction in the number of homozygotes implying that the mutated genes have semilethal effects.

Soares (1978) detected a *Ldh-1* variant by a change of electrophoretic mobility of the enzyme in F_1 offspring of a cross between mice of the DBA/2J and C57BL/6J strains. Genetic analyses revealed normal transmission for this mutation. Homozygous mutants are fertile and show no apparent deleterious effects. Additionally, visual analysis of the zymogram patterns

Table 2. *Distribution of progeny in backcrosses among homozygous wild-types and heterozygous mutants (B) and in intercrosses of heterozygous mutants (I), respectively*

LDH mutant no.	Type of cross	Average litter size	Offspring (*n*)			Transmission (%)
			Wild types	Hetero-zygotes	Homo-zygotes	
1049	B	6·6	302	321	—	103
	I	4·9	108	170	0*	—
1592	B	6·2	440	453	—	101
	I	6·7	347	599	267	—
1623	B	7·1	153	171	—	106
	I	6·5	34	89	46	—
1962	B	6·7	227	200	—	94
	I	6·1	53	119	21*	—
2014	B	6·1	224	219	—	99
	I	5·0	50	104	15*	—
9546	B	6·7	227	231	—	101
	I	6·0	194	366	4*	—
10866	B	5·8	137	148	—	104
	I	7·1	32	47	22	—
29804	B	6·2	266	276	—	102
	I	6·5	68	115	68	—

* Significantly different from the 1:2:1 Mendelian ratio (χ^2 test).

https://doi.org/10.1017/S001667230002797X Published online by Cambridge University Press

Table 3. *Three point crosses to localize* Ldh-1 *alleles on chromosome 7 of the mouse. Segregation of* chinchilla (*c*), pink-eyed dilution (*p*) *and* Lactate dehydrogenase-1 (*L*) *in offspring of the parental mating* (*cp*+/*cp*+) × (++*L*/*cp*+)

	Progeny class/phenotype (*n*)										
	Non-recombinant		Single recombinant 1		Single recombinant 2		Double recombinant		Recombination (%)[a]		
LDH mutant no.	++*L*	*cp*+	+*p*+	*c*+*L*	+++	*cpL*	+*pL*	*c*++	$RF_1 \pm$S.E.	$RF_2 \pm$S.E.	$RF_3 \pm$S.E.
1049	27	27	4	2	1	1	0	0	9·7±3·8	3·2±2·2	12·9±4·3
1592	121	115	31	23	5	6	1	0	18·2±2·2	4·0±1·1	21·5±2·4
1623	90	93	16	12	9	9	0	0	12·2±2·2	7·9±1·8	20·1±2·6
1962	98	87	12	16	11	15	1	1	12·4±2·1	11·6±2·1	22·4±2·7
2014	118	132	16	15	7	9	0	0	10·4±1·8	5·4±1·3	15·8±2·1
9546	80	85	10	20	5	7	0	1	14·9±2·5	6·3±1·7	20·2±2·8
10866	109	122	20	22	18	12	0	3	14·7±2·0	10·8±1·8	23·5±2·4
29804	99	118	18	33	4	6	0	1	18·6±2·3	3·9±1·2	21·9±2·5
Total	742	779	127	143	60	65	2	6	14·4±0·8	6·9±0·6	20·5±0·9

[a] RF_1, recombination frequency between *c* and *p*; RF_2, recombination frequency between *p* and *Ldh-1*; RF_3, recombination frequency between *c* and *Ldh-1*.

from the homozygous mutants reveal a considerable reduction in the intensity of staining suggesting a LDH activity deficiency (similar to mutants 1592 and 1962). Linkage analyses have located *Ldh-1* on chromosome 7 between *Gpi-1* (RF = 15·5±4·8%, *n* = 59) and *Hbb* (RF = 25·3±6·0%, *n* = 64).

In mutant no. 1592 polyacrylamide gel electrophoresis was performed with various tissues. It could be shown that the mutation affects the electrophoretic mobility and staining intensity of isozymes containing LDH-A polypeptides, while the mobility and intensity of the band corresponding to subunit B remain constant (Charles & Pretsch, 1981). Due to these observations and because mainly *Ldh-1* is expressed in blood it is concluded that the mutation affects *Ldh-1* and linkage tests were performed for this locus in all eight LDH mutants.

The present linkage results (Table 3) indicate all LDH mutants to be *Ldh-1* alleles and that the order of the three loci is as described in the literature (Davisson & Roderick, 1981; Lyon, 1988). The combined data yield a RF of 14·4±0·8% between *c* and *p*. This value is in good agreement with that described in the literature (Davisson & Roderick, 1981). The RFs for *c*–*Ldh-1* and *p*–*Ldh-1* were calculated as 20·5±0·9% and 6·9±0·6% (*n* = 1924), respectively. In the last edited mouse chromosome atlas the map distance of *p* and *Ldh-1* is recorded as 2 cM (Lyon, 1988). Based on the present linkage experiments this value seems too small. It is suggested that 7 cM is more accurate.

Since there are homologies between mouse and man for the examined locus, experiments with mouse LDH mutation can supply important information for humans. For instance, heritable enzyme alterations are often connected with clinical symptoms in man. One of the LDH mutants (no. 1592) is afflicted with a severe haemolytic anaemia associated with extreme reticulocytosis and splenomegaly when homozygous (Kremer *et al.* 1987; Datta *et al.* 1988). These mutants may, therefore, be model animals for this hereditary disease in humans. In transplantation experiments with the same mutant, no functional difference between pluripotent stem cells of wild type and homozygous mutant mice was detected (Datta & Dörmer, 1987). These findings underline the relevance of the LDH mutant mouse as a model for studies of normal stem cell regulation.

The studies were supported in part by contract No. BI6-E-156-D of the Commission of the European Communities.

References

Charles, D. J. & Pretsch, W. (1981). A mutation affecting the lactate dehydrogenase locus *Ldh-1* in the mouse. I. Genetical and electrophoretical characterization. *Biochemical Genetics* **19**, 301–309.

Charles, D. J. & Pretsch, W. (1987). Linear dose-response relationship of erythrocyte enzyme-activity mutations in offspring of ethylnitrosourea-treated mice. *Mutation Research* **176**, 81–91.

Datta, T. & Dörmer, P. (1987). Establishment of permanent chimerism in a lactate dehydrogenase-deficient mouse mutant with hemolytic anemia. *Experimental Hematology* **15**, 1158–1162.

Datta, T., Kremer, J.-P., Hültner, L. & Dörmer, P. (1988). The role of the spleen in a lactate dehydrogenase mutant mouse (*Ldh-1*^{e}/*Ldh-1*^{e}) with hemolytic anemia. *Experimental Hematology* **16**, 281–284.

Davisson, M. T. & Roderick, T. H. (1981). Recombination percentages. In *Genetic Variants and Strains of the Laboratory Mouse* (ed. M. C. Green), pp. 283–313. Stuttgart, New York: Fischer.

Kremer, J.-P., Datta, T., Pretsch, W., Charles, D. J. & Dörmer, P. (1987). Mechanisms of compensation of hemolytic anemia in a lactate dehydrogenase mouse mutant. *Experimental Hematology* **15**, 664–670.

Lalley, P. A., Minna, J. D. & Francke, U. (1978). Conservation of autosomal gene synteny groups in mouse and man. *Nature* **274**, 160–163.

Lyon, M. F. (1988). Mouse chromosome atlas. *Mouse News Letter* **81**, 20–41.

O'Brien, D., Linnenbach, A. & Croce, C. M. (1978). Assignment of the gene for lactic dehydrogenase A to mouse chromosome 7 using mouse-human hybrids. *Cytogenetics and Cell Genetics* **21**, 72–76.

Peters, J. & Andrews, S. J. (1985). Linkage of lactate dehydrogenase-2, *Ldh-2*, in the mouse. *Biochemical Genetics* **23**, 217–225.

Pretsch, W. & Charles, D. (1980). Genetical and biochemical characterization of a dominant mutation of mouse lactate dehydrogenase. In *Electrophoresis '79* (ed. B. J. Radola), pp. 817–824. Berlin, New York: Walter de Gruyter.

Pretsch, W. & Charles, D. J. (1984). Detection of dominant enzyme mutants in mice: model studies for mutations in man. In *Monitoring Human Exposure to Carcinogenic and Mutagenic Agents* (IARC Scientific Publications No. 59) (ed. A. Berlin, M. Draper, K. Hemminki and H. Vainio), pp. 361–369. Lyon: International Agency for Research on Cancer.

Radola, B. J. (1980). Ultrathin-layer isoelectric focusing in 50–100 μm polyacrylamide gels on silanized glass plates or polyester films. *Electrophoresis* **1**, 43–56.

Soares, E. R. (1978). Genetic and linkage tests. *Mouse News Letter* **59**, 11–12.