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

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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 \(c^{-144} \pm 0.8\% \pm 69 \pm 0.6\% - Ldh-1\).

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 \(\times\) human and mouse \(\times\) 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 F1 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 *cch*, 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 *cch* and *p*. These two loci are linked on chromosome 7 with a recombination frequency (RF \(\pm\) s.e.) of 14.7 \(\pm\) 0.3% (Davisson & Roderick, 1981). Recombination frequencies were calculated in offspring of backcrosses of the triple heterozygous mutants with *cchp/cchp* 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* | Blood LDH activity (%) | LDH-PAGIF banding pattern | References |
|---------------|-------------|------------------------|---------------------------|------------|
|               |             | Heterozygotes | Homozygotes |                  |            |
| 1049          | 250 mg ENU  | 55          | —           | Shiftedc       | Pretsch & Charles (1984) |
| 1592          | 600 mg PHC1 | 60          | 10          | Shifted        | Pretsch & Charles (1980) |
| 1623          | 160 mg ENU  | 95          | 85          | Normal         | Charles & Pretsch (1987) |
| 1962          | 160 mg ENU  | 65          | 25          | Normal         | Charles & Pretsch (1987) |
| 2014          | 250 mg ENU  | 65          | 30          | Normal         | Charles & Pretsch (1987) |
| 9546          | 250 mg ENU  | 50          | 2           | Shifted        | Pretsch & Charles (1984) |
| 10866         | 160 mg ENU  | 100         | 100         | Normal         | Charles & Pretsch (1987) |
| 29804         | 80 mg ENU   | 60          | 25          | Normal         | Charles & Pretsch (1987) |

* Paternal treatment expressed as dose of the mutagen/kg body weight. ENU, 1-ethyl-1-nitrosourea; PHC1, procarbazine hydrochloride.

A Specific activity in homozygous wild types set at 100%.

* 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 remaining mutant lines (1623, 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 F̄ 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) | | |
|---------------|--------------|-------------------|--------------|---|---|---|---|
|               |              |                   | Wild types   | Heterozygotes | Homozygotes | Transmission (\%) |
| 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            | 6-7               | 224          | 219          | —           | 99 |
|               | I            | 6-5               | 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 (χ² test).

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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 (Davission & Roderick, 1981; Lyon, 1988). c and p. This value is in good agreement with that described in the literature (Davission & 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.

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