Tpi-1 and Gapd are linked very closely on mouse chromosome 6

WALTER PRETSCH*, ANGELIKA NEUHÄUSER-KLAUS AND SIEGBERT MERKLE
GSF-Institut für Säugetiergenetik, Ingolstädter Landstr. 1, D-8042 Neuherberg, Federal Republic of Germany

(Received 14 February 1990 and in revised form 30 April 1990)

Summary
Mutations in the structural genes for triosephosphate isomerase and glyceraldehyde-3-phosphate dehydrogenase activity in the mouse, selected after mutagen treatment, were used to estimate the map distance between the two loci. It is shown that Tpi-1 and Gapd are closely linked on chromosome 6, with a recombination frequency of 0.1 ± 0.1%.

1. Introduction
In the mouse Tpi-1 and Gapd are the structural loci for triosephosphate isomerase (TPI; EC 5.3.1.1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; EC 1.2.1.12), respectively. Both loci were shown to be located on chromosome 6 by use of Chinese hamster × mouse somatic cell hybrid clones (Leinwand, Kozak & Ruddle, 1978; Minna et al. 1978; Bruns et al. 1979). A genetic variant with low erythrocyte TPI activity was utilized in linkage studies in the mouse to place Tpi-1 in the distal half of the chromosome 4 cM proximal to Ldh-2, lactate dehydrogenase-2 (Peters & Andrew, 1985; Bulfield, Ball & Peters, 1987; Davison & Roderick, 1989). Due to the absence of suitable mutants, localization of Gapd has not been possible.

Various mutagenicity experiments were previously carried out to screen for genetically inherited enzyme-activity alterations. Mutants with decreased TPI or GAPDH activity were detected (Charles & Pretsch, 1986, 1987) and were assumed to be due to mutations affecting the structural loci Tpi-1 and Gapd, respectively.

In this paper we describe location tests on these mutations, with a view to answering the questions (1) are all the TPI and GAPDH mutations we have recovered located respectively at the Tpi-1 and Gapd structural loci on mouse chromosome 6? (2) Are Tpi-1 and Gapd closely linked in the mouse? In all mammalian species so far studied, the loci for TPI and GAPDH show syntenic autosomal homologies which appear to be conserved (Lalley & McKusick, 1985).

A detailed characterization of the TPI mutants has been published elsewhere (Merkle & Pretsch, 1989).

2. Materials and methods
The mutations used in this study originated in different mutagenicity experiments. Male (102/El × C3H/El)F1 mice were treated and then immediately caged with untreated Test-stock females (Charles & Pretsch, 1986, 1987). The mutant alleles of the mutant lines TPI 2161, TPI 2312, TPI 3502, TPI 9606, GAPD 525, GAPD 577, GAPD 3284, and GAPD 28003 were designated Tpi-PmNeu to Tpi-Pm4Neu and GapdmNeu to Gapdm4Neu, respectively.

For linkage studies, heterozygous TPI and GAPDH animals, with an approximately 50% decreased TPI or GAPDH activity, respectively, were mated. Double heterozygotes, deficient for both enzyme activities, were selected and backcrossed with C3H/El wild-type inbred mice. Offspring of these crosses were classified for TPI and GAPDH activity and used to calculate the recombination frequency between Tpi-1 and Gapd.

In order to test whether the TPI mutations map to the Tpi-1 structural locus on chromosome 6, one of these mutations (Tpi-1m4Neu) was tested for recombination with wa-1, waved-1, and Mip*, Microphthalmia (Neuhäuser-Klaus, Schäffer & Pretsch, 1987), a new mutation with a phenotype similar to Mip.

Determination of the specific activities of TPI and GAPDH was performed at 334 nm with an Eppendorf ACP 5040 analyzer (Eppendorf, Hamburg, FRG) (Charles & Pretsch, 1987). Wild-type and heterozygous
3. Results and discussion

(i) Backcrossing and intercrossing of heterozygous mutants

Table 1 presents the results of the genetic characterization studies. Backcrossing heterozygous mutants with wild-type C3H/El animals revealed homozygous wild-type and heterozygous mutant offspring in a ratio of approximately 1:1. No fitness effects of the mutations in heterozygotes on litter size of backcrosses could be observed by taking the mean litter size of strain C3H/El production stocks in Neuherberg as a standard. For backcrossing of heterozygous mutants, C3H/El wild-type animals were alternately used as males or females to exclude a fitness influence of one parental type on the litter size.

To investigate the homozygous viability of the mutants, heterozygotes were crossed inter se. In 7 of 8 mutant lines intercrossing resulted in only wild types and animals with 50% reduced activity in an approximate 1:2 ratio. No third class of animals was recovered. Litter size of intercrosses was significantly reduced compared to the litter size of backcrosses. The absence of a third class of animals apart from wild-type and heterozygous animals and the decreased litter size of intercrosses suggest that homozygotes are lethal. This hypothesis was proved genetically by the absence of homozygotes among offspring of intercrosses. In each mutant line twenty randomly chosen animals resulting from intercrosses with altered activity were crossed inter se to determine their genotypes. Wild types in the progeny of each of these matings excluded homozygosity of the parents. According to Favor (1984) in this case the probability not to detect possible homozygotes is less than 0.001.

For the mutant line Gapd\textsuperscript{m3Neu} a very small number of homozygotes with roughly 10% GAPDH activity compared to the wild type could be recovered. It has not yet been clarified why only in this mutant line homozygotes originate even if their number is negligible. The only three homozygous males arose in the first intercross after mutation induction. This suggests that the genetic background of the maternal Test-stock was responsible for this effect (Charles & Pretsch, 1987). After transferring the mutant gene to a standard genetic C3H/El background the mutation Gapd\textsuperscript{m3Neu} became fully lethal in later generations.

(ii) Linkage tests with TPI and GAPDH mutant alleles

To test for linkage between the TPI and GAPDH mutant alleles, the crosses shown in Table 2 were made, mice heterozygous in the repulsion phase for a mutant allele of each locus being crossed to wild-type animals. The results indicate that the two loci are very closely linked: only one recombinant was observed among the 961 offspring scored, giving a recombination frequency of 0.1 ± 0.1% for Gapd and Tpi-1.

To test whether the TPI mutations map to the Tpi-1 structural locus on chromosome 6, a three-point linkage test was carried out with the mutation

Table 1. Distribution of mutant progeny in backcrosses between homozygous C3H/El wild-types and heterozygous mutants (B) and in intercrosses of heterozygous mutants (I), respectively

| Mutant     | Type of cross | Litter size | Offspring (n) | Ratio homozygotes/wild-type animals |
|------------|---------------|-------------|---------------|-----------------------------------|
| Tpi-1\textsuperscript{-m3Neu} | B             | 6.4 ± 0.4  | 256 | 243 | 0.95 |
|            | I             | 5.2 ± 0.4  | 92  | 218 | 0   | 2.37 |
| Tpi-1\textsuperscript{-m5Neu} | B             | 6.6 ± 0.4  | 235 | 246 | 0.95 |
|            | I             | 5.2 ± 0.5  | 90  | 135 | 0   | 1.50 |
| Tpi-1\textsuperscript{-m5Neu} | B             | 6.9 ± 0.5  | 331 | 329 | 0.99 |
|            | I             | 5.3 ± 0.5  | 97  | 143 | 0   | 1.47 |
| Tpi-1\textsuperscript{-m4Neu} | B             | 6.5 ± 0.4  | 245 | 252 | 1.03 |
|            | I             | 5.0 ± 0.5  | 85  | 152 | 0   | 1.79 |
| Gapd\textsuperscript{m3Neu} | B             | 6.6 ± 0.2  | 225 | 195 | 0.87 |
|            | I             | 6.0 ± 0.1  | 60  | 113 | 0   | 1.88 |
| Gapd\textsuperscript{m2Neu} | B             | 6.6 ± 0.2  | 144 | 142 | 0.99 |
|            | I             | 5.2 ± 0.3  | 31  | 41  | 0   | 1.32 |
| Gapd\textsuperscript{m3Neu} | B             | 6.8 ± 0.2  | 193 | 206 | 1.07 |
|            | I             | 5.8 ± 0.2  | 76  | 170 | 3.3* | 2.24 |
| Gapd\textsuperscript{m3Neu} | B             | 7.4 ± 0.2  | 196 | 162 | 0.83 |
|            | I             | 5.4 ± 0.1  | 42  | 75  | 0   | 1.79 |

* Data are given as mean ± s.e.m. of 30 litters. Significant differences (P < 0.01) between litter size of backcrosses and intercrosses (t test) are marked by *.

* One male genetically confirmed; two males sterile.
Linkage of Tpi-1 and Gapd in the mouse

Table 2. Segregation of Tpi-1 and Gapd in offspring from matings of double heterozygous mutants (T+ / +G) with wild-type C3H/El animals*

| Gapd allele | Tpi-1 allele | Tpi-1 allele |
|-------------|--------------|--------------|
| a-m1Neu     | a-m2Neu      | a-m3Neu      |
| m1Neu       | T++ +G+ +TG++ | T++ +G+ +TG++ | T++ +G+ +TG++ |
| m2Neu       | 31 32 0 0     | 26 16 0 0     | 26 16 0 0     |
| m3Neu       | 12 13 0 0     | 34 39 0 0     | 34 39 0 0     |
| m4Neu       | — — — —       | — — — —       | — — — —       |
| Total       | T++ +G+ +TG++ | T++ +G+ +TG++ | T++ +G+ +TG++ |

* T++ +G+ +TG++ : TPI deficient (non-recombinant) offspring.
+G+ + : GAPDH deficient (non-recombinant) offspring.
TG++ +G+ + : TPI and GAPDH deficient (recombinant) offspring.
+++/ + + : wild type (recombinant) offspring.

Tpi-1^m1Neu and the testmarkers wa-I and Mi* (Table 3). The map order and distance of the three tested genes is

wa-I - 4.4 ± 1.6 - Mi* - 13.8 ± 2.7 - Tpi-1.

The recombination percentage between wa-I and Mi* agrees with the mouse linkage map (Lyon, 1989) and confirms that Mi* and Mi are allelic. The distance between Mi* and Tpi-1 is in accordance with the mapping data of Bulfield, Ball & Peters (1987) confirming that Tpi-1^m1Neu is a mutation at the structural locus for TPI.

In the ten mammalian species in which Tpi-1, Gapd and Ldh-2 have been assigned, there has been linkage conservation of these genes (Lalley & McKusick, 1985). This linkage group could have been maintained randomly or by selective action. Nadeau & Taylor (1984) have proposed that many long chromosomal

Table 3. Recombination of Tpi-1^m1Neu, triosephosphate isomerase-1, Mi*, microphthalmia, and wa-I, waved-1
(partial mating: Tpi-1 Mi* +/ + wa-I × ++ + wa-I/ + + wa-I)

| Type of recombinant | Progeny class/phenotype | Number |
|---------------------|-------------------------|--------|
| Non-recombinant     | Tpi-1 Mi* +             | 72     |
|                     | + + wa-I                | 61     |
| Single recombinant 1| Tpi-1 + wa-I            | 11     |
|                     | + Mi* +                 | 9      |
| Single recombinant 2| Tpi-1 Mi* wa-I          | 5      |
|                     | + + +                   | 2      |
| Double recombinant  | Tpi-1 + +               | 2      |
|                     | + Mi* wa-I              | 0      |
|                     |                        | 160    |

Recombination (RF ± S.E.)

|                  | Number |
|------------------|--------|
| Tpi-1-Mi*        | 22/160 | 13.8 ± 2.7 |
| Mi*-wa-I         | 9/160  | 5.6 ± 1.8  |
| Tpi-1-wa-I       | 27/160 | 16.9 ± 3.0 |

Downloaded from https://www.cambridge.org/core. IP address: 35.160.27.221, on 01 May 2022 at 10:15:49, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0016672300029013
segments are expected to be conserved regardless of the function of loci with each segment. However, the fact that the three enzymes TPI, GAPDH and LDH are all involved in the same metabolic pathway of glycolysis supports an evolutionary relationship of these functionally related genes.

We would like to express our appreciation to Drs E. Reeve, U. H. Ehling and J. Favor for suggestions and helpful criticisms of the manuscript. The competent technical assistance of M. Ellendorff and S. Wolf is gratefully acknowledged. This research was supported in part by Contract B16-E-156-D from the Commission of the European Communities.

References

Bruns, G., Gerald, P. S., Lalley, P., Francke, U. & Minna, J. (1979). Gene mapping of the mouse by somatic cell hybridization. *Cytogenetics and Cell Genetics* 25, 139.

Bulfield, G., Ball, S. T. & Peters, J. (1987). An allele at the triose phosphate isomerase, *Tpi-1* locus on chromosome 6 recovered from feral mice. *Genetical Research, Camb.* 50, 239–243.

Charles, D. J. & Pretsch, W. (1986). Enzyme-activity mutations detected in mice after paternal fractionated irradiation. *Mutation Research* 160, 243–248.

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

Davisson, M. T. & Roderick, T. H. (1989). Linkage map. In *Genetic Variants and Strains of the Laboratory Mouse* (ed. M. F. Lyon and A. G. Searle), pp. 416–427. Oxford: Oxford University Press.

Favor, J. (1984). Characterization of dominant cataract mutations in mice: penetrance, fertility and homozygous viability of mutations recovered after 250 mg/kg ethynitrosourea paternal treatment. *Genetical Research, Camb.* 44, 183–197.

Lyon, M. F. (1989). Mouse chromosome atlas. *Mouse News Letter* 84, 24–45.

Lalley, P. A. & McKusick, V. A. (1985). Report of the committee on comparative mapping. *Cytogenetics and Cell Genetics* 40, 536–566.

Leinward, L. A., Kozak, C. A. & Ruddle, F. H. (1978). Assignment of the genes for triose phosphate isomerase to chromosome 6 and tripeptidase-1 to chromosome 10 in *Mus musculus* by somatic cell hybridization. *Somatic Cell Genetics* 4, 231–240.

Merkle, S. & Pretsch, W. (1989). Characterization of triosephosphate isomerase mutants with reduced enzyme activity in *Mus musculus*. *Genetics* 123, 837–844.

Minna, J. D., Bruns, G. A. P., Krinsky, A. H., Lalley, P. A., Francke, U. & Gerald, P. S. (1978). Assignment of a *Mus musculus* gene for triosephosphate isomerase to chromosome 6 and for glyoxalase-I to chromosome 17 using somatic cell hybrids. *Somatic Cell Genetics* 4, 241–252.

Nadeau, J. H. & Taylor, B. A. (1984). Lengths of chromosomal segments conserved since divergence of man and mouse. *Proceedings of the National Academy of the USA* 81, 814–818.

Neuhauser-Klaus, A., Schäffer, E. & Pretsch, W. (1987). Characterization of a newly recovered Mi mutation. *Mouse News Letter* 78, 64.

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