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

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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 $0{\cdot}1 \pm 0{\cdot}1$ %.

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; Davisson & 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).

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2. Materials and methods

The mutations used in this study originated in different mutagenicity experiments. Male (102/El × C3H/El)F_1 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, GAPDH 525, GAPDH 577, GAPDH 3284, and GAPDH 28003 were designated $Tpi\text{-}1^{a\text{-}m1Neu}$ to $Tpi\text{-}1^{a\text{-}m4Neu}$ and $Gapd^{m1Neu}$ to $Gapd^{m4Neu}$, 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 ($Tpi\text{-}1^{a\text{-}m2Neu}$) was tested for recombination with *wa-1*, waved-1, and *Mi**, Microphthalmia (Neuhäuser-Klaus, Schäffer & Pretsch, 1987), a new mutation with a phenotype similar to Mi^b.

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

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animals could be clearly distinguished based on their different enzyme activities.

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 $Gapd^{m3Neu}$ 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 $Gapd^{m3Neu}$ 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 $0·1 \pm 0·1$% 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[a]	Offspring (*n*) Wild types	Hetero-zygotes	Homo-zygotes	Ratio heterozygotes/ wild-type animals
$Tpi\text{-}1^{a\text{-}m1Neu}$	B	$6·4 \pm 0·4$	256	243	—	0·95
	I	$5·2 \pm 0·4$*	92	218	0	2·37
$Tpi\text{-}1^{a\text{-}m2Neu}$	B	$6·6 \pm 0·4$	235	246	—	1·05
	I	$5·2 \pm 0·5$*	90	135	0	1·50
$Tpi\text{-}1^{a\text{-}m3Neu}$	B	$6·9 \pm 0·5$	331	329	—	0·99
	I	$5·3 \pm 0·5$*	97	143	0	1·47
$Tpi\text{-}1^{a\text{-}m4Neu}$	B	$6·5 \pm 0·4$	245	252	—	1·03
	I	$5·0 \pm 0·5$*	85	152	0	1·79
$Gapd^{m1Neu}$	B	$6·6 \pm 0·2$	225	195	—	0·87
	I	$6·0 \pm 0·1$*	60	113	0	1·88
$Gapd^{m2Neu}$	B	$6·6 \pm 0·2$	144	142	—	0·99
	I	$5·2 \pm 0·3$*	31	41	0	1·32
$Gapd^{m3Neu}$	B	$6·8 \pm 0·2$	193	206	—	1·07
	I	$5·8 \pm 0.2$*	76	170	3[b]	2·24
$Gapd^{m4Neu}$	B	$7·4 \pm 0·2$	196	162	—	0·83
	I	$5·4 \pm 0·1$*	42	75	0	1·79

[a] 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 *

[b] One male genetically confirmed; two males sterile.

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

Gapd allele	*Tpi-1* allele: a-m1Neu T+/++	+G/++	TG/++	++/++	a-m2Neu T+/++	+G/++	TG/++	++/++
m1Neu	31	32	0	0	26	16	0	0
m2Neu	—	—	—	—	34	39	0	0
m3Neu	12	13	0	0	—	—	—	—
m4Neu	—	—	—	—	—	—	—	—
Total	T+/++: 511				+G/++: 449			

Gapd allele	*Tpi-1* allele: a-m3Neu T+/++	+G/++	TG/++	++/++	a-m4Neu T+/++	+G/++	TG/++	++/++
m1Neu	—	—	—	—	79	101	0	0
m2Neu	6	10	0	0	122	83	0	1
m3Neu	—	—	—	—	97	113	0	0
m4Neu	—	—	—	—	104	42	0	0
Total	TG/++: 0				++/++: 1			

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

Tpi-1$^{a\text{-}m2Neu}$ and the testmarkers *wa-1* and *Mi** (Table 3). The map order and distance of the three tested genes is

wa-1–4.4 ± 1.6–*Mi**–13·8 ± 2·7–*Tpi-1*.

The recombination percentage between *wa-1* 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*$^{a\text{-}m2Neu}$ 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*$^{a\text{-}m2Neu}$*, triosephosphate isomerase-1, Mi*, microphthalmia, and wa-1, waved-1*
(parental mating: Tpi-1 Mi +/+ +wa-1 × ++ wa-1/++ wa-1)*

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

Recombination (*RF* ± S.E.)		
*Tpi-1–Mi**	22/160	13·8 ± 2·7
Mi–wa-1*	9/160	5·6 ± 1·8
Tpi-1–wa-1	27/160	16·9 ± 3·0

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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.

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