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An allele at the triose phosphate isomerase, *Tpi-1* locus on chromosome 6 recovered from feral mice

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Summary

An animal with low erythrocyte triose phosphate isomerase (TPI) activity was found amongst mice trapped on a farm in Leicestershire. The low TPI activity was caused by the segregation of a single co-dominant gene which also affected the K_m for glyceraldehyde-3-phosphate and heat stability of the enzyme. We designate the gene *Tpi-1*, the structural locus for TPI, with the *a* allele in the common inbred strains and the *b* allele derived from the wild-caught mouse. *Tpi-1* was known to be on chromosome 6 by somatic cell techniques and, as shown in a preliminary report (Peters & Bulfield, 1984), we have confirmed and extended this finding using three chromosome-6 marker genes giving the order: *Sig*-28-*Lc*-11-*Mi*^{wh}-16-*Tpi-1*.

1. Introduction

During the screening of wild-caught mice for genetic variation in the activity of 14 erythrocyte enzymes (Bulfield, Hall & Tsakas, 1984) a female was found with low activity of triose phosphate isomerase (TPI; EC 5.3.1.1). In this paper we describe the genetical and biochemical characterization of this variant and its assignment to the structural locus, *Tpi-1* on chromosome 6.

2. Materials and Methods

(i) *Mice*

The original female mouse with low erythrocyte TPI activity (designated: X2) was a *Mus musculus domesticus* trapped on a farm in Leicestershire; 17 other animals were trapped in the same area but none had low TPI activity (Bulfield *et al.* 1984).

(ii) *Triose phosphate isomerase assay*

This was performed at 340 nm on either a LKB reaction rate analyser (Bulfield & Moore, 1974) or on an Eppendorf ACP 5040 analyser (Charles & Pretsch, 1987). For the LKB instrument 100 μl heparinized blood samples were diluted in a buffer containing 0·05 M Tris, pH 7·4, 1 mM EDTA, 1 mM dithiothreitol, 0·1% Triton X-100. Tissues were homogenized in a buffer containing 0·25 M sucrose, 0·05 M Tris., pH 7·4, 1 mM EDTA, 1 mM dithiothreitol and centrifuged at 60000 rev./min for 1 h; the supernatant was used for the enzyme assay. TPI was assayed in 1 ml volume containing 0·1 M Tris, pH 7·4, 0·2 i.u. glycerophosphate dehydrogenase, 0·15 mM NADH, 2·5 mM glyceraldehyde 3-phosphate (injected last). The enzyme activity was expressed as mM NADH utilized min^{-1} g^{-1} wet wt. tissue or ml^{-1} blood at 30 °C.

For the Eppendorf instrument samples were prepared and assayed as described by Charles & Pretsch (1987). Blood samples of 100 μl were taken and the red cells lysed by a 1:11 dilution of the whole blood with a buffer containing 0·1 M triethanolamine pH 7·4, 1 mM EDTA, 5 mM dithiothreitol, 0·005% saponin. After standing for 10 min at 4 °C the haemolysates were centrifuged at 6000 rev./min (Beckman Microfuge 12) for 10 min; and the supernatants were diluted 1:1 in a buffer identical to the lysing buffer except that it did not contain saponin. Five μl of this 1:22 dilution was used for assay of TPI. Assays were carried out at 25 °C in a final volume of 355 μl containing 0·1 M Triethanolamine pH 7·4, 1 mM EDTA, 5 mM dithiothreitol, 2·4 i.u. glycerophosphate dehydrogenase, 0·41 mM NADH and 8·4 mM glyceraldehyde 3-phosphate (Ba salt) (added last). The enzyme activity was expressed as μmol NADH utilized min^{-1} g^{-1} haemoglobin. Haemoglobin was estimated in the ACP 5040 using the cyanmethaemoglobin method according to the manufacturer's instructions (Boehringer Corporation (London) Ltd).

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The variant enzyme was characterized by its heat stability, Michaelis Menten kinetics and pI. Heat stability of TPI was determined in blood or tissue homogenates for varying times at 50 °C, the K_m values for glyceraldehyde 3-phosphate were calculated by regression analysis. Erythrocyte and liver homogenates were electrofocused using a LKB Multiphor at 20 W for 4 h. The gels were stained with a 2% agar overlay using the same reaction mixture as for the enzyme assay; at least four isoenzymes were present in both tissues.

(iii) *Genetical analysis and mapping*

The original wild-caught female mouse (X2) was crossed to a C57BL/10 inbred male; the offspring were classified for TPI activity. The offspring with lower TPI activities were then intercrossed and a true-breeding low-TPI strain produced. Animals of this strain were than used to perform that formal segregation analysis; they were crossed to C57BL/10 animals and the F_1's backcrossed to both C57BL/10 and low TPI stock parents. For the linkage studies males of the TPI stock were crossed to females of the position testing stock SIG and F_1 females were backcrossed to TPI males. The SIG stock carries three chromosome-6 visible marker genes *Sig*, sightless, *Lc* lurcher, and *Mi*^{wh}, white.

3. Results

(i) *Segregation analysis*

As described in the Materials and Methods section, a true-breeding low-TPI-activity strain was produced. These animals were mated to C57BL/10 animals and the F_1's backcrossed to both parental strains. The distribution of erythrocye enzyme activities in back-cross animals (Fig. 1) shows a clear monogenic segregation of codominant alleles. The locus will be called *Tpi-1*, the allele in C57BL/10 (and all inbred strains of mice so far examined) *a*, and the allele causing low TPI activity, *b*. It is nccessary first to decide whether *Tpi-1* affects TPI structure and kinetics and hence is the structural locus, and secondly to map it.

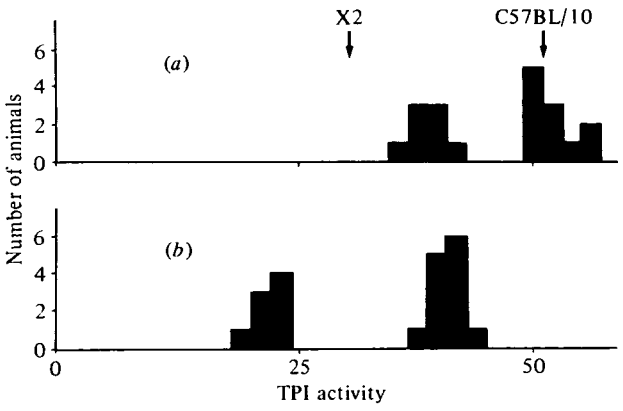

Fig. 1. Segregation analysis of erythrocyte TPI activities (*a*) backcross to C57BL/10 and (*b*) backcross to low TPI homozygotes; see text for details. Arrows show mean values of C57BL/10 animals and the original wild-caught mouse, X2 expressed as NADH utilized min^{-1} ml^{-1} blood at 30 °C.

(ii) *Biochemical characterization*

The activity and kinetics of TPI were analysed in homozygous *b/b* and *a/a* (C57BL/10) animals. The activity of TPI in *b/b* animals is 42% of that in *a/a* animals (Table 1; Bulfield *et al.* 1984) with K_m's for glyceraldehyde 3-phosphate differing significantly: $3{\cdot}51 \pm 0{\cdot}37$ mM (*b/b*; $n = 4$) and $1{\cdot}63 \pm 0{\cdot}14$ mM (*a/a*; $n = 4$).

There was also a significant difference in heat stability of TPI in tissue homogenates from *b/b* and *a/a* animals; this difference in stability holds for both erythrocyte and liver homogenates, even though there is a tissue difference in overall enzyme stability (Fig. 2). The difference in both K_m and heat stability between *a/a* and *b/b* homozygotes leads to the conclusion that *Tpi-1* is the structural locus for TPI.

(iii) *Mapping of Tpi-1*

For linkage testing, crosses were made of females of the SIG stock (*Sig Lc Mi*^{wh} *Tpi-1*^{a}/+ + + *Tpi-1*^{a}) and males of the low TPI stock. A four-point linkage test

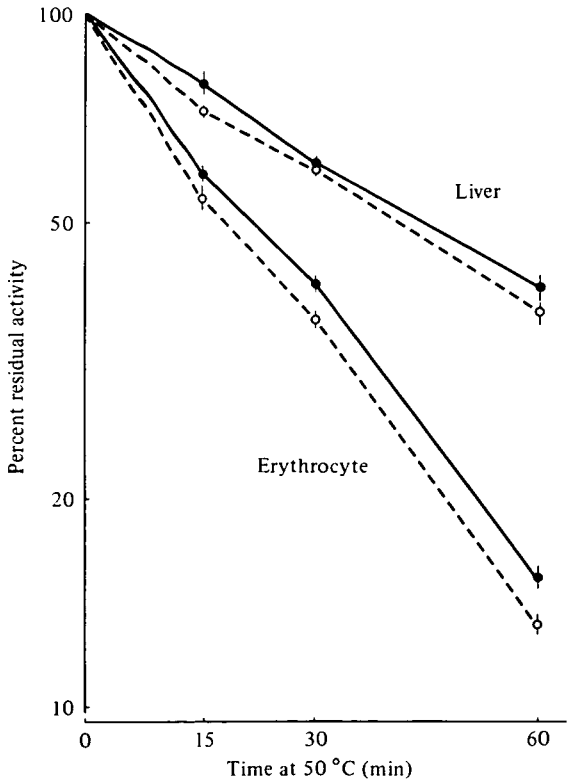

Fig. 2. Heat-stability of TPI from *Tpi-1*^{a}/*Tpi-1*^{a} (C57BL/10, ●) and *Tpi-1*^{b}/*Tpi-1*^{b} (○). See text for details.

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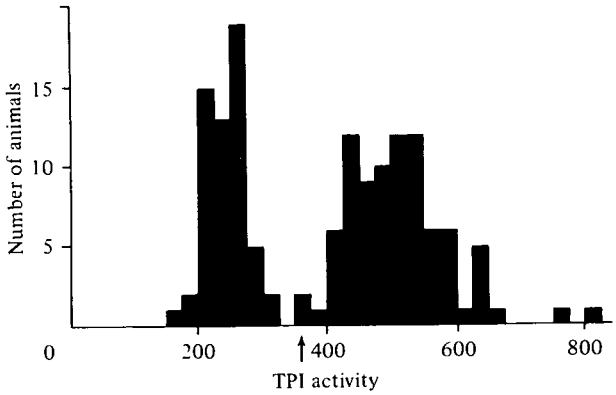

Fig. 3. TPI activities in erythrocytes from mice resulting from the backcross to the TPI stock. The arrow shows the values for the two mice excluded from the linkage analysis. Enzyme activities expressed as μmol NADH utilized min^{-1} g^{-1} haemoglobin at 25 °C.

was carried out by selecting *Sig Lc* Mi^{wh} *Tpi-1*^{a}/+ + + *Tpi-1*^{b} female offspring and backcrossing them to + + + *Tpi-1*^{b}/+ + + *Tpi-1*^{b} males of the TPI stock. The activity levels of erythrocyte TPI in the backcross offspring formed a bimodal distribution (Fig. 3). Of the 142 offspring analysed for TPI activity levels, 83 were classified as belonging to the high-activity TPI-1AB group and 57 to the low-activity TPI-1B group. Two mice could not be classified with certainty because the enzyme activity level fell in the region between the activities generally found in TPI-1B and TBI-1AB mice. These two were excluded from the linkage analysis. The mean activities ± S.E. were 513 ± 8·5 U g^{-1} Hb for TPI-1AB and 243 ± 4·2 U g^{-1} Hb for TPI-1B. An excess of offspring which were TPI-1AB was found, particularly amongst the male offspring,

Table 1. *Activity of TPI in tissue homogenates from C57BL/10 and mutant mice*

Tissue	*Tpi-1*^{a}/*Tpi-1*^{a} (C57BL/10)	*Tpi-1*^{b}/*Tpi-1*^{b}	Ratio *bb*/*aa*	Significance (P) (*t* test)
Blood	52·1 ± 1·79	21·8 ± 0·35	0·42	0·001
Liver	1063 ± 34·4	1174 ± 4·65	1·10	N.S.
Kidney	1409 ± 15·6	1285 ± 98·9	0·91	N.S.
Brain	655 ± 26·5	729 ± 40·5	1·11	N.S.

[a] Mean ± S.E. for five animals expressed as mM NADH utilized min^{-1} g^{-1} tissue of ml blood at 30 °C.

Table 2. *Recombination of* Sig, sightless, Lc, lurcher, Mi^{wh}, white *and Tpi-1, triose phosphate isomerase-1.*
(Parental mating *Sig*$_b$*Lc* Mi^{wh} *Tpi-1*^{a}/+ + + *Tpi-1*^{b} × + + + *Tpi-1*^{b}/+ + + *Tpi-1*^{b}.)

Progency class	Progeny genotype *Sig*	*Lc*	Mi^{wh}	*Tpi-1*	Number
Parental or non-recombinant	*Sig*+	*Lc*+	Mi^{wh}+	*ab*	39
	++	++	++	*bb*	30
Single recombinant 1, *Sig-Lc*	*Sig*+	++	++	*bb*	12
	++	*Lc*+	Mi^{wh}+	*ab*	23
Single recombinant 2, *Lc-*Mi^{wh}	*Sig*+	*Lc*+	++	*bb*	3
	++	++	Mi^{wh}+	*ab*	9
Single recombinant 3, Mi^{wh}*-Tpi-1*	*Sig*+	*Lc*+	Mi^{wh}+	*bb*	8
	++	++	++	*ab*	10
Double recombinant 1, *Sig-Lc*, *Lc-*Mi^{wh}	*Sig*+	++	Mi^{wh}+	*ab*	0
	++	*Lc*+	++	*bb*	1
Double recombinant 2, *Sig-Lc*, Mi^{wh}*-Tpi-1*	*Sig*+	++	++	*ab*	0
	++	*Lc*+	Mi^{wh}+	*bb*	2
Double recombinant 3, *Lc-*Mi^{wh}, Mi^{wh}*-Tpi-1*	*Sig*+	*Lc*+	++	*ab*	1
	++	++	Mi^{wh}+	*bb*	1
Triple recombinant, *Sig-Lc*, *Lc-*Mi^{wh} MI^{wh}*-Tpi-1*	*Sig*+	++	Mi^{wh}+	*bb*	0
	++	*Lc*+	++	*ab*	1
Total					140

	Recombination RF ± SE	
Sig-Lc	39/140	27·8 ± 32·7
Lc-Mi^{wh}	16/140	11·4 ± 2·7
Mi^{wh}*-Tpi-1*	23/140	16·4 ± 3·1

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where there were 48 TPI-1AB:28 TPI-1B ($\chi^2 = 5{\cdot}26$, 1 D.F., $P = 0{\cdot}0218$). The reason(s) for this are unclear.

The results of the linkage test (Table 2) indicated that the order of the four genes was as shown. Subsequent to an initial report of this experiment (Peters & Bulfield, 1984) three additional mice have been scored and corrections to the numbers of the recombinants between *Sig* and *Lc* have been made. With this order all except six offspring could be explained by the occurrence of only a single crossover. Five of the remaining six would require a double crossover and the sixth would require a triple crossover. With any other order of loci the number of postulated crossovers would be greater. The recombination percentages between *Sig* and *Lc* and between *Lc* and Mi^{wh} are in agreement with the mouse linkage maps (Davisson & Roderick, 1986). The data give a recombination between Mi^{wh} and *Tpi-1* in females of 23/140 or $16{\cdot}4 \pm 3{\cdot}1\%$ and the map order is *Sig*–28–*Lc*–11–Mi^{wh}–16–*Tpi-1*.

(iv) *Tissue distribution*

The activity of TPI has been determined in four tissues from *a/a* and *b/b* homozygotes (Table 1). The activity of TPI is altered only in erythrocytes of *b/b* animals remaining unaltered in liver, kidney and brain homogenates.

4. Discussion

We report the recovery and characterization of an allele at the triose phosphate isomerase structural locus, *Tpi-1*, from a wild caught mouse. This allele is rare in British *Mus musculus domesticus* as it is not present in the 17 other mice trapped in the same area of Leicestershire nor in another 114 *M. m. domesticus* or 41 M. m. musculus wild-caught animals, 173 in total, a frequency of 0·0058 (Bulfield *et al.* 1984). This compares with an incidence of 0·0104 for low activity TPI variants in man (Mohrenweiser, 1981).

Although homozygotes for the variant *b* allele had 42% of the erythrocyte TPI activity of laboratory animals (C57BL/10) they did not show any clinical signs of anaemia or any significant alterations in haematological parameters (G. Bulfield, unpublished observations). This is to be expected as in most cases of human TPI deficiency with clinical symptoms the erythrocyte enzyme activity is below 20% of normal (Schneider *et al.* 1968*a*, *b*; reviews of cases by Eber *et al.* 1979; Rosa *et al.* 1985).

Homozygotes for the *b* alleles not only have 42% of normal erythrocyte TPI activity but they also have a lower enzyme heat stability in both erythrocytes and liver and a higher K_m for glyceraldehyde 3-phosphate than *a/a* animals. These alterations in enzyme structure in *b/b* animals indicate that *Tpi-1* is the structural locus for the enzyme.

Tpi-1 had been assigned to mouse chromosome 6 by somatic cell genetic techniques (Leinwand *et al.* 1978; Minna *et al.* 1978) and the linkage data reported here and in a preliminary account of this experiment (Peters & Bulfield, 1984), place *Tpi-1* in the distal half of the chromosome in a similar region to *Ldh-2* lactate dehydrogenase-2 (Peters & Andrews, 1985). *Tpi-1* and *Ldh-2* are linked in all sixteen mammalian species studied so far (Lalley & McKusick, 1985), and in addition, in the eleven mammalian species in which *Gapd* (glyceraldehyde 3-phosphate dehydrogenase) has been assigned, there has been linkage conservation of *Tpi-1*, *Gapd* and *Ldh-2* (Lalley & McKusick, 1985). At least 40 conserved autosomal sequences with two or more loci are known in mouse and man (Searle *et al.* 1987). The conserved sequence on the short arm of chromosome 12 in man and on chromosome 6 in the mouse contains *Kras*-2 (Kirsten rat sarcoma virus oncogene) as well as *Tpi*-1, *Gapd* and *Ldh*-2. The linear order of these three genes on human chromosome 12 has been established by Law & Kao (1982) to be TPI1-GAPD-LDHB-centromere. The order of the three genes remains to be determined in the mouse, and is a point of interest in studies of the genetic changes that have occurred during the evolution of the murine and human genomes. Conserved sequences may contain genes in the same or a different linear order in the two species or intercalation of one or more loci from other chromosomes within a conserved group may occur.

One unusual feature of *b/b* homozygotes is that, although they have low erythrocyte TPI activity they do not have altered activity in liver, kidney or brain. This is despite the evidence that there is a single active structural gene for TPI in man (Peters, Hopkinson & Harris, 1973; Decker & Mohrenweiser, 1981; Asakawa & Iida, 1985; Brown *et al.* 1985) and presumably also in the mouse, and that the enzyme in *b/b* animals has an altered heat stability both in liver and in erythrocytes (Fig. 2). It is quite possible that the difference in TPI activity in *b/b* animals between liver and erythrocytes is due to active enzyme synthesis and turnover in the liver and lack of synthesis in erythrocytes. This situation has been previously found in *c/c* homozygotes of *Gpi-1* which have a heat-labile enzyme (glucosephosphate isomerase) with low steady-state enzyme activity in erythrocytes and near normal activities in liver, kidney and brain (Padua, Bulfield & Peters, 1978).

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