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Phosphofructokinase Activity in Normal Diploid Mice during Development and in Trisomy 16 Fetal Mice

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ABSTRACT

Although several of the genes mapped to human chromosome 21 have been assigned to mouse chromosome 16, it has not yet been possible to do this for the gene for the phosphofructokinase liver type subunit (PFKL). The goal of this study was to determine if there is a 1.5-fold increase of the PFK activity in fetal trisomy 16 mice, which, if present, would be indicative of a gene doasage effect. However, rather than an increase, an almost 100% decrease of the mean PFK activity was observed in fetal trisomy 16 liver at both 14 and 17 days of gestation when compared to littermate controls. This is the first biochemical abnormality detected in trisomy 16 fetal liver. In contrast, no significant differences in the mean PFK activity in homogenized whole fetus or brain trisomy 16 and diploid controls were observed. A developmental maturational effect of the PFK activity was observed in fetal liver from normal diploid mice, with a 3-fold increase of the activity from day 14 to day 18 of gestation and a further 2-fold increase to adulthood. The decreased PFK activity in fetal trisomy 16 liver may therefore be the result of delayed maturation of the liver in trisomic fetuses.

INTRODUCTION

The enzyme, phosphofructokinase (PFK) (EC 2.7.1.11), is under the control of three structural loci coding for muscle (M), liver (L), and platelet (P) subunits. The respective genes have been mapped to three different human chromosomes, PFKP to chromosome 10 (19), PFKM to chromosome 1 (22) and PFKL to chromosome 21 (21). The last has been mapped to the distal part of the long arm of chromosome 21 (5, 17). This is of special interest since trisomy for this part of chromosome 21 is associated with the phenotype of Down syndrome (13). The enzyme activity of PFK has been shown to be 50% increased in red cells (21, 3, 4, 14) and in fibroblasts (1).

Several of the genes mapped to human chromosome 21 have also been assigned to mouse chromosome 16. These loci include superoxide dismutase-1 (Sod-1) (6, 15), the

interferon-a/B receptor (Ifrc) (6, 15), phosphoribosylglycinamide synthetase (Prgs) (7) and the oncogene Ets-2 (18). Although there is significant homology between the distal part of mouse chromosome 16 and distal part of human chromosome 21, it has not yet been possible to map the gene for PFKL to the mouse chromosome 16 or any other mouse chromosome. Therefore, since the mouse with trisomy 16 has been developed as an animal model for Down syndrome (10), we studied the enzyme activity of PFK in different organs of fetal trisomy 16 mouse (brain, whole embryo, and liver) and in littermate diploid controls. The goal of this investigation was to determine if there is a 1.5-fold increase of the PFK activity in fetal liver of trisomy 16 mice, which, if present, could be indicative of a gene doasage effect. Such an increase has been found for superoxide dismutase-1 (1). We have also studied the activity in liver from normal diploid mice of different gestational ages in order to evaluate the variation of the PFK with age, since trisomy 16 mice are developmentally delayed (10).

MATERIAL and METHODS

Breeding of mice and preparation of tissue

Male mice, double heterozygous for two different Robertsonian translocation chromosomes, (Rb(16.17)32Lub and Rb(11.16)2H, each of which contains chromosome 16 within a metacentric chromosome, were mated to superoulated CD1 female mice containing a normal set of acrocentric chromosomes (10, 12). After 14 or 17 days of gestation, the pregnant mice were sacrificed. Trisomy 16 fetuses were identified by their phenotypic abnormalities, which included reduced size and massive edema (10). The whole fetus or fetal brain or liver was homogenized in ice-cold *Tris-HCl buffer (50mM, pH 7.6) at a tissue to buffer ratio of 19:1 (wt/vol) (2-4 mg protein/ml).

Phosphofructokinase (PFK) activity assay

Assay of PFK was performed with a Gilford response spectrophotometer at 26°C as described by Vora et al. (20). The reaction mixture (1.0 ml) contained 50 mM tris(hydroxymethyl)methylglycin at pH 8.4, 5 mM $MgCl_2$, 0.15 mM NADH, 0.1 mM dithiothreitol, 0.25 mM ATP, 2.4 mM Fru-6-P, 0.18 unit of aldolase, 0.6 units of triose phosphate isomerase, and 0.1 units of glycerophosphate dehydrogenase. The reaction was started by adding 0.1 ml of the enzyme preparation to 0.9 ml of the assay mixture. The decrease in absorbance at 340 nm was recorded for 15 min against a blank from which PFK was omitted. PFK was expressed against a standard of rabbit liver PFK (Sigma, F 8134) as U/g total protein. Protein was measured according to the micromethod of Lowry et al. (16) with bovine serum albumin as standard.

RESULTS

The PFK activities in littermate diploid and trisomy 16 fetuses and in fetal brain and liver are shown in Figure 1. There were no significant differences in the mean PFK

activity in homogenized whole fetus or brain between trisomy 16 and diploid controls. However, an almost 100% decrease of the mean PFK activity was observed in fetal trisomy 16 liver of both 14 ($p<0.0001$) and 17 ($p<0.001$) day gestational ages when compared to littermate controls. The specific PFK activity in normal diploid mice of 17 day gestation was 6-times greater in the whole fetus and 3-times greater in the brain than in the liver.

To determine if the decreased PFK activity in livers of trisomy 16 fetuses was due to a developmental maturational effect, PFK activity was studied in liver from fetuses of different gestational ages and in adult liver (Fig. 2). The PFK activity increased 3-fold from day 14 to day 18 of gestation ($p<0.0001$), and there was a further 100% increase in the PFK activity in liver from gestational day 18 to adulthood ($p<0.0001$).

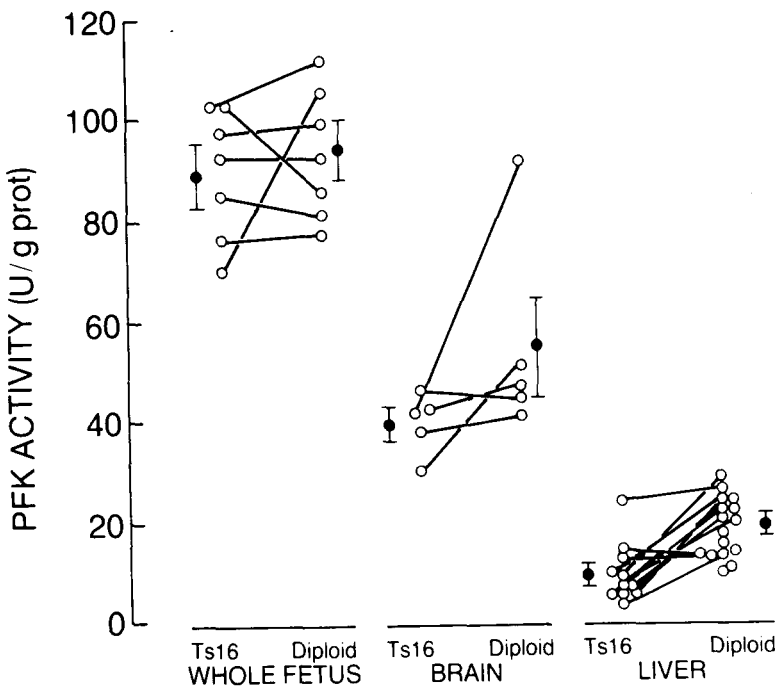

Fig. 1. The PFK activity (U/g protein) of homogenized whole body, brain and liver of trisomy 16 and matched littermate fetuses at 17 days of gestation. The means ± S.E. are shown.

DISCUSSION

The ratios of the levels of PFK activity in mouse of homogenized whole fetus, brain and liver were 6:3:1, which is consistent with the finding in rat of the ratios of the PFK level in muscle, brain and liver of 10:7:1 (9). In mice there was no difference between trisomy 16 and diploid fetuses of the PFK activity of either the whole fetus or

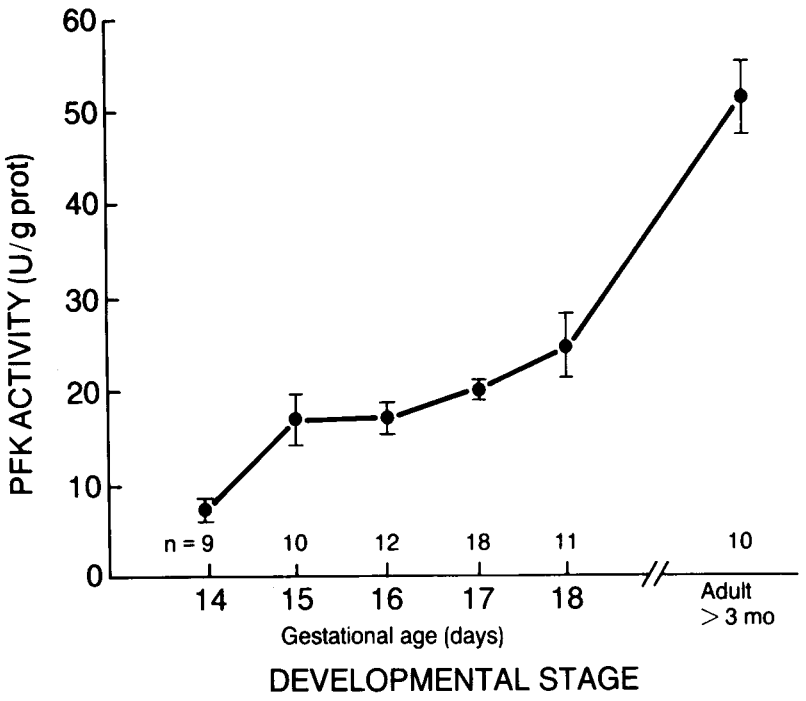

Fig. 2. Developmental pattern of the PFK activity in homogenized liver of normal diploid mice. The means ± S.E. are shown.

the brain. However, this result cannot be considered as indicating that the gene for PFKL is not on chromosome 16, since M subunits predominate in muscle (whole fetus) and M and P subunits predominate in brain (9).

PFK activity was decreased in fetal trisomy 16 mouse liver compared to littermate controls. This decreased activity may be related to the enormous increase in PFK activity with fetal age in liver, coupled with the delayed maturation of the trisomy 16 fetuses. It seems reasonable to assume that the increasing PFK activity with age is principally due to a change of the PFKL subunit and not to changes with age of the other two subunits. In humans, Davidson et al. (8) have shown that there is a developmental change with increasing age, especially in muscle in which all PFK subunits are represented in early development but only the PFKM subunit is represented in the adult. In human liver, however, the PFKL subunit predominates throughout all prenatal stages of development.

Even though this study has not established the location of the gene for PFKL subunit relative to chromosome 16 in mouse, it has shown that the trisomic condition does have a negative effect on the activity of PFKL in the fetal liver. This may be attributed partly, if not wholly, to delayed maturation of the liver. This is the first

biochemical abnormality detected in the fetal trisomy 16 liver, which has already been shown to be deficient in a variety of hematopoetic stem cells (11).

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