Genet. Res., Camb. (1993), **62**, *pp.* 39–43 *With 4 text-figures*

Parental origin of mutant allele does not explain absence of gene dose in X-linked *Hyp* mice

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Summary

The expectation for a gene dose effect in an X-linked phenotype is that the corresponding metrical trait in heterozygous females will lie between values for affected hemizygous males and unaffected males and females. We made sequential measurements (at 30, 60, 90, 120 and 150 days) of serum phosphate concentration and tail length in mice with X-linked hypophosphatemia (genotypes: *Hyp*/Y, *Hyp*/+ and *Hyp*/*Hyp*) and in their normal litter-mates (genotypes: +/Y, +/+). We also measured renal mitochondrial 25-hydroxyvitamin D_3-24-hydroxylase (24-hydroxylase) activity in 5 to 7-month-old mice fed control and low phosphate diets and representing all five genotypes. The animals were obtained by controlled breeding under uniform environmental conditions. The mutant animals all had uniformly and significantly lower serum phosphate levels, shorter tail length and higher 24-hydroxylase activity relative to unaffected litter-mates. There was no evidence of a gene dose effect because values were not significantly different among the three mutant genotypes. We also studied the influence of gamete of origin on serum phosphate, tail length and renal mitochondrial 24-hydroxylase activity in the *Hyp*/+ offspring of affected males (*Hyp*/Y) or affected females (*Hyp*/+ or *Hyp*/*Hyp*). We found no effect on the distribution of trait values. We conclude that parental origin of mutant allele does not explain the absence of a gene dose effect in *Hyp* mice.

1. Introduction

X-linked hypophosphatemia (XLH), the most common type of genetic hypophosphatemia in humans, is inherited as a dominant phenotype (Rasmussen & Tenenhouse, 1989; Scriver & Tenenhouse, 1990; Scriver *et al.* 1991). The gene maps to the X chromosome, region Xp22.1-p22.2 (Thakker *et al.* 1990; Econs *et al.* 1992), but the product of the gene is still unknown. The associated functional defect results in the inability of the proximal renal tubule to reabsorb filtered phosphate efficiently with phosphate wasting, severe hypophosphatemia and impaired mineralization of bone and teeth. The disease is characterized by short stature, rickets in the child and osteomalacia in the adult. Both plasma calcium and PTH concentrations are normal in patients with XLH (Arnaud *et al.* 1971).

The *Hyp* mutation, which maps to a homologous region on the mouse X chromosome, produces a phenotype similar to that of the human disease and thus provides a useful animal model to examine the genetic and biochemical mechanisms for hypophosphatemic rickets (Eicher *et al.* 1976; Scriver & Tenenhouse, 1990; Tenenhouse & Scriver, 1992). *Hyp* mice are characterized by a specific defect in Na^+-dependent phosphate transport at the renal brush-border membrane (Tenenhouse & Scriver, 1978) which is not dependent on PTH (Cowgill *et al.* 1979) and can account for the hypophosphatemia. In addition, *Hyp* mice exhibit abnormal regulation of renal vitamin D metabolism which is associated with increased renal mitochondrial 25-hydroxyvitamin D_3-24-hydroxylase (24-hydroxylase) activity (Tenenhouse *et al.* 1988). The abnormality in 24-hydroxylase in *Hyp* mice is exacerbated by phosphate deprivation which, in normal litter-mates, has no effect on enzyme activity (Tenenhouse & Jones, 1990). 24-Hydroxylase catalyzes the first reaction of the C-24 oxidation pathway which is responsible for the degradation of 1,25-dihydroxyvitamin D_3, the hormonally active form of vitamin D (Jones *et al.* 1987).

The expectation for a gene dose effect in an X-linked trait is that carrier females have a more variable and less severe phenotype than affected males.

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Phenotypic variation in carrier females is attributed to random inactivation of the X chromosome (Lyon, 1988). XLH patients show some evidence of a gene dose effect in their clinical signs, radiographic features (Winters *et al.* 1958; Reid *et al.* 1991) and secondary dental development (Shields *et al.* 1990). But apparent absence of a gene dose effect is the more prominent observation both in XLH patients and in *Hyp* mice (Scriver & Tenenhouse, 1990). For example, the age-specific serum phosphate values are not truly different in untreated affected male and female patients (Winters *et al.* 1958), and in male and female *Hyp* mice (Eicher *et al.* 1976). Direct measures of Na^+-phosphate cotransport in renal brush-border membrane vesicles yield similarly depressed values in *Hyp*/+ and *Hyp/Hyp* female mice (Scriver & Tenenhouse, 1990). The reason for absence of a gene dose effect on these kidney-related parameters in XLH patients and *Hyp* mice is still unknown.

In this study, we measured the serum phosphate concentration, tail length and renal 24-hydroxylase activity in three *Hyp* genotypes (*Hyp*/Y, *Hyp*/+, *Hyp/Hyp*) and in their normal litter-mates (+/Y, +/+) under uniform environmental conditions. We also compared serum phosphate values, tail length and 24-hydroxylase activity in the *Hyp*/+ offspring of affected male (*Hyp*/Y) or affected female (*Hyp*/+ or *Hyp/Hyp*) transmitting parents. We show that there is no gene dose effect on serum phosphate values, tail length, and renal 24-hydroxylase activity and that gamete of origin does not explain this finding.

2. Materials and methods

(i) *Animals*

All mice were bred and raised at the Animal Research Centre of the Montreal Children's Hospital. The original breeding pairs (*Hyp*/+ × +/Y on C57BL/6J background) were obtained from the Jackson Laboratory (Bar Harbour, ME). The mice were kept on a 12-h light/dark cycle at constant room temperature (72 °C). Animals were fed Teklad-Wayne Breeder Blox (no. 8626, Teklad, Madison, WI) and received tap water *ad libitum*. To examine the effect of gene dose on renal mitochondrial 24-hydroxylase activity, mice were fed control (1% phosphate) or low phosphate (0·03% phosphate) diets (test diets 86128 and 86129, Teklad, Madison, WI) for 5 days before killing. To generate progeny and maximize animal husbandry standards, we used harem breeding in standard mouse shoe-box cages and filter bonnets. Mice were weaned at the age of 25 days and genotyped by serum phosphate levels.

(ii) *Breeding strategies*

To measure the gene dose effect and to determine whether gamete of origin or the transmitting parent influences phenotype, we used the following breeding strategies:

(*a*) for normal animals: +/+ × +/Y;
(*b*) for normal and affected animals: *Hyp*/+ × +/Y;
(*c*) for *Hyp*/+ offspring of *Hyp*/Y: +/+ × *Hyp*/Y;
(*d*) for *Hyp*/+ offspring of *Hyp/Hyp*: *Hyp/Hyp* × +/Y;
(*e*) for homozygous mutant females: *Hyp/Hyp* × *Hyp*/Y.

Body weight, tail length and serum phosphate levels were measured at 30, 60, 90, 120 and 150 days of age on 6–12 mice per genotype. To show whether maternal hypophosphatemia affects serum phosphate and tail length in normal offspring, we compared parameters between normal males and between normal females derived from breeding strategies (*a*) and (*b*). To test for a gene dose effect, we compared parameters in normal females (+/+), normal males (+/Y), heterozygous females (*Hyp*/+), and mutant hemizygotes (*Hyp*/Y) obtained from breeding strategy (*b*) and homozygous *Hyp* females (*Hyp/Hyp*) from strategy (*e*). The effect of gamete of origin was studied in heterozygotes derived from a transmitting dam [strategies (*b*) and (*d*)] or sire [strategy (*c*)].

(iii) *Measurement*

(*a*) *Analytical.* To minimize circadian variation, bloods, obtained by retro-orbital puncture, were always drawn in the afternoon. We measured tail length on restrained mice and body weight on a Mettler 6000 balance. We used a kit for the quantitative determination of serum inorganic phosphate (Stanbio Lab. Inc., Texas). For measurement of 24-hydroxylase activity, mitochondria, prepared from renal cortex of individual mice, were incubated with 50 nM [^{3}H]-25-hydroxyvitamin D_3 (Amersham, Canada) under initial rate conditions as described previously (Tenenhouse & Jones, 1987).

(*b*) *Statistical.* All data were analyzed using a Statview 512 program by a single factor factorial Anova Scheffe *F* test. The level of significance was set at $P < 0{\cdot}05$.

3. Results

(i) *Serum phosphate*

Normal offspring of normal females (+/+) and heterozygotes (*Hyp*/+) have similar serum phosphate values at 30 days and thereafter (data not shown). From these data, we concluded that maternal phenotype does not affect the serum phosphate concentration of normal males and females after weaning. Sequential analysis of serum phosphate over 150 days demonstrated that all mutant mice have serum

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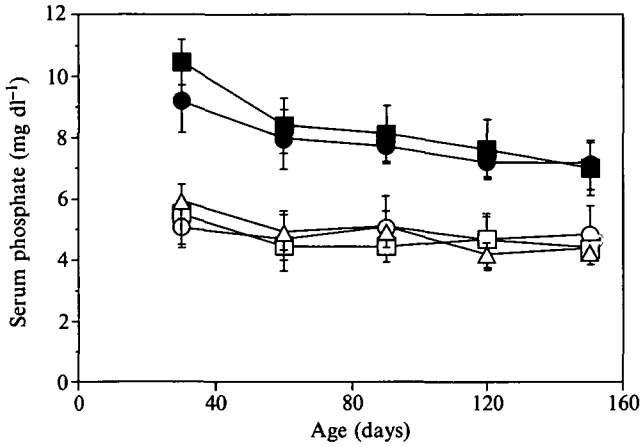

Fig. 1. Serum phosphate concentration in +/Y (■), +/+ (●), *Hyp*/Y (□), *Hyp*/+ (○) and *Hyp*/*Hyp* (△) mice. Means ± S.D. derived from at least 6 mice are shown. Values are significantly different between normal and mutant genotypes but not among mutant genotypes.

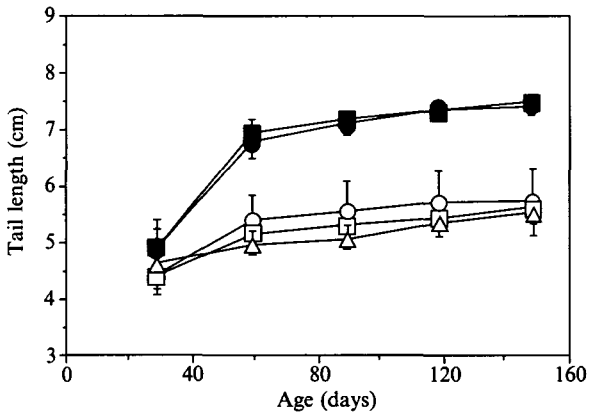

Fig. 2. Tail length of +/Y (■), +/+ (●), *Hyp*/Y (□), *Hyp*/+ (○) and *Hyp*/*Hyp* (△) mice. Means ± S.D. derived from at least 6 mice are shown. Values are significantly different between normal and mutant genotypes but not among mutant genotypes.

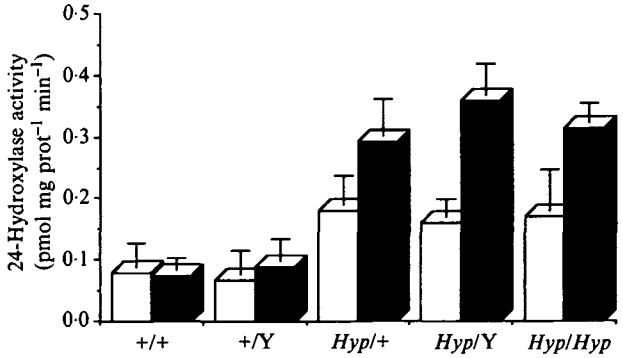

Fig. 3. Renal mitochondrial 24-hydroxylase activity in +/Y, +/+, *Hyp*/Y, *Hyp*/+ and *Hyp*/*Hyp* mice fed the control (□) or low phosphate (■) diets. Each bar depicts the mean ± S.D. derived from 5 mice. Values are significantly different between normal and mutant genotypes but not among mutant genotypes.

phosphate values significantly lower than unaffected animals (Fig. 1). However, values at all ages are not significantly different in *Hyp*/Y, *Hyp*/+ and

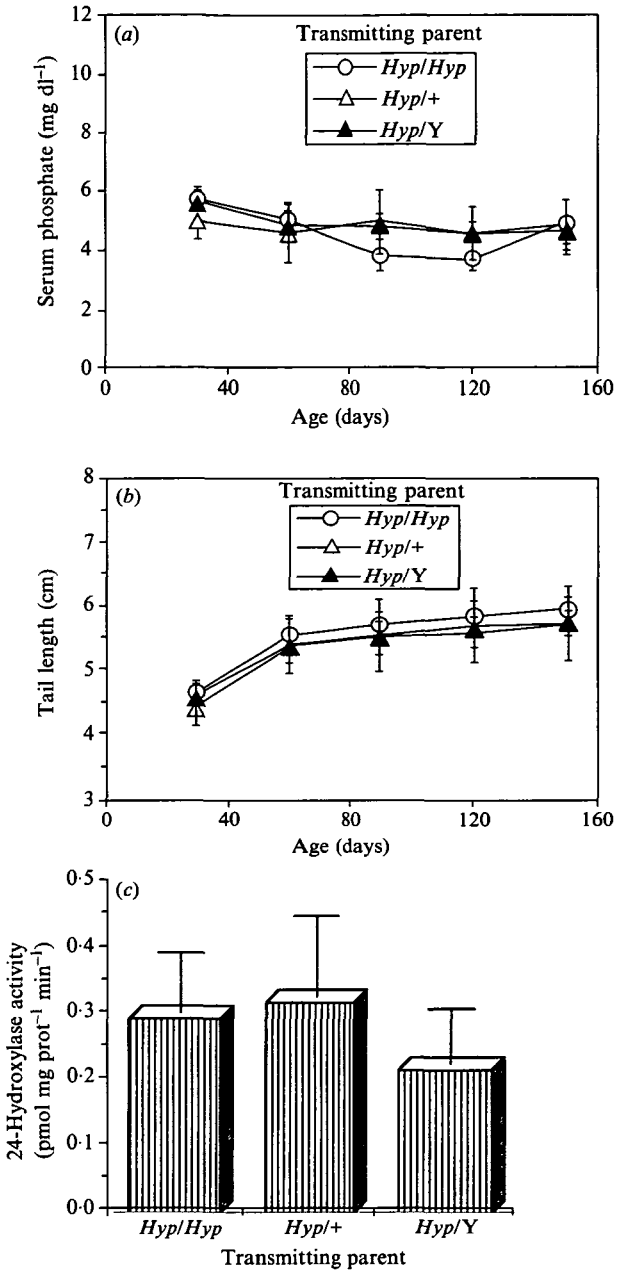

Fig. 4. Serum phosphate concentration (*a*), tail length (*b*) and renal mitochondrial 24-hydroxylase activity (*c*) in *Hyp*/+ offspring of +/+ × *Hyp*/Y (▲), *Hyp*/+ × +/Y (△) and *Hyp*/*Hyp* × +/Y (○). Means ± S.D. derived from at least 6 mice are shown. 24-Hydroxylase activity was measured in renal mitochondria of *Hyp*/+ mice fed the low phosphate diet.

Hyp/*Hyp* animals (Fig. 1). Absence of a gene dose effect on serum phosphate levels is apparent in these findings.

(ii) *Tail length*

Mutant mice have significantly shorter tails than normal mice after the age of 30 days and there is no evidence of a gene dose effect, i.e. tail length is similar in all three mutant genotypes (Fig. 2).

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(iii) *Renal mitochondrial 24-hydroxylase activity*

Renal 24-hydroxylase activity is significantly higher in mutant animals than in normals fed either control or low phosphate diets (Fig. 3). While the low phosphate diet has no effect on 24-hydroxylase activity in normal mice (+/Y, +/+), the mutant mice (*Hyp*/Y, *Hyp*/+ and *Hyp*/*Hyp*) respond to phosphate deprivation with a significant increase in 24-hydroxylase activity (Fig. 3); a gene dose effect is not apparent.

(iv) *Gamete of origin*

To determine whether absence of gene dose in *Hyp* mice could be ascribed to parental origin of the mutant allele, we compared serum phosphate values, tail length and renal 24-hydroxylase activity in the *Hyp*/+ offspring of either affected males (*Hyp*/Y) or affected females (*Hyp*/+ or *Hyp*/*Hyp*). Serum phosphate (Fig. 4*a*), tail length (Fig. 4*b*) and renal 24-hydroxylase activity (Fig. 4*c*) were similar in all three groups of *Hyp*/+ females. The finding implies that gamete of origin does not influence the metrical trait.

4. Discussion

In the present study, we investigated the effect of gene (*Hyp*) dose in mice with X-linked hypophosphatemia. Sequential post-weaning measurements of serum phosphate concentration and tail length showed that mutant hemizygotes, heterozygotes and mutant homozygotes all have values that are deviant from normal but not different among themselves. In addition, we demonstrated that renal mitochondrial 24-hydroxylase activity is equivalently elevated in all three mutant genotypes when compared to activity in normal mice. Absence of a gene dose effect is again apparent. A gene dose effect in the *Hyp* mouse has not been extensively investigated heretofore but its absence in three different parameters corroborates our own preliminary earlier findings (Scriver & Tenenhouse, 1990), the provocative evidence of Eicher *et al.* (1976) and the observations of Kay *et al.* (1991).

Several possible mechanisms for absence of a gene dose effect in *Hyp* mice have been offered (Scriver & Tenenhouse, 1990). These include (i) functional inactivation by a dominant negative mutation (Herskowitz, 1987), (ii) random inactivation of X chromosomes followed by selection of the mutant clone (Broadhead *et al.* 1986), (ii) allelic restriction (Coleclough *et al.* 1981), and (iv) selective imprinting determined by parental origin of the mutant allele (Sapienza *et al.* 1987). Here we show that the latter mechanism does not account for the absence of a gene dose effect in *Hyp* mice. The second hypothesis is unlikely to explain data consistent in a population. The first and third are remote possibilities here.

The present findings remain consistent with a humoral basis for X-linked hypophosphatemia. Parabiosis (Meyer *et al.* 1989*a*, *b*) and renal transplantation (Nesbitt *et al.* 1992) studies have provided evidence for a transacting factor which inhibits renal phosphate transport and disturbs the regulation of renal vitamin D metabolism in *Hyp* mice. The nature of the factor and whether the factor is the product of the X-linked *Hyp* gene remain unknown at present.

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