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Recovery of Normal Growth in Spontaneous Dwarf Rats (*dr*) by Targeted Expression of the Human GH Transgene to the Pituitary Gland

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A STRAIN of spontaneous dwarf rat (gene symbol ***dr***) with autosomal recessive inheritance was discovered in Japan [1], and has been well characterized by others [2–4] and ourselves [5, 6]. The genetic cause of isolated GH deficiency in the strain of ***dr*** was identified as a point mutation at the splice acceptor site of the rat GH gene, i.e., at the junction between the third intron and the forth exon [7, 8]. We treated ***dr*** by germline incorporation of a chimeric gene of the mouse metallothionein-1 gene promoter and human GH gene [9], which was ubiquitously expressed in ***dr*** and resulted in rat gigantism [10].

Previous experiments in transgenic mice have shown that a chimeric gene (designated as PGGB) of the rGH gene 5′-flanking promoter (1.7 kbp upstream gene segment from the start point of the rGH gene transcription) and the genomic hGH gene (2.1 kbp) was required to increase the hGH transgene expression of the pituitary to levels approaching that of the mouse GH gene [11, 12]. We have established a strain of transgenic Sprague-Dawley rats (PGGB-SD), which bears PGGB in the germ cell [13]. Our studies have demonstrated that the upstream 1.7 kbp fragment of the rat GH gene increases the hGH transgene expression to levels approaching that of the rGH gene, and suggested a posibility that the targeted and regulated transgene expression in the pituitary is mandatory for normal growth recovery in ***dr***.

Here we report a successful treatment of dwarf phenotype in ***dr*** by targeting expression of the human GH gene to the pituitary gland of ***dr***.

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Materials and Methods

A chimeric gene (PGGB) of the rat GH gene promoter with human GH was first transmitted from PGGB-bearing Sprague-Dawley rats (PGGB-SD) to ***dr*** by crossing a male PGGB-SD (Tg ID 2846) with homozygous female ***dr*** (***dr*** +/+, ***dr*** ID 322) (Fig. 1). A strain of 2846 showed the hGH gene expression specific to somatotrophs and mammosomatotrophs, but not thyrotrophs, as assessed by the immunohistochemistry for hGH [13]. Heterozygous ***dr*** (***dr*** +/−), which bore both PGGB- and ***dr***-gene, was then crossed with homozygous ***dr*** (***dr*** +/+). Since GH secretion is pulsatile in nature and shows age- and sex-related developmental changes [14], a line of both male and female PGGB-bearing transgenic ***dr*** (PGGB-***dr***) was established and maintained by measuring both rGH and hGH levels in plasma under light ether anesthesia on day 21 and day 50 after birth [15].

We assayed for rGH and hGH by specific RIAs using reagents supplied by the NIDDK [16]. The least detectable values for both RIAs were 2 ng/ml. PGGB-***dr*** of both sexes showed detectable levels of

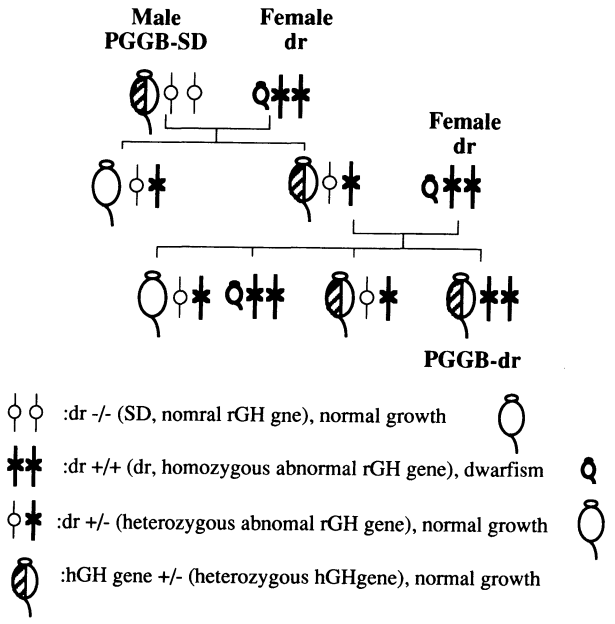

Fig. 1. A schematic pedigree of the generation of PGGB-*dr*. Rats that carry the heterozygous PGGB gene (a chimeric gene of the −1.7 kbp rGH gene promoter and the 2.1 kbp of the hGH gene) (PGGB-SD) and the homozygous normal rGH gene were crossed with female *dr* that carry the homozygous mutant rGH gene). The heterozygous PGGB-*dr* that carry the heterozygous PGGB gene and the heterozygous *dr* gene were then crossed with *dr*. A line of homozygous PGGB-*dr* was selected and maintained by measuring rGH and hGH levels in plasma on day 21 and day 50 after birth.

hGH in plasma with at least one blood sampling either on day 21 or day 50, while no rGH was detected in plasma on any sampling days. The second and third generations of PGGB-*dr* were used for the analysis of body growth. Aged and sex matched SD and *dr* were used as controls. Individual rats of each group were weighed at weaning on day 21 and there after at 10 days intervals until 2 months of age. Group differences in growth curves of both sexes were respectively subjected to a one-way analysis of variance with repeated measures using a computer program StatView 4.5J for Macintosh computer. A probability of $p<0.05$ was considered significant.

Results

Growth curve of PGGB-dr

The postnatal growth curves in each group are shown in Fig. 2. Growth in PGGB-*dr* was indistinguishable from that in SD, but significantly greater than that in *dr*. The dwarf phenotype of *dr* was completely corrected by the targeted incorporation of the hGH transgene to the pituitary gland of *dr*.

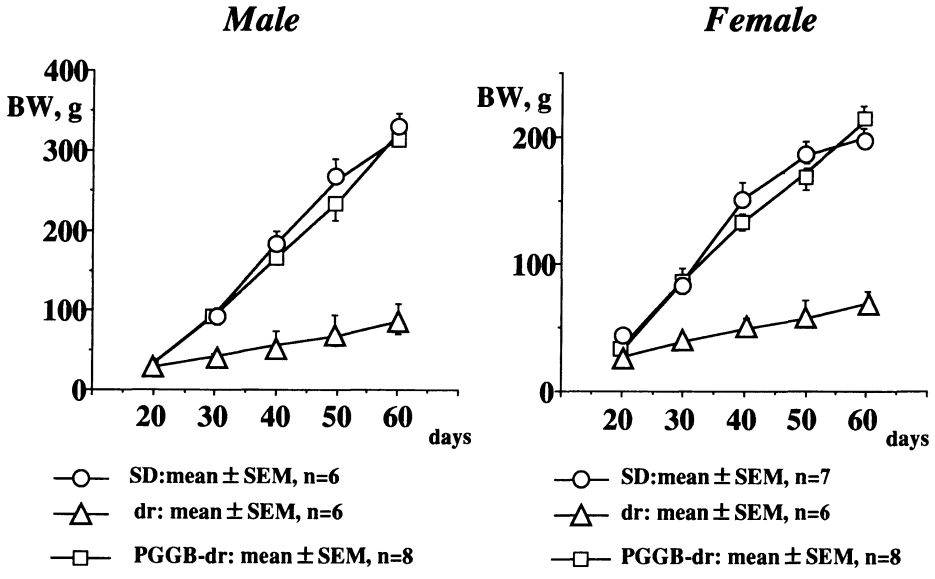

Fig. 2. Postnatal growth curves in SD, *dr* and PGGB-*dr* of both sexes. The mean ± SEM are shown. The number of animals in each group is shown. Growth in PGGB-*dr* is indistinguishable from that in SD, but significantly greater than that in *dr* (*dr* v.s. PGGB-*dr* or SD, $p<0.001$).

Discussion

We established a line of transgenic rats, which are deficient in endogenous rGH, but grow indistinguishably from control SD rats by expressing exogenous hGH transgene in the pituitary gland.

Our previous attempt to correct the dwarf phenotype of ***dr*** by germline incorporation of a chimeric hGH gene coupled with the mouse metallothionein I promoter has resulted in rat gigantism [10] as reported in a strain of dwarf mouse [9]. The mouse metallothionein I promoter-driven hGH gene was ubiquitously expressed in the giant ***dr***. There were no differences in hGH gene expression, as reflected in plasma hGH levels, between prepubertal and adult rats, or between males and females. By contrast, the hGH gene targeted (PBBG) to the pituitary resulted in normal recovery of growth in ***dr*** of both sexes, suggesting that the strain of PGGB-***dr*** shows physiologically regulated hGH gene expression in response to the onset of puberty and sexually dimorphic growth rates. The detailed hGH secretory dynamics and hGH immunohistochemistry are currently under investigation in PGGB-***dr*** of both sexes.

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