Dwarfism and early death in mice lacking C-type natriuretic peptide

Hideki Chusho*†, Naohisa Tamura*†, Yoshihiro Ogawa*‡, Akihiro Yasoda*, Michio Suda*, Takashi Miyazawa*, Kenji Nakamura§, Kazuki Nakao§, Tatsuya Kurihara¶, Yasato Komatsu*, Hiroshi Itoh*, Kiyoshi Tanaka*, Yoshihiko Saito*, Motoya Katsuki§, and Kazuwa Nakao*

*Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan; §Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo, Tokyo 108-0071, Japan; and ¶Suntory Institute for Biomedical Research, 1-1-1 Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618-0024, Japan

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Longitudinal bone growth is determined by endochondral ossification that occurs as chondrocytes in the cartilaginous growth plate undergo proliferation, hypertrophy, cell death, and osteoblastic replacement. The natriuretic peptide family consists of three structurally related endogenous ligands, atrial, brain, and C-type natriuretic peptides (ANP, BNP, and CNP), and is thought to be involved in a variety of homeostatic processes. To investigate the physiological significance of CNP *in vivo*, we generated mice with targeted disruption of CNP (*Nppc*$^{-/-}$ mice). The *Nppc*$^{-/-}$ mice show severe dwarfism as a result of impaired endochondral ossification. They are all viable perinatally, but less than half can survive during postnatal development. The skeletal phenotypes are histologically similar to those seen in patients with achondroplasia, the most common genetic form of human dwarfism. Targeted expression of CNP in the growth plate chondrocytes can rescue the skeletal defect of *Nppc*$^{-/-}$ mice and allow their prolonged survival. This study demonstrates that CNP acts locally as a positive regulator of endochondral ossification *in vivo* and suggests its pathophysiological and therapeutic implication in some forms of skeletal dysplasia.

There are two major mechanisms of bone formations, membranous and endochondral ossifications. The former occurs when mesenchymal precursor cells directly differentiate into bone-forming osteoblasts, a process by which all flat bones are formed. The latter involves the conversion of an initial cartilage template into bone and is responsible for the formation of long bones and vertebrae.

The natriuretic peptide system consists of a family of three structurally related endogenous ligands, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) (1), and three membrane-bound receptors, two of which are guanylyl cyclase (GC)-coupled receptors (GC-A and GC-B) that mediate the biological actions of the ligands, and one of which is a biologically silent receptor (C-receptor) implicated in the metabolic clearance of the ligands (2, 3). ANP and BNP are cardiac hormones that are produced predominantly by the atrium and ventricle, respectively (4–6), and are thought to play important roles in the regulation of cardiovascular homeostasis, primarily through GC-A (7, 8). On the other hand, CNP occurs in a wide variety of central and peripheral tissues (9–12) and may act locally as an autocrine/paracrine regulator through GC-B (7, 8).

We have created transgenic mice overexpressing BNP under the control of the liver-specific serum amyloid P component promoter and demonstrated that they exhibit blood pressure reduction (13). They also show marked skeletal overgrowth accompanied by the activation of endochondral ossification (14). It has been also reported that C-receptor-deficient mice (*Npr3*$^{-/-}$ mice) show skeletal abnormalities similar to those seen in transgenic mice overexpressing BNP (15, 16). These observations suggest that natriuretic peptides can affect endochondral ossification. In this context, previous studies have revealed no such skeletal abnormalities in ANP (*Nppa*)-deficient mice (17) or BNP (*Nppb*)-deficient mice (18), indicating that neither ANP nor BNP is involved in endochondral ossification under physiological conditions. In this study, we generated mice with targeted disruption of CNP (*Nppc*$^{-/-}$ mice) and demonstrated that they show severe dwarfism and early death as a result of impaired endochondral ossification. Targeted expression of CNP in the growth plate chondrocytes can rescue the skeletal defect of *Nppc*$^{-/-}$ mice and allow their prolonged survival. This study demonstrates that CNP is a bona fide endogenous natriuretic peptide in the bone, where it activates endochondral ossification.

Materials and Methods

Gene Targeting and Generation of Transgenic Mice. The 129/Sv mouse *Nppc* was isolated from a 129/Sv mouse genomic library in λFixII (Stratagene, La Jolla, CA). A targeting vector was constructed, in which exons 1 and 2 of the 129/Sv mouse *Nppc* that encode the entire coding sequences of mouse preproCNP were replaced by the neomycin resistance gene (Fig. 1*a*). The targeting vector was introduced into embryonic stem cells by electroporation (18). Double selection in G418 and ganciclovir produced seven homologously recombinant embryonic stem cell clones that were analyzed by Southern blot analysis with the 5′ and 3′ external probes indicated (Fig. 1 *a* and *b*). Male chimeras derived from two independent clones with germ-line transmission of the disrupted allele were bred to C57BL/6J or 129/SvJ females.

Generation of transgenic mice (Tg mice) with targeted expression of CNP in the growth plate chondrocytes under the control of the mouse pro-α_1(II) collagen (*Col2a1*) promoter [provided by B. de Crombrugghe at the M. D. Anderson Cancer Center, Houston, TX (19)] will be reported elsewhere (A.Y., Y.K., H.C., T.M., Y.O., and Kazuwa Nakao, unpublished observations). The transgene expression was detected only in the chondrocytes (19). To perform genetic rescue of *Nppc*$^{-/-}$ mice, Tg mice were mated with *Nppc*$^{+/-}$ mice, and F1 offspring heterozygous for the transgene and for the *Nppc* allele ablation were bred to generate *Nppc*$^{-/-}$ mice with the transgene expression (Tg/*Nppc*$^{-/-}$ mice). The care of the animals and all experiments were conducted in accordance with the institutional guidelines of Kyoto University Graduate School of Medicine.

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Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide; GC-B, guanylyl cyclase-B; Tg, transgenic; *Nppc, Npr2, Col2a1, Gapd*, mouse genes for CNP, GC-B, pro-α_1(II) collagen, and glyceraldehyde-3-phosphate dehydrogenase, respectively.

†H.C. and N.T. contributed equally to this study.

‡To whom reprint requests should be addressed. E-mail: ogawa@kuhp.kyoto-u.ac.jp.

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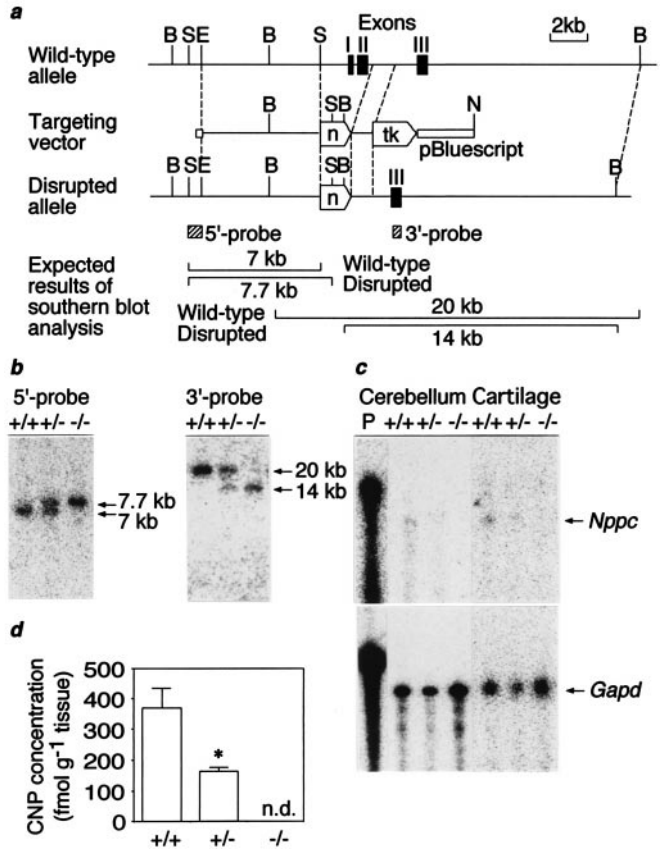

Fig. 1. Targeted disruption of the mouse *Nppc*. (*a*) Restriction maps of the wild-type 129/Sv mouse *Nppc* allele, targeting vector, and the predicted disrupted allele. Closed boxes indicate exons (I–III). Locations of 5′ and 3′ external probes are shown as hatched bars. B, *Bam*HI; S, *Sph*I; E, *Eco*RI; N, *Not*I; tk, Herpes simplex virus thymidine kinase gene; n, neomycin resistance gene. (*b*) Southern blot analysis of genomic DNAs from F2 offspring with 5′ and 3′ probes upon digestion with *Sph*I and *Bam*HI, respectively. (*c*) RNase protection analysis of *Nppc* and *Gapd* transcript in the cerebellum and tibial epiphyseal cartilage. The cerebellum and tibial cartilage are obtained from mice at 20 weeks and 7 days of age, respectively. Ten micrograms and 1 μg of total RNA were used to analyze *Nppc* and *Gapd* transcripts, respectively. (*d*) Cerebellar CNP concentrations at 20 weeks of age ($n = 4$). n.d., not detectable. *, $P < 0.05$ vs. $Nppc^{+/+}$ mice.

RNA and Peptide Analysis. Total RNA was extracted from various tissues from $Nppc^{+/+}$, $Nppc^{+/-}$, and $Nppc^{-/-}$ mice. In the cerebellum and epiphyseal cartilage, *Nppc* mRNA expression was assessed by RNase protection assay. Ten micrograms and 1 μg of total RNA were used to analyze *Nppc* and glyceraldehyde-3-phosphate dehydrogenase (*Gapd*) transcripts, respectively. ^{32}P-labeled antisense *Nppc* and *Gapd* riboprobes were generated from a 5′-rapid amplification of cDNA ends-based 129/Sv mouse *Nppc* cDNA fragment and mouse *Gapd* cDNA fragment (a gift from M. B. Prystowsky at the Albert Einstein College of Medicine), respectively. In other tissues, *Nppc* and *Gapd* mRNA expressions were assessed by reverse transcription (RT)-PCR and Southern blot analysis (*Nppc*: sense primer, 5′-AAAAAGGGTGACAAGACTCCAGGCAG-3′; antisense primer, 5′-GGTGTTGTGTATTGCCAGTA-3′; antisense probe, 5′-CCTTC-TTGTTGCCGCCTTTGAT-3′; *Gapd*: sense and antisense primers, a mouse G3PDH control amplimer set, CLONTECH; antisense probe, 5′-GCCTTGACTGTGCCGT-TGAATTTGCCGTGA-3′). Cerebellar CNP concentrations were determined by a RIA for CNP (9).

Skeletal Preparation and Histology. Skeletal preparation and histological analysis were performed as described (14). Briefly, mice were killed, skinned, eviscerated, and subjected to soft x-ray analysis (23 kVp, 5 mA for 1 min; Softron Type SRO-M5, Softron, Tokyo). Bones from 7-day-old mice were fixed in 4% paraformaldehyde in 0.01 M PBS (pH 7.4), decalcified in 10% EDTA, and embedded in paraffin. Five-micrometer-thick sections were sliced and stained with Alcian blue (pH 2.5) and hematoxylin/eosin. The slices were also analyzed for apoptosis by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling assay according to the manufacturer's protocol (Takara Shuzo, Shiga, Japan).

Detection of BrdUrd-Labeled Cells. Mice at 1 week of age were injected intraperitoneally with BrdUrd (100 μg/g body weight) and were killed 6 h later. Tibiae were fixed in 4% paraformaldehyde in 0.01 M PBS (pH 7.4), decalcified in 10% EDTA, and embedded in paraffin. Detection of BrdUrd-positive cells in the growth plate was performed by BrdUrd labeling and with a detection kit II (Roche Diagnostics).

***In Situ* Hybridization.** Digoxigenin-labeled antisense and sense riboprobes were obtained from reverse transcription–PCR products for *Nppc*, rat GC-B (nucleotides 762-1394 of rat GC-B cDNA; GenBank M26896), mouse pro-α_1(I) collagen (*Col1a1*) (nucleotide 693-1139 of *Col1a1* cDNA; GenBank M14423) and rat pro-α_1(X) collagen (a gift from B. R. Olsen at Harvard Medical School, Boston, MA), mouse pro-α_1(II) collagen (*Col2a1*) (a gift from Y. Yamada at the National Institutes of Health, Bethesda, MD), and mouse Indian hedgehog (*Ihh*) (a gift from K. Lee at Massachusetts General Hospital, Boston, MA) cDNA fragments with the use of a digoxigenin RNA labeling kit (Roche Diagnostics). Tibiae from 7-day-old mice were fixed in 4% paraformaldehyde, 0.5% glutaraldehyde, 1 mM $CaCl_2$ in 0.1 M phosphate buffer (pH 7.2) for 24 h, embedded in paraffin, and sliced into 5-μm-thick sections. *In situ* hybridization analysis was performed as described (20).

cGMP Assay. The tail bone cGMP concentrations in 7-day-old mice were determined by a RIA for cGMP as described (21).

Statistical Analysis. Data are expressed as means ± SE. The statistical significance of differences in mean values was assessed by Student's *t* test. The difference in survival rates among genotypes was assessed by Kaplan–Meier analysis.

Results and Discussion

Generation of $Nppc^{-/-}$ Mice. To investigate the physiological significance of CNP *in vivo*, we generated mice with a disrupted *Nppc* allele by gene targeting in 129/Sv mouse-derived embryonic stem cells (Fig. 1*a*). Male chimeras with germ-line transmission of the disrupted allele were bred to C57BL/6J and 129/SvJ females, and $Nppc^{+/+}$ mice (wild type), $Nppc^{+/-}$ mice (heterozygous for the disrupted allele), and $Nppc^{-/-}$ mice (homozygous for the disrupted allele) were obtained (Fig. 1*b*). The *Nppc* mRNA levels were decreased by ≈50% in the cerebellum and tibial epiphyseal cartilage from $Nppc^{-/-}$ mice relative to those of $Nppc^{+/+}$ mice (Fig. 1*c*). Cerebellar CNP concentrations were also decreased in $Nppc^{+/-}$ mice relative to $Nppc^{+/+}$ mice ($P < 0.05$, $n = 4$) (Fig. 1*d*). No *Nppc* mRNA or CNP was detected in the cerebellum or cartilage from $Nppc^{-/-}$ mice (Fig. 1 *c* and *d*). The *Nppc* transcript was also detected in other tissues, including the cerebrum, pituitary, heart, kidney, ovary, and uterus, in $Nppc^{+/+}$ mice but not in $Nppc^{-/-}$ mice (data not shown).

Analysis of 96 intercrosses between 129/B6 hybrids heterozygous for the disrupted allele revealed that the genotype ratio of +/+:+/−:−/− at weaning (at 4 weeks of age) is 1.00:2.13:0.37 ($n = 648$), indicating a deviation from the expected Mendelian proportions ($P < 0.001$ by χ^2 test). On the other hand, the

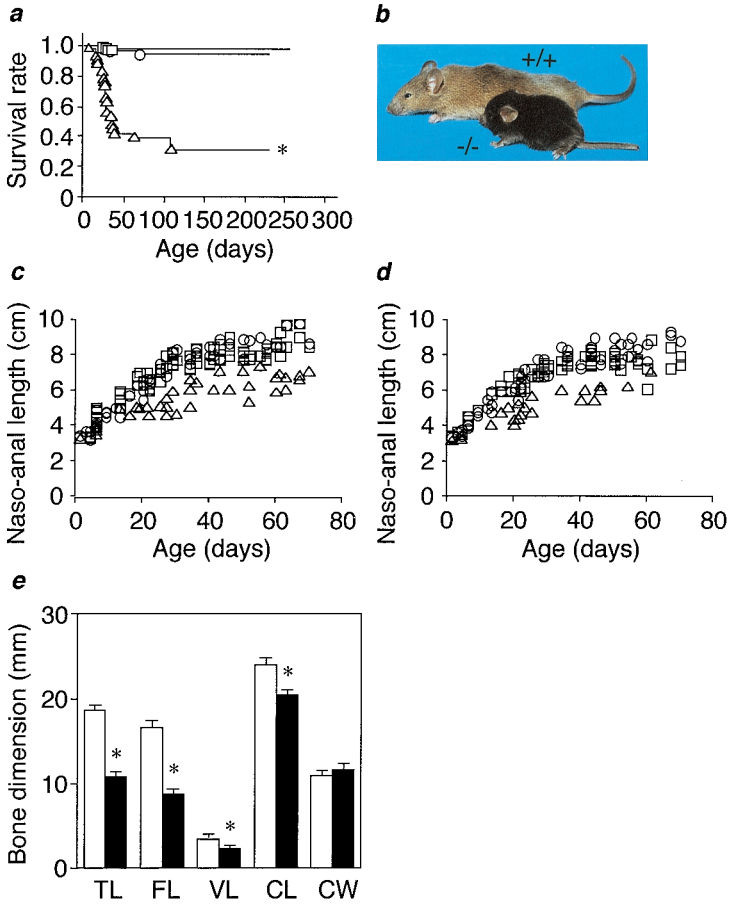

Fig. 2. Dwarfism and mortality in *Nppc*$^{-/-}$ mice. (*a*) Survival curves of *Nppc*$^{+/+}$ (○), *Nppc*$^{+/-}$ (□), and *Nppc*$^{-/-}$ (△) mice. (*b*) Gross appearance of *Nppc*$^{+/+}$ and *Nppc*$^{-/-}$ mice at 10 weeks of age. (*c* and *d*) Growth curves of *Nppc*$^{+/+}$ (○), *Nppc*$^{+/-}$ (□), and *Nppc*$^{-/-}$ (△) mice. (*c*) Males. (*d*) Females. (*e*) Soft x-ray analysis of *Nppc*$^{+/+}$ and *Nppc*$^{-/-}$ mice at 10 weeks of age. Bone dimensions in *e* are of *Nppc*$^{+/+}$ (open bars) and *Nppc*$^{-/-}$ (closed bars) mice ($n = 4$). TL, tibial length; FL, femoral length; VL, fifth lumbar vertebral length; CL, naso-occipital length of the calvarium; CW, maximal interparietal distance of the calvarium. *, $P < 0.05$ vs. *Nppc*$^{+/+}$ mice.

genotype ratio of +/+:+/−:−/− at 16.5 days post coitus was 1.0:1.5:1.4 ($n = 65$), suggesting that *Nppc*$^{-/-}$ mice are not embryonically lethal. More than half of *Nppc*$^{-/-}$ mice died before weaning, and only 30% survived at 100 days of age and thereafter (Fig. 2*a*). No appreciable differences in the genotype ratio and survival rate were noted between the 129/B6 hybrid and 129/Sv pure backgrounds.

At birth, *Nppc*$^{-/-}$ pups had a body weight and a naso-anal length about 90% of those of *Nppc*$^{+/+}$ pups. In *Nppc*$^{-/-}$ mice, dwarfism with short tails and extremities became prominent as they grew (Fig. 2*b*). The naso-anal lengths of male and female *Nppc*$^{-/-}$ mice were 60–70% of those of *Nppc*$^{+/+}$ mice at 4–10 weeks of age (Fig. 2 *c* and *d*). Body weights of *Nppc*$^{-/-}$ mice were about 70% of those of *Nppc*$^{+/+}$ mice, and no significant differences in visceral organ/body weight ratios were noted between genotypes at 20 weeks of age. No other gross abnormalities were found in *Nppc*$^{-/-}$ mice.

Soft x-ray analysis revealed that the longitudinal growth of vertebrae and tail and limb bones is affected in *Nppc*$^{-/-}$ mice. The longitudinal lengths of femurs, tibiae, and vertebrae in *Nppc*$^{-/-}$ mice were 50–80% of those in *Nppc*$^{+/+}$ mice (Fig. 2*e*). The naso-occipital length of the calvarium, which depends on the growth of occipital and sphenoidal bones formed through endochondral ossification, was also reduced significantly in *Nppc*$^{-/-}$ mice relative to *Nppc*$^{+/+}$ mice ($n = 4$, $P < 0.05$) (Fig. 2*e*). On the other hand, in *Nppc*$^{-/-}$ mice, there were no appreciable changes in the shape and interparietal width of the skull vault, which are formed by membranous ossification. These observations indicate that loss of CNP affects endochondral ossification but not membranous ossification *in vivo*.

Longitudinal bone growth is determined by endochondral ossification in the cartilaginous growth plate, which is located at both ends of vertebrae and long bones (22). The cartilaginous growth plate consists of the resting, proliferative, and hypertrophic zones of chondrocytes. Histologically, *Nppc*$^{-/-}$ mice displayed striking narrowing of the growth plate of vertebrae and long bones compared with *Nppc*$^{+/+}$ mice at 7 days of age (Fig. 3 *a* and *b*). The heights of the proliferative and hypertrophic zones were markedly reduced in *Nppc*$^{-/-}$ mice, whereas no significant differences in the resting zone were noted between genotypes. *In situ* hybridization analysis revealed no appreciable difference in the intensity of *Col2a1* and type X collagen (*Col10a1*) mRNA expression between genotypes (Fig. 3 *c–f*). No ectopic expression of *Col1a1* mRNA was found in the growth plate from *Nppc*$^{-/-}$ mice (data not shown). These findings suggest that chondrocyte precursors are capable of differentiating into hypertrophic chondrocytes in the growth plate from *Nppc*$^{-/-}$ mice. However, the band width of *Ihh*-expressing cells [cells committing to differentiation into hypertrophic chondrocytes (23)] was narrowed in *Nppc*$^{-/-}$ mice relative to *Nppc*$^{+/+}$ mice (Fig. 3 *g–j*), although the intensity of *Ihh* expression was not

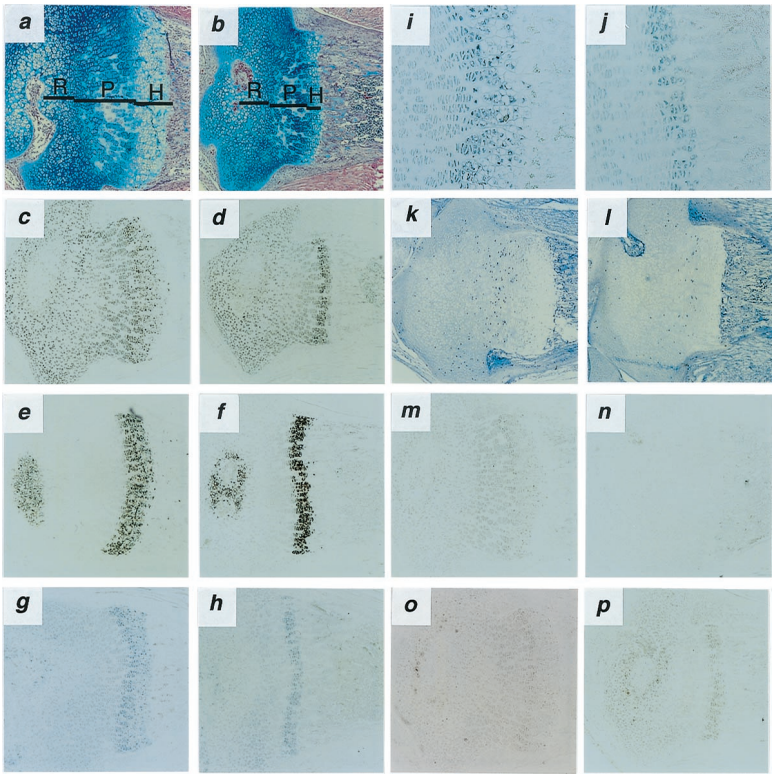

Fig. 3. Histological analysis of proximal epiphyseal cartilages of the tibia from 7-day-old *Nppc*$^{+/+}$ and *Nppc*$^{-/-}$ mice. (*a* and *b*) Alcian blue-hematoxylin/eosin staining of tibial epiphyseal cartilages from *Nppc*$^{+/+}$ (*a*) and *Nppc*$^{-/-}$ (*b*) mice. Resting (R), proliferating (P), and hypertrophic (H) zones are indicated. (*c–j*, *m–p*) *In situ* hybridization analysis of tibial epiphyseal cartilages from *Nppc*$^{+/+}$ (*c*, *e*, *g*, *i*, *m*, *o*) and *Nppc*$^{-/-}$ (*d*, *f*, *h*, *j*, *n*, *p*) mice showing expression of mRNA for *Col2a1* (*c*, *d*), *Col10a1* (e, *f*), *Ihh* (*g-j*), *Nppc* (*m, n*), or *Npr2* (*o, p*). (*k* and *l*) Immunohistochemical detection of BrdUrd-labeled chondrocytes in the tibial growth plate from *Nppc*$^{+/+}$ (*k*) and *Nppc*$^{-/-}$ (*l*) mice. (Magnification: *a–h*, *k–p*, ×40; *i, j,* ×100.)

remarkably different between genotypes. Notably, the ratio of the height of the hypertrophic zone to the height of the proliferative zone was decreased by ≈50% in *Nppc*$^{-/-}$ mice compared with *Nppc*$^{+/+}$ mice (Fig. 3 *a* and *b*). These observations suggest that the rate of cell differentiation into hypertrophic chondrocytes is reduced in *Nppc*$^{-/-}$ mice. The number of hypertrophic chondrocytes positive for terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling was not significantly different between genotypes (data not shown), suggesting that chondrocyte apoptosis is unaffected in *Nppc*$^{-/-}$ mice.

We also assessed the consequence of *Nppc* ablation on the proliferation of the growth plate chondrocytes. There was a significant reduction of BrdUrd-labeled cells in *Nppc*$^{-/-}$ mice relative to *Nppc*$^{+/+}$ mice (Fig. 3 *k* and *l*), demonstrating that CNP promotes chondrocyte proliferation *in vivo*.

In *Nppc*$^{+/+}$ mice, *Nppc* mRNA was detected in proliferative and prehypertrophic chondrocytes in the growth plate but not detected in *Nppc*$^{-/-}$ mice (Fig. 3 *m* and *n*). Expression of mRNA for GC-B (*Npr2*) was detected predominantly in the proliferative and prehypertrophic chondrocytes in both *Nppc*$^{-/-}$ and *Nppc*$^{+/+}$ mice (Fig. 3 *o* and *p*). The tail bone cGMP concentrations in *Nppc*$^{-/-}$ mice were markedly smaller than those in *Nppc*$^{+/+}$ mice at 7 days of age (9.7 ± 2.2 vs. 25.6 ± 2.7 pmol/g tissue, $n = 6$, $P < 0.01$), suggesting that CNP is a major determinant of cGMP in the bone. Evidence has indicated that CNP increases the height of the proliferative and hypertrophic zones of chondrocytes and cartilage matrix production in the organ cultures of fetal mouse and rat long bones via cGMP production (21, 24). It is therefore likely that CNP acts primarily on proliferative and prehypertrophic chondrocytes expressing GC-B, thus stimulating growth plate chondrocyte proliferation and differentiation *in vivo* through cGMP-mediated pathway. Histology of other organs was not remarkable in *Nppc*$^{-/-}$ mice at 20 weeks of age (data not shown).

Transgenic Rescue of Nppc$^{-/-}$ Mice. To determine whether local expression of CNP in the bone can rescue the dwarfism of *Nppc*$^{-/-}$ mice *in vivo*, they were crossed with mice with transgenic expression of CNP in the growth plate chondrocytes (Tg mice). The genetically "rescued" animals [or *Nppc*$^{-/-}$ mice with the transgene expression (Tg/*Nppc*$^{-/-}$ mice)] were of normal appearance (Fig. 4*a*), and their skeletons were indistinguishable from those of *Nppc*$^{+/+}$ mice. During postnatal development, no significant difference in the naso-anal length was observed between Tg/*Nppc*$^{-/-}$ and *Nppc*$^{+/+}$ mice (Fig. 4*b*). Body weights of Tg/*Nppc*$^{-/-}$ mice were not different from those of *Nppc*$^{+/+}$ mice at 10 weeks of age (24.3 ± 1.9 vs. 21.9 ± 1.1 g, $n = 6$). Histologically, no appreciable differences in tibial growth plate cartilage were noted between Tg/*Nppc*$^{-/-}$ and *Nppc*$^{+/+}$ mice (Fig. 4 *c–e*). The tail bone cGMP concentrations in Tg/*Nppc*$^{-/-}$ mice (17.2 ± 4.9 pmol/g tissue, $n = 6$) were also roughly comparable to those in *Nppc*$^{+/+}$ mice (25.6 ± 2.7 pmol/g tissue, $n = 6$, $P > 0.05$). These findings demonstrate that CNP, when expressed locally in the growth plate chondrocytes, can rescue the skeletal defect of *Nppc*$^{-/-}$ mice via the cGMP-mediated mechanism *in vivo*. Although CNP is expressed in a variety of central and peripheral tissues, the data of this study indicate that CNP acts locally as a positive regulator of endochondral ossification. Targeted expression of CNP in the growth plate chondrocytes also resulted in prolonged survival of *Nppc*$^{-/-}$ mice; most Tg/*Nppc*$^{-/-}$ mice examined (11 of 13 mice) survived up to

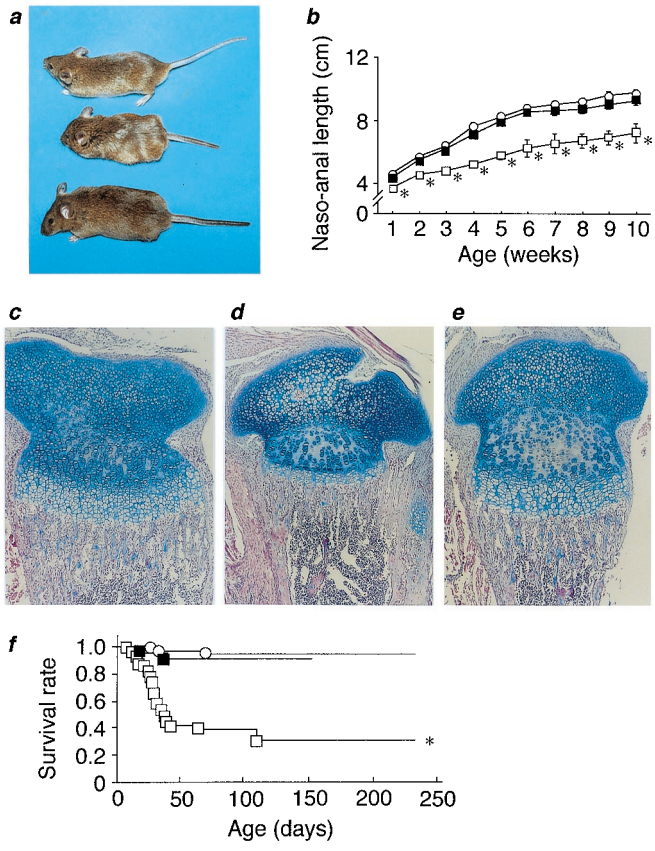

Fig. 4. Genetic rescue of *Nppc*$^{-/-}$ mice by crosses with transgenic mice with targeted expression of CNP in the growth plate chondrocytes. (*a*) Gross appearance of *Nppc*$^{+/+}$ mice, *Nppc*$^{-/-}$ mice without the transgene expression, and *Nppc*$^{-/-}$mice with the transgene expression (Tg/*Nppc*$^{-/-}$ mice) at 20 weeks of age. (*From Top to Bottom*) *Nppc*$^{+/+}$, *Nppc*$^{-/-}$, and Tg/*Nppc*$^{-/-}$ mice. (*b*) Growth curves of *Nppc*$^{+/+}$ (○), *Nppc*$^{-/-}$ (□), and Tg/*Nppc*$^{-/-}$ (■) mice. *$P <$ 0.05 vs. *Nppc*$^{+/+}$ mice. (*c–e*) Alcian blue-hematoxylin/eosin stainings of tibial epiphyseal cartilages from 7-day-old *Nppc*$^{+/+}$ (*c*), *Nppc*$^{-/-}$ (*d*), and Tg/*Nppc*$^{-/-}$ (*e*) mice. (*f*) Survival curves of *Nppc*$^{+/+}$ (○), *Nppc*$^{-/-}$ (□), and Tg/*Nppc*$^{-/-}$ mice (■). *, $P < 0.05$ vs. *Nppc*$^{+/+}$ mice.

20 weeks of age (Fig. 4*f*). Therefore, *Nppc*$^{-/-}$ mice are short-lived because of their skeletal abnormalities.

Transgenic mice with elevated plasma BNP concentrations show skeletal overgrowth due to increased endochondral ossification (13, 14), whereas mice with targeted disruption of BNP exhibit no skeletal abnormalities (18). Thus, BNP, a hormone derived from the cardiac ventricle, is not involved in endochondral ossification under physiological conditions. This study has established CNP as an endogenous ligand to activate the cGMP production in the bone, thereby regulating endochondral ossification. In this context, mice with targeted disruption of cGMP-dependent protein kinase II, an intracellular mediator of cGMP that is expressed in late proliferative and early hypertrophic chondrocytes in the growth plate, show dwarfism due to impaired endochondral ossification (25). We postulate that the CNP/GC-B/cGMP-dependent protein kinase II signaling cascade plays a critical role in endochondral ossification. Recently, *Npr3*$^{-/-}$ mice have been reported to show skeletal overgrowth and increased endochondral ossification (15, 16). It is likely that the metabolic clearance of CNP produced locally is delayed in *Npr3*$^{-/-}$ mice, thereby activating endochondral ossification.

The *Nppc*$^{-/-}$ mice exhibit gross phenotypes and histologic features similar to those found in patients with achondroplasia (26) and mouse models for achondroplasia (27, 28). Several gain-of-function mutations in the fibroblast growth factor receptor 3 gene have been found in most achondroplastic patients and those with two other distinct skeletal dysplasias, hypochondroplasia and thanatophoric dysplasia (29–32). Our data suggest that CNP may be one of the causative genes for such skeletal dysplasias of unknown origin and may be useful for treatment of achondroplasia.

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