復経口投与予備毒性試験

塩酸メクリジンをラット(Crl:CD(SD), 雌雄各6匹/群)に100、150、300及び1000mg/kgの用量で1週間反復経口投与し，認められる毒性について確認する。対照群には媒体(0.1%Tween 80添加0.5%カルボキシメチルセルロースナトリウム溶液)を投与する。

〔確認項目〕一般状態観察、体重測定、摂食量、血液学的検査、血液生化学的検査、尿検査、剖検、器官重量

本試験は株式会社 LSI メディエンスに外注する。

2. イヌにおけるメクリジン 1 週間反復経口投与予備毒性試験

塩酸メクリジンをイヌ（雌雄各1匹/群）に 100、150、300 及び 1000mg/kg の用量で1週間反復経口投与し、認められる毒性について確認する。対照群には媒体 (0.1%Tween 80 添加 0.5% カルボキシメチルセルロースナトリウム溶液)を投与する。

〔確認項目〕一般状態観察、体重測定、摂食量、血液学的検査、血液生化学的検査、尿検査、剖検、器官重量

本試験は株式会社 LSI メディエンスに外注する。

（倫理面への配慮）

メクリジンの 1 週間反復経口投与予備毒性試験は株式会社 LSI メディエンスの「動物実験に関する指針」に基づき、動物実験委員会審査及び試験研究センター長の承認を得て実施する。

「動物の愛護及び管理に関する法律の一部を改正する法律」(平成17年法律第68号)、「実験動物の飼養及び保管並びに苦痛の軽減に関する基準」（平 成18年4月28日環境省告示第88号)、「研究機関等における動物実験等の実施に関する基本指針（平成18年6月1日文部科学省告示第71号)、動物実験の適正な実施に向けたガイドライン(日本学術会議 平成18年6月1日作成）等、必要な省令、通知を遵守して実験を施行する。また、非臨床試験の受注業者は医薬品GLP 適合性評価「A」取得及び AAALAC International 完全認証取得施設であることを選定条件とする。

C. 研究結果

ラットにおける反復投与の結果、1000mg/kg 群の雌雄全例、および300mg/kg群の雄4例、雌2例が第5～8日に死亡した。100mg/kg、150mg/kg の群における死亡例はなく、これら濃度では一般状態観察試験でも明らかな異常は認めなかった。一方、尿検査、血液学的検査、血液生化学的検査などでは肝肥大、ASAT および ALAT の高値などの異常所見を認めた。

イヌにおける反復投与では、1000mg/kg 群の雌雄でのみ嘔吐が散見された。また、1000mg/kg群の雄で摂餌量の低下および軽度の体重減少を認めた。その他、1000mg/kg 群の雌雄、300mg/kg 群の雄で総コレステロール値の低下を認めたが、いずれも重篤ではなかった。150mg/kg群および100mg/kg群では異常を認めなかった。

D. 考察

メクリジンの 1 週間反復経口投与予備毒性試験に関しては、ラットとイヌでは薬剤感受性が著しく異なっていた。ラットでは300mg/kg以上の用量群において死亡が発現し、100mg/kg 以上の用量

では肝臓への影響が示唆されたため、より長期の反復投与試験を実施する場合、最高用量には300mg/kgよりも低用量を設定するのが望ましいと考えられた。一方、イヌでは1000mg/kgまでの用量において重篤な所見を認めなかったことから、より長期間の反復投与毒性試験の高用量として、1000mg/kgを設定することは可能であると思われた。

今回実施したラットおよびイヌにおけるメクリジンの 1 週間反復経口投与予備毒性試験結果を基に、次年度実施するGLP適用2週間反復投与毒性試験の実施などについてPMDAと薬事戦略相談で議論しながら、治験実施の必要な最低限の非臨床試験の実施について決定する予定としている。

E.　結論

ラットおよびイヌにおけるメクリジン 1 週間反復経口投与予備毒性試験を施行し、塩酸メクリジンの毒性に関するデータを取得した。

GLP適用の2週間反復投与毒性試験実施のための用量設定根拠となるデータを取得した。

F.　研究発表

1. 論文発表

なし

2. 学会発表

なし

G.　知的財産権の出願・登録状況（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

厚生労働科学研究委託費（早期探索的・国際水準臨床研究事業）
委託業務成果報告（業務項目）

モデルマウスでの薬効薬理試験

担当責任者　松下雅樹　名古屋大学医学部附属病院　整形外科　病院助教

研究要旨
軟骨無形成症(achondroplasia ： ACH)は骨の成長抑制因子である線維芽細胞増殖因子受容体3（fibroblast growth factor receptor 3 ： FGFR3)の機能獲得型変異による過剰なFGFR3の活性化が原因であり、低身長に加えて脊柱管狭窄症・大後頭孔狭窄などの重篤な合併症も生じる。ACHにおけるFGFR3の活性を抑える根本的治療法はない。我々は既存薬のスクリーニングにより、一般用医薬品（OTC医薬品）の乗り物酔い防止薬で抗ヒスタミン薬であるメクリジンがFGFR3下流のシグナル系を抑え、異常に活性化したFGFR3シグナルを抑制することを見出した。メクリジンは変異FGFR3導入細胞ならびにFGF2刺激軟骨細胞を濃度依存的に増殖かつ分化させた。また、胎生期マウス脛骨器官培養系においてもメクリジンは骨伸長作用を示した。さらに、成長期のACHモデルマウスにOTC医薬品としての承認用量に相当するメクリジンを経口投与したところ、長管骨を中心としてモデルマウスの骨伸長を促進した。メクリジンの内服治療は患者の負担が軽く、医療費低減や小児への長期投与におけるコンプライアンスも期待できる。また、FGFR3の異常活性化によって生じる他の骨系統疾患（タナトフォリック骨異形成症、軟骨低形成症）や、ACHにおける大後頭孔や脊柱管の狭窄などの重篤な合併症への適応拡大の可能性がある。

A. 研究目的

軟骨無形成症(achondroplasia：ACH)は骨の成長抑制因子である線維芽細胞増殖因子受容体 3（fibroblast growth factor receptor 3 ： FGFR3)の恒常的活性化により骨伸長は抑制され、低身長に加え大後頭孔狭窄や脊柱管狭窄の重篤な合併症も生じる。ACH における FGFR3 の活性を抑える根本的治療法はない。我々は既存薬のスクリーニングにより同定した乗り物酔い止め薬としての一般用医薬品（OTC 医薬品）であるメクリジンが FGFR3 シグナルを抑制し、軟骨無形成症モデルマウスにおいて長管骨の成長抑制をレスキューすることを証明した。本研究では、大後頭孔および脊柱管の成長に与えるメクリジンの効果を検討する。

B. 研究方法

FVBバックグラウンドの *Fgfr3*^{ach} マウスを疾患モデルマウスとして用い、ヘテロ接合型を実験に使用した。0.4g のメクリジンを 1kg の餌に混ぜ、メクロジンを混餌経口投与した。妊娠 16.5 日よりマウス母体にメクリジン混餌（0.4 g/kg）投与開始し，得られた 5 日齢の仔 *Fgfr3*^{ach} マウスの全身骨を採取しアリザリンレッドとアルシアンブルー染色による透明骨格標本を作成した。頭蓋底および胸腰椎を摘出し、蝶形骨-基底後頭骨および基底後頭骨-側後頭骨の軟骨結合の骨性架橋、第 12 胸椎から第 3 腰椎までの椎弓軟骨結合の骨化を評価した。完全骨化を 2 点、不全骨化を 1 点とスコア化し軟骨結合の骨化を定量した。

さらに妊娠 14.5 日のマウス母体にメクリジン混餌（0.4 g/kg）を 3 日間投与後に胎仔を採取しホモジネートし組織中メクリジン濃度を測定した。

（倫理面への配慮）

「動物の愛護及び管理に関する法律の一部を改正する法律」（平成 17 年法律第 68 号）、「実験動物の飼養及び保管並びに苦痛の軽減に関する基準」（平 成 18 年 4 月 28 日環境省告示第 88 号）、「研究機関等における動物実験等の実施に関する基本指針（平成 18 年 6 月 1 日文部科学省告示第 71 号）、動物実験の適正な実施に向けたガイドライン（日本学術会議 平成 18 年 6 月 1 日作成）および名古屋大学の動物実験委員会の名古屋大学における動物実験等に関する取扱規程（平成 19 年 3 月 12 日規程第 71 号）等、必要な省令、通知を遵守して実験を施行した。

C. 研究結果

頭蓋底における軟骨結合の骨性架橋スコアは，コントロール群（n = 5）は 5.0 ± 1.0、メクリジン投与群（n = 3）は 2.7 ± 0.6 とメクリジンは *Fgfr3*^{ach} マウスにおける大後頭孔周囲の軟骨結合の早期骨化を有意に抑制した（$p < 0.05$）。（図 1）

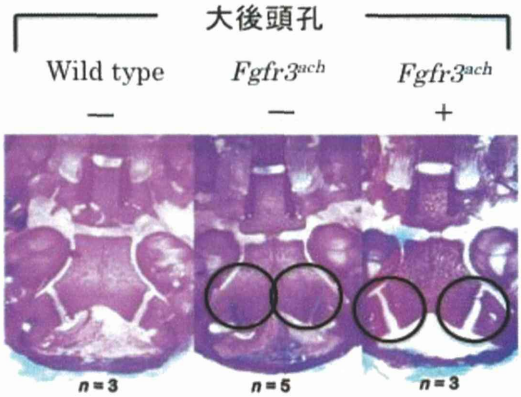

図 1. 大後頭孔のアリザリンレッド染色による透明骨格標本

また、胸腰椎移行部における椎弓軟骨結合の骨化スコアは、コントロール群（n = 5）は 7.0 ± 0.7、メクリジン投与群（n = 3）は 4.0 ± 2.6 とメクリジンは椎弓軟骨結合の早期骨化を有意に抑制した（$p < 0.05$）。（図 2）

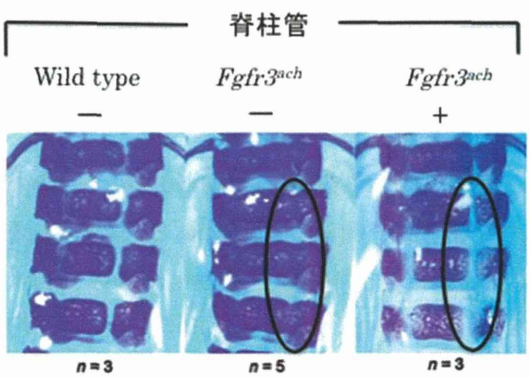

図 2. 脊柱管のアルシアンブルー染色による透明骨格標本

一方、胎仔の組織中メクリジン濃度(n = 4) は 56.91 ± 20.05 ng/g とメクリジンの胎盤移行性を確認した。

D. 考察

メクリジンは *Fgfr3*^{ach} マウスにおいて長管骨に加え、大後頭孔および脊柱管の成長抑制をレスキューすることが示唆された。軟骨無形成症における大後頭孔狭窄や脊柱管狭窄をレスキューするためには、頭蓋底や椎弓の軟骨結合の骨化以前にメクリジンを投与開始する必要がある。

E. 結論

胎生期よりメクリジン治療を開始すれば ACH における大後頭孔や脊柱管の狭窄をレスキューできる可能性がある。

F. 研究発表

1. 論文発表

1) Matsushita M, Hasegawa S, Kitoh H, Mori K, Ohkawara B, Yasoda A, Masuda A, Ishiguro N, Ohno K : Meclozine Promotes Longitudinal Skeletal Growth in Transgenic Mice with Achondroplasia Carrying a Gain-of-Funciton Mutation in the *FGFR3* Gene. Endocrinology. 2015 Feb;156(2):548-542.

2. 学会発表

1) 松下雅樹、鬼頭浩史、三島健一、西田佳弘、石黒直樹、大野欽司：Meclozine はオフラベル効能により FGFR3 シグナルを抑制し軟骨無形成症の根本的治療薬となりうる. 第 28 回日本軟骨代謝学会、2015.3.6-7 (東京)

2) Masaki Matsushita, Hiroshi Kitoh, Kenichi Mishima, Yoshihiro Nishida, Naoki Ishiguro, Kinji Ohno : Meclozine enhances skeletal growth in transgenic achondroplasia mice with a constitutive active FGFR3 mutation. Annual meeting of Orthopaedic Research Society, 2015.Mar.28-31 (Las Vegas)

G. 知的財産権の出願・登録状況 (予定を含む)

1. 特許取得

なし

(ただし、既に本開発治療薬であるメクリジンに関する特許の PCT 出願を行った)

2. 実用新案登録

なし

3. その他

なし

Ⅲ. 学会等発表実績

研究成果の学会等発表及び刊行に関する実績一覧表

1．学会等における発表

発表者氏名	発表題目 （口頭・ポスター）	発表場所 （学会等名）	発表 時期	国内・ 国外
Masaki Matsushita, Hiroshi Kitoh, Kenichi Mishima, Yoshihiro Nishida, Naoki Ishiguro, Kinji Ohno	Meclozine enhances skeletal growth in transgenic achondroplasia mice with a constitutive active FGFR3 mutation （ポスター発表）	Annual meeting of Orthopaedic Research Society	2015年 3月 28-31日	国外
松下雅樹、鬼頭浩史、三島健一、西田佳弘、石黒直樹、大野欽司	meclozine はオフラベル効能により FGFR3 シグナルを抑制し軟骨無形成症の根本的治療薬となりうる （口頭発表）	第28回日本軟骨代謝学会	2015年 3月 6-7日	国内

2．学会誌・雑誌等における論文掲載

発表者氏名	論文タイトル	発表雑誌	巻号:頁	発行年
Matsushita M, Hasegawa S, Kitoh H, Mori K, Ohkawara B, Yasoda A, Masuda A, Ishiguro N, Ohno K	Meclozine promotes longitudinal skeletal growth in transgenic mice with achondroplasia carrying a gain-of-function mutation in the FGFR3 gene.	Endocrinology	156(2):548-542	2015
Olney RC, Prickett TC, Espiner EA, Mackenzie WG, Duker AL, Ditro C, Zabel B, Hasegawa T, Kitoh H, Aylsworth AS, Bober MB	C-type natriuretic peptide plasma levels are elevated in subjects with achondroplasia, hypochondroplasia, and thanatophoric dysplasia.	J Clin Endocrinol Metab	Epub ahead of print	2015 (Feb)

Ⅳ．研究成果の刊行物・別刷

Meclozine Promotes Longitudinal Skeletal Growth in Transgenic Mice with Achondroplasia Carrying a Gain-of-Function Mutation in the *FGFR3* Gene

Masaki Matsushita, Satoru Hasegawa, Hiroshi Kitoh, Kensaku Mori, Bisei Ohkawara, Akihiro Yasoda, Akio Masuda, Naoki Ishiguro, and Kinji Ohno

Division of Neurogenetics, Center for Neurological Diseases and Cancer (M.M., S.H., B.O., A.M., K.O.), Department of Orthopaedic Surgery (M.M., H.K., N.I.), Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan; Department of Media Science (K.M.), Graduate School of Information Science, Nagoya University, Nagoya 466-8550, Japan; and Department of Diabetes, Endocrinology and Nutrition (A.Y.), Kyoto University Graduate School of Medicine, Kyoto 606-8501, Japan

Achondroplasia (ACH) is one of the most common skeletal dysplasias causing short stature owing to a gain-of-function mutation in the *FGFR3* gene, which encodes the fibroblast growth factor receptor 3. We found that meclozine, an over-the-counter drug for motion sickness, inhibited elevated FGFR3 signaling in chondrocytic cells. To examine the feasibility of meclozine administration in clinical settings, we investigated the effects of meclozine on ACH model mice carrying the heterozygous $Fgfr3^{ach}$ transgene. We quantified the effect of meclozine in bone explant cultures employing limb rudiments isolated from developing embryonic tibiae from $Fgfr3^{ach}$ mice. We found that meclozine significantly increased the full-length and cartilaginous primordia of embryonic tibiae isolated from $Fgfr3^{ach}$ mice. We next analyzed the skeletal phenotypes of growing $Fgfr3^{ach}$ mice and wild-type mice with or without meclozine treatment. In $Fgfr3^{ach}$ mice, meclozine significantly increased the body length after 2 weeks of administration. At skeletal maturity, the bone lengths including the cranium, radius, ulna, femur, tibia, and vertebrae were significantly longer in meclozine-treated $Fgfr3^{ach}$ mice than in untreated $Fgfr3^{ach}$ mice. Interestingly, meclozine also increased bone growth in wild-type mice. The plasma concentration of meclozine during treatment was within the range that has been used in clinical settings for motion sickness. Increased longitudinal bone growth in $Fgfr3^{ach}$ mice by oral administration of meclozine in a growth period suggests potential clinical feasibility of meclozine for the improvement of short stature in ACH. **(*Endocrinology* 156: 548–554, 2015)**

Fibroblast growth factor receptor 3 (FGFR3) is a negative regulator of endochondral bone growth. Gain-of-function mutations in the *FGFR3* gene cause several short-limbed skeletal dysplasias, including achondroplasia (ACH) (1, 2), hypochondroplasia (3), severe ACH with developmental delay and acanthosis nigricans (4), and thanatophoric dysplasia types I and II (5). In contrast, loss-of-function mutations in *FGFR3* lead to camptodactyly, tall stature, and hearing loss syndrome (6). ACH is the most common short-limbed skeletal dysplasia, with an incidence of 1 in 16 000–26 000 live births (7). Clinical features of ACH include severe short stature with rhizomelic shortening of the extremities, relative macrocephaly with frontal bossing, midface hypoplasia, and increased lumbar lordosis. In addition, foramen magnum stenosis, hydrocephalus, and spinal canal stenosis are potentially serious complications of ACH (8).

Growth hormone has been administered to children with ACH for treatment of short stature in some countries (9), but the response to this therapy is moderate and the long-term effects remain controversial. Limb-lengthening procedures are another therapeutic option to gain bone

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Abbreviations: ACH, achondroplasia; CNP, C-type natriuretic peptide; CT, computed tomography; FGFR3, Fibroblast growth factor receptor 3; Micro-CT, microcomputed tomography.

length (10) but involves significant time and effort. Inhibition of FGFR3 signaling is a therapeutic strategy for ACH and other FGFR3-related skeletal dysplasias (11–13); however, no effective treatments are currently available for these disorders. C-type natriuretic peptide (CNP) is a potent antagonist of *FGFR3* signaling, which alleviates the short-limbed phenotype of transgenic ACH mice through inhibition of the intracellular MAPK pathway (14). CNP, however, has a short half-life, and continuous iv infusion is required for in vivo experiments (15). A CNP analog with an extended half-life, BMN-111 has recently been developed, and significant bone growth recovery was demonstrated in a mouse model of ACH by sc administration of BMN-111 (16). Recent studies using induced pluripotent stem cells established from patients with FGFR3-related skeletal dysplasias revealed that statins also rescue the mouse phenotypes (17).

In our previous study, we found that meclozine, an over-the-counter H1 receptor inhibitor used to treat motion sickness, inhibited elevated FGFR3 signaling and promoted chondrocyte proliferation and differentiation using various chondrocytic cell lines. We also confirmed that meclozine alleviates fibroblast growth factor 2 (FGF2)-mediated longitudinal growth inhibition of embryonic tibiae in bone explant cultures (18). In the present study, we orally administered meclozine to immature transgenic mice with ACH and investigated the effects of this drug on longitudinal bone growth and bone-related complications.

Materials and Methods

Mice

Fgfr3^{ach} mice (FVB background) were provided by Dr David M. Ornitz at Washington University (19). In brief, *Fgfr3*^{ach} mice express activated *FGFR3* in the growth plate using the *Col2a1* promoter. In all experiments, we used transgenic mice carrying the heterozygous *Fgfr3*^{ach} transgene. Due to unavailability of a sufficient number of wild-type FVB mice, we employed BL6J mice to investigate the effect of meclozine on wild-type mice. All experimental procedures were approved by the Animal Care and Use Committee of our institution.

Bone explant cultures

For bone explant cultures, tibiae from *Fgfr3*^{ach} mice embryos were dissected under the microscope on embryonic day 16.5, placed in a 48-well plate, and cultured in BGJb medium (Invitrogen) supplemented with 0.2% BSA and 150 mg/ml ascorbic acid in the presence or absence of 20 μM meclozine (MP Biomedicals). The embryonic tibiae were cultured for 6 days with daily replenishment of the medium. The longitudinal length of the bone, which was defined as the length between the proximal and distal articular cartilage, was measured using ImageJ (18).

Oral administration of meclozine to mice

Food containing meclozine, which was prepared by mixing 0.4 g of meclozine with 1 kg of food (Oriental Yeast Co.), was administered ad libitum to 1) wild-type mice from 2 weeks of age, 2) *Fgfr3*^{ach} and littermate mice from 3 weeks of age, or 3) pregnant mice carrying wild-type embryos from 14 days of gestation. Body length was measured every week.

Calculation of feed intake

Fgfr3^{ach} male mice and wild-type female mice were allowed to mate and the 3-week-old littermates were divided into treated and untreated groups. Each group shared one cage for 3 weeks, and total food intake for each cage was measured. The measurements were divided by the total body weight of the mice in each cage, and the calculated values between the two groups were compared.

Radiological analysis

At the end of the treatment, *Fgfr3*^{ach} mice were subjected to microcomputed tomography (Micro-CT) scans (0.5 mm Al fil-

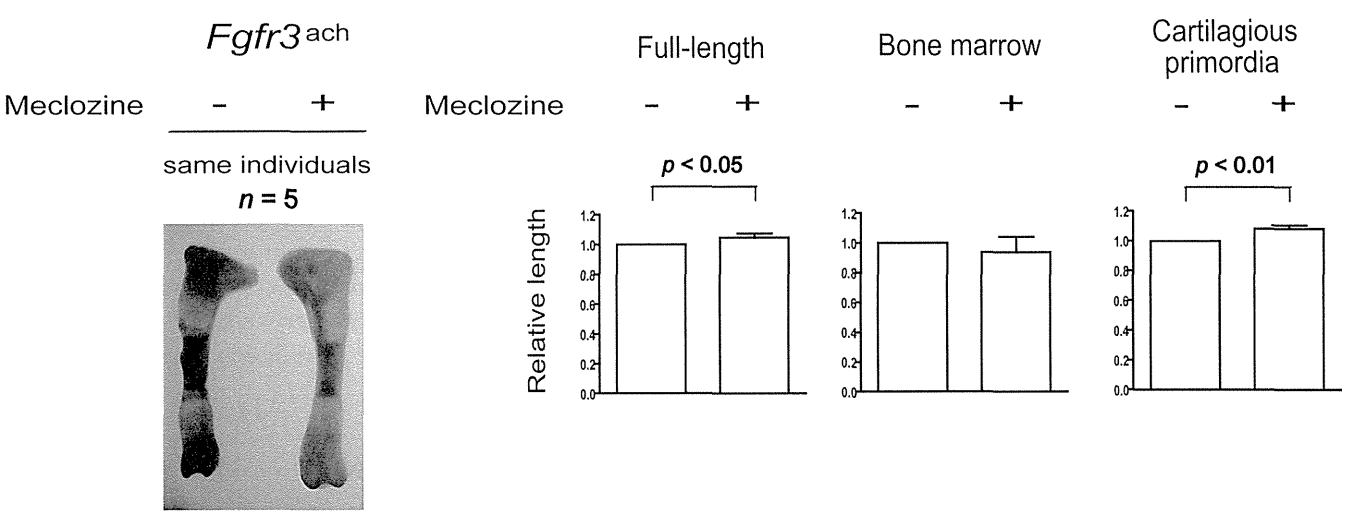

Figure 1. Meclozine enhances bone growth of embryonic tibiae in *Fgfr3*^{ach} mice in explant cultures. Meclozine significantly increased the full-length and cartilaginous primordia of tibiae in *Fgfr3*^{ach} mice but had no effect on the length of bone marrow.

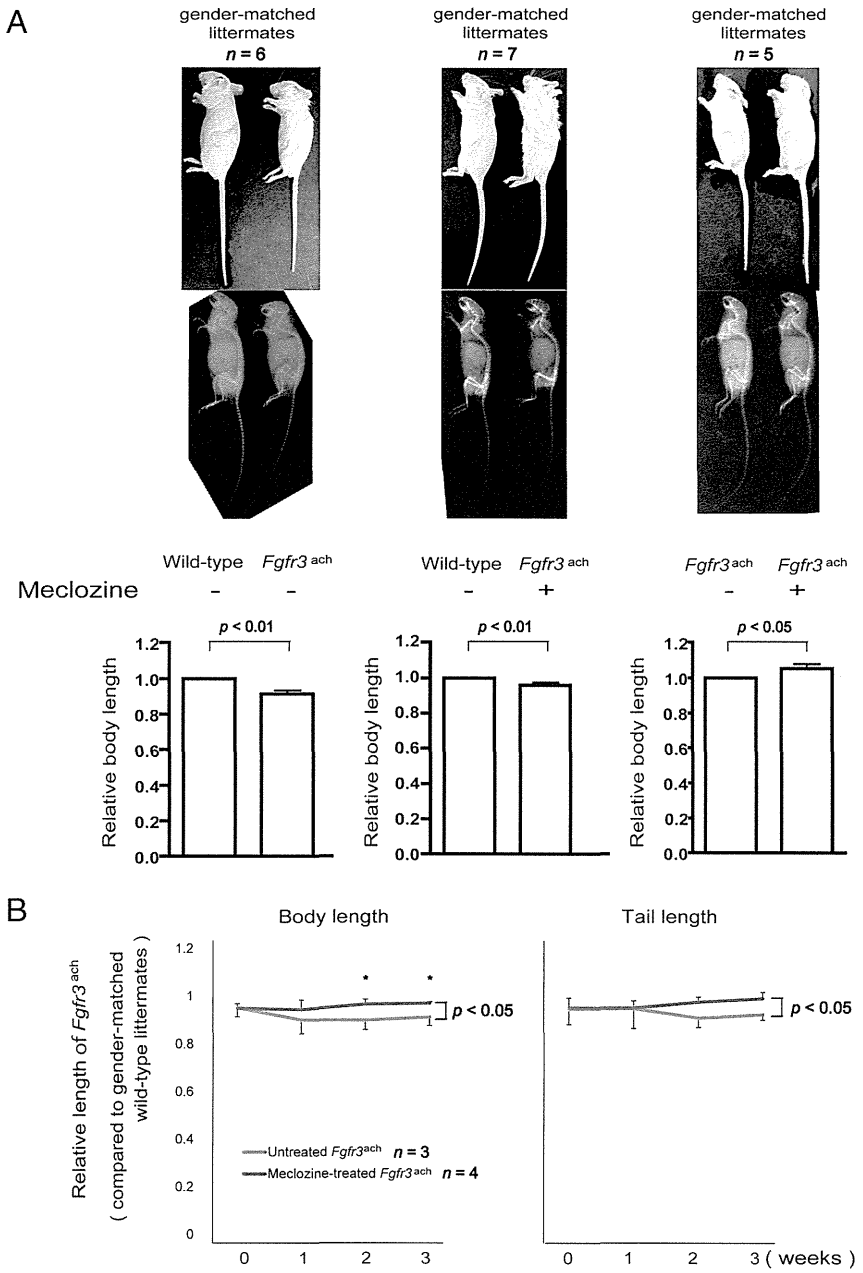

Figure 2. Meclozine reverses the dwarfed phenotype in *Fgfr3*^{ach} mice. A, Meclozine treatment was initiated without prior knowledge of the genotype and administered for 3 weeks. The 3 indicated pairs of sex-matched littermates were examined. Body length of the mice was normalized to that of the indicated sex-matched wild-type littermate. Body lengths of *Fgfr3*^{ach} mice without meclozine treatment were 0.913 ± 0.023 of the sex-matched wild-type littermates (left graph). The body lengths of *Fgfr3*^{ach} mice with meclozine treatment were 0.960 ± 0.018 of the sex-matched wild-type littermates (middle graph). Meclozine significantly increased the body length of *Fgfr3*^{ach} mice ($P < .05$ by unpaired t test, not shown). In 5 kinships in which sex-matched *Fgfr3*^{ach} mice were treated with or without meclozine, meclozine increased the body length of *Fgfr3*^{ach} mice to 1.054 ± 0.027 of the untreated *Fgfr3*^{ach} mice (right graph). Left panel, a male pair; middle panel, a female pair and right panel, a male pair. Mean and SD are indicated in the graphs and the legends. The statistical differences shown on each graph were analyzed by paired t test. B, The body and tail lengths of meclozine-treated *Fgfr3*^{ach} mice were significantly longer than those of untreated *Fgfr3*^{ach} mice. The measured lengths of the *Fgfr3*^{ach} mice were normalized to those of the sex-matched wild-type littermates. Statistical significance by two-way ANOVA is shown on the right side of each graph. *, $P < .05$ by Fisher's LSD test for each pair.

ter, 50 kV, 500 μA for 0.054 s; SkyScan 1176, Bruker) as well as a soft x-ray (30 kV, 5 mA for 20 s; SOFTEX Type CMB-2; SOFTEX). Three-dimensional images from the computed tomography (CT) scan were reconstructed by an in-house volume-rendering software (20). This software enabled us to render 3D views of the CT scan from arbitrary viewpoints and directions, as well as measure the distance between two specific points. The lengths of various bones, including the cranium, humerus, radius, ulna, femur, tibia, and vertebrae (L1–5) were measured on both the soft x-ray films and the reconstructed 3D images. The areas of the foramen magnum and spinal canal from L3, L4, and L5 vertebrae were measured from the reconstructed 2D images with the CT-Analyzer (Bruker). The total bone volume of each mouse was also measured from the reconstructed 3D images.

Skeletal preparation

Whole skeletons were collected, stored in 90% ethanol for 3 days, and followed by acetone treatment for 2 days (21). Specimens were then stained using Alizarin red to analyze ossified bones, and Alcian blue to analyze cartilage, for 3 days at 37°C. Following incubation, the samples were transferred to 1% potassium hydroxide (KOH) and incubated at room temperature for 2 days. The specimens were serially washed with decreasing concentrations (1–0%) of KOH and increasing concentrations (0–100%) of glycerol by monitoring the intensity of the stain and the amount of tissue remaining on the specimens. Longitudinal bones, including the humerus, radius, ulna, femur, and tibia were dissected under the microscope and their lengths were measured using ImageJ.

Measurement of plasma meclozine concentrations

Cardiac blood samples were collected under anesthesia from 8-week-old BL6J mice fed 0.2 or 0.4 g meclozine per kilogram food ad libitum for 72 hours, and plasma concentrations of meclozine were measured (Tanabe R&D Service Co.).

Statistical analysis

Data are expressed as mean ± SD. Statistical analyses were carried out using the paired and unpaired Student t tests, or two-way ANOVA followed by Fisher's LSD test.

Results

Meclozine attenuated the growth defect in the *Fgfr3*^{ach} tibiae in bone explant culture

We quantified the effect of meclozine in bone explant cultures by using developing embryonic tibiae isolated from limb rudiments in *Fgfr3*^{ach} mice (n = 5) at embryonic day 16.5 (E16.5). We added 20mM meclozine to the culture medium and compared the length of the treated tibia with that of the contralateral untreated tibia from the same mouse. Meclozine significantly increased the full-length and cartilaginous primordia of the tibiae from *Fgfr3*^{ach} mice by 4.6% ($P < .05$) and 8.3% ($P < .01$) compared with the contralateral untreated tibiae, respectively. In contrast, the bone marrow in the meclozine-treated tibiae was similar to that in untreated tibiae (Figure 1).

Meclozine ameliorated the short stature of *Fgfr3*^{ach} mice

We next examined the effects of meclozine on longitudinal skeletal growth in *Fgfr3*^{ach} mice. Three-week-old littermates comprised of *Fgfr3*^{ach} and wild-type mice, which were born from *Fgfr3*^{ach} male and wild-type female, were randomly divided into two groups (a meclozine-treated group and an untreated group). Thus, a variable combination of four groups was generated for each kinship: 1) meclozine-treated *Fgfr3*^{ach} mice, 2) meclozine-treated wild-type mice, 3) untreated *Fgfr3*^{ach} mice, and 4) untreated wild-type mice. *Fgfr3*^{ach} and wild-type littermates were not differentiated when meclozine treatment was started because we genotyped mice using tails at the end of the experiment to consecutively measure the total body length. The absolute values of the body length and bone length were compared within a kinship but not between kinships. For comparison across kinships, we used values normalized with wild-type littermates in the same kinship. We analyzed a total of eight kinships, including 12 *Fgfr3*^{ach} mice and 19 wild-type mice. Food containing 0.4 g meclozine per kilogram was administered ad libitum to 3-week-old littermates in the treated group for 3 weeks. At 6 weeks, the body length of the untreated *Fgfr3*^{ach} mice was much shorter than that of the sex-matched wild-type littermates. In contrast, the body lengths of the treated *Fgfr3*^{ach} mice were closer to those of sex-matched wild-type littermates. Quantitative measurements demonstrated that meclozine increased the body length of *Fgfr3*^{ach} mice by 5.4% ($P < .05$; Figure 2A). Temporal analyses demonstrated that meclozine had a statistically significant effect on body length after 2 weeks (Figure 2B). Intake of food by individual mice (per 1 g body weight) was similar between the treated and untreated groups (Supplemental Figure 1).

Meclozine increased the bone length of *Fgfr3*^{ach} mice

We next quantified the effects of meclozine on the bone growth of *Fgfr3*^{ach} mice using radiological analysis. After 3 weeks of meclozine administration, *Fgfr3*^{ach} mice were subjected to soft x-ray and Micro-CT scan. We measured individual bone lengths based on the 3D images reconstructed from the Micro-CT scan. The bone lengths, including the cranium, radius, ulna, femur, tibia, and vertebrae (L1–5) were significantly longer in meclozine-treated *Fgfr3*^{ach} mice than in the untreated *Fgfr3*^{ach} mice (Figure 3A). Similar results were obtained from measurements using the soft x-ray films (Supplemental Figure 2). The bone volumes of the *Fgfr3*^{ach} mice, measured from osseous voxels on the reconstructed images, were significantly increased by meclozine treatment (Figure 3B).

We further analyzed the area of the foramen magnum and lumbar spinal canal in *Fgfr3*^{ach} mice. Meclozine did not in-

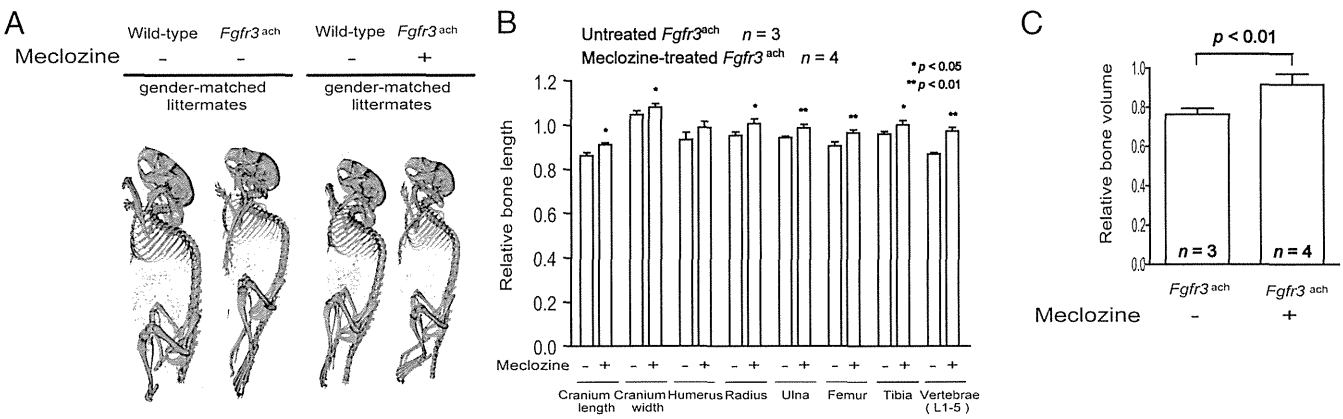

Figure 3. Meclozine increases the longitudinal bone growth of *Fgfr3*^{ach} mice. A, Reconstructed CT images. Left panel, a male pair; right panel, a female pair. B, Meclozine significantly increased the length of the cranium, radius, ulna, femur, tibia, and vertebrae in *Fgfr3*^{ach} mice. C, The total bone volume of meclozine-treated *Fgfr3*^{ach} mice was significantly increased compared with that of untreated *Fgfr3*^{ach} mice. B and C, Bone lengths and total bone volumes of *Fgfr3*^{ach} mice were normalized to those of the sex-matched wild-type littermates. Statistical significance was analyzed by the unpaired *t* test.

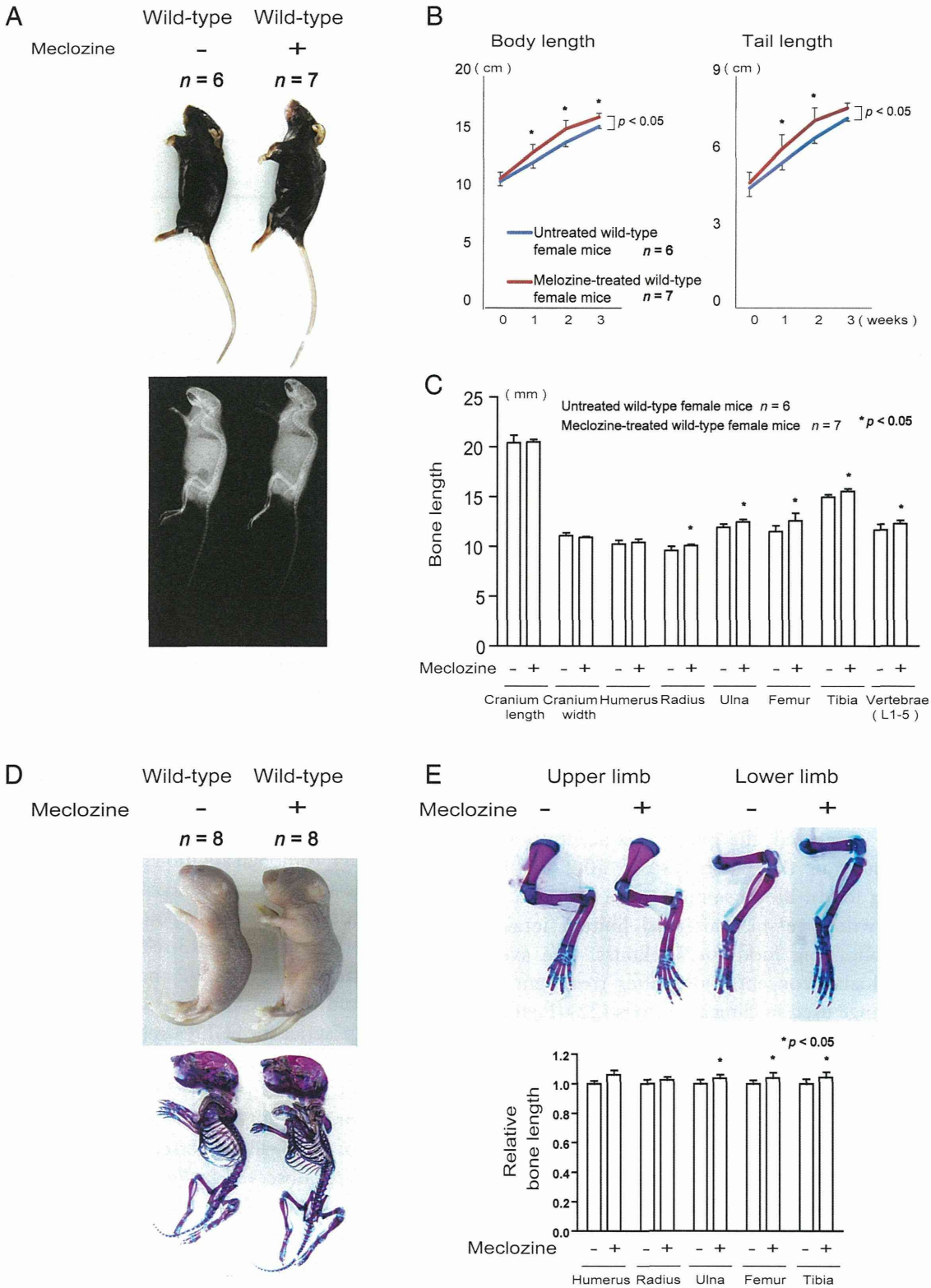

Figure 4. A–C, Wild-type mice were treated with meclozine 2 weeks after birth for 3 weeks. A, Visual images and soft x-ray images of wild-type female mice with or without meclozine. Meclozine-treated mice were larger than the untreated mice. B, Body and tail lengths of meclozine-treated wild-type female mice were significantly longer than those of untreated wild-type female mice. Statistical significance analyzed by two-way ANOVA is shown on the right side of each graph. *, $P < .05$ by Fisher's LSD test for each pair. C, The lengths of the radius, ulna, femur, tibia, and vertebrae on the soft x-ray films were significantly increased by meclozine treatment by unpaired *t* test. D and E, Pregnant mice were treated with meclozine from embryonic day 14. D, Visual images and skeletons stained with Alizarin red and Alcian blue of wild-type mice at postnatal day 5, with or without meclozine. Meclozine-treated offspring were larger than untreated offspring. E, The lengths of the ulna, femur, and tibia measured using stained skeletons were significantly increased after meclozine treatment, as assessed by unpaired *t* test.

crease the area of foramen magnum and lumbar spinal canal in *Fgfr3*^{ach} mice (Supplemental Figures 3 and 4).

Meclozine increased the bone growth in wild-type mice

We further examined the effects of meclozine on bone growth in wild-type mice. Meclozine was administrated to 2-week-old wild-type mice for 3 weeks. Because wild-type mice were weaned at 2 weeks after birth, we started meclozine treatment 1 week earlier than that for *Fgfr3*^{ach} mice. The body length of meclozine-treated mice was significantly longer than that of untreated mice after 1 week (Figure 4, A and B). The bone lengths of the radius, ulna, femur, tibia, and vertebrae (L1–5) of the meclozine-treated mice were significantly longer than the corresponding bone lengths in the untreated mice (Figure 4C).

We next administered meclozine to pregnant wild-type mice to examine the effects of the drug on fetal bone development. After administration of meclozine to wild-type mice at 14 days of gestation, their offspring were subjected to transparent specimen analysis at postnatal day 5. The meclozine-treated offspring were larger than the untreated offspring (Figure 4D). The lengths of the long tubular bones, including the ulna, femur, and tibia, were significantly longer in the meclozine-treated mice than in the untreated-mice (Figure 4E).

The plasma meclozine concentration in the present study is clinically relevant

We next measured the plasma concentrations of meclozine used in the current study (0.2 or 0.4 g meclozine/kg food). Blood samples were collected from 8-week-old wild-type mice after ad libitum intake of meclozine-containing food for 72 hours. The average plasma meclozine concentrations after treatment were within the range used in clinical settings (22) (Figure 5).

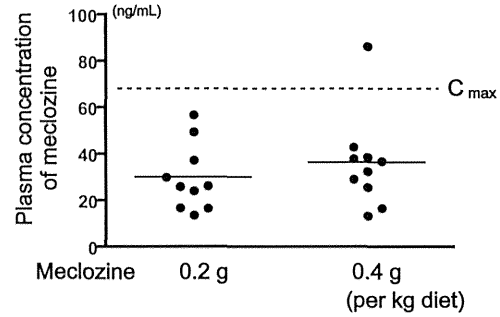

Figure 5. Plasma concentrations of meclozine in 8-week-old wild-type mice fed 0.2 or 0.4 g meclozine/kg diet for 72 h. The average plasma concentrations were 30.30 ± 14.27 ng/ml and 36.58 ± 20.12 ng/ml for 0.2 and 0.4 g/kg diet, respectively. The mean peak drug concentration (C_{max}) after a single dose of 25 mg of meclozine in human subjects was 68.42 ng/ml (22).

Discussion

The small compounds, NF449 (11), A31 (12), and P3 (13), have recently been reported to improve short-stature phenotypes in animal models with accelerated FGFR3 signaling. The safety of these novel compounds in humans, however, remains to be elucidated. Meclozine, an over-the-counter H1 inhibitor, has been safely used for motion sickness for more than 50 years, and its optimal dose and adverse effects have already been established. A previous study suggests that the peak plasma concentration of meclozine is 68.42 ng/ml after a single clinical dose in humans (22). We have demonstrated that oral administration of clinically attainable concentrations of meclozine increased longitudinal bone growth in *Fgfr3*^{ach} mice during the observed growth period. This effect was most likely due to the inhibition of activated FGFR3 signaling, which was previously observed in cultured rat chondrosarcoma cells (18). Currently, distraction osteogenesis is the only available surgical procedure to increase bone length; however, it is a long and invasive treatment that compromises the patient's quality of life. The healing index is 36.2 days per cm (23); thus, the patients are forced to attach external fixators for 362 days to increase their height by 10 cm. In contrast, meclozine increased body length of *Fgfr3*^{ach} mice by 5.4% in our study. If the same effect could be observed in patients with ACH, the patients could be expected to increase 6.7–7.1 cm in height, based on the average height of adults with ACH. Although additional studies are needed to investigate the adverse effects associated with long-term drug administration, meclozine is a potential therapeutic compound for ACH and other FGFR3-related disorders.

To date, CNP, the CNP analog BMN-111, and statins are the only promising compounds that can be applied in clinical practice for FGFR3-related skeletal disorders. Yasoda et al (15) reported that continuous iv administration of CNP rescued the short-limbed phenotype in *Fgfr3*^{ach} mice. Lorget et al (16) similarly demonstrated that sc injection of BMN-111 reversed the dwarfism-related clinical features observed in *Fgfr3*$^{Y367C/+}$ mice. In addition, Yamashita et al (17) identified that ip injection of statins, which have been used to lower serum cholesterol levels, rescued the phenotype of *Fgfr3*^{ach} mice using a screen with patient-derived induced pluripotent stem cells. Currently, CNP and BMN-111 administration is restricted to injection, which potentially poses physical and psychological burdens on young children. Given that meclozine and statins can be administered orally, they are more convenient for children to take. Although the site of action of statins has not been dissected (17), meclozine and statins may

have an additive effect on abnormally activated FGFR3 signaling in patients with ACH.

Some ACH patients have neurological complications such as gait disturbance, leg paralysis, hydrocephalus, and central hypopnea, in addition to short stature, which is caused by stenosis of the foramen magnum or spinal canal. In the current study, meclozine failed to enhance the growth of the foramen magnum and lumbar spinal canal. Similarly, BMN-111 could not increase the sagittal and lateral diameters of the foramen magnum in *Fgfr3*$^{Y367C/+}$ mice (16). Stenosis of the spinal canal and foramen magnum in patients with ACH is most likely due to premature synchondrosis closure, and any growth-promoting treatment for these stenoses must precede the timing of the synchondrosis closure (24). Jin et al (13) demonstrated that the thanatophoric phenotypes of *Fgfr3*$^{K644E/+}$ mice were rescued by ip injection of P3 to the mother. Prenatal treatments are generally unrealistic even if the patients get a definite diagnosis of ACH during prenatal periods. Therefore, we tested the effect of meclozine from 3 weeks of age in *Fgfr3*^{ach} mice. In future, we will examine the effect of maternal administration of meclozine using mouse models to treat devastating phenotypes in thanatophoric dysplasia and possibly stenosis of the spinal canal and foramen magnum in ACH.

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C-Type Natriuretic Peptide Plasma Levels Are Elevated in Subjects With Achondroplasia, Hypochondroplasia, and Thanatophoric Dysplasia

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Context: C-type natriuretic peptide (CNP) is a crucial regulator of endochondral bone growth. In a previous report of a child with acromesomelic dysplasia, Maroteaux type (AMDM), caused by loss-of-function of the CNP receptor (natriuretic peptide receptor-B [NPR-B]), plasma levels of CNP were elevated. In vitro studies have shown that activation of the MAPK kinase (MEK)/ERK MAPK pathway causes functional inhibition of NPR-B. Achondroplasia, hypochondroplasia, and thanatophoric dysplasia are syndromes of short-limbed dwarfism caused by activating mutations of fibroblast growth factor receptor-3, which result in overactivation of the MEK/ERK MAPK pathway.

Objective: The purpose of this study was to determine whether these syndromes exhibit evidence of CNP resistance as reflected by increases in plasma CNP and its amino-terminal propeptide (NTproCNP).

Design: This was a prospective, observational study.

Subjects: Participants were 63 children and 20 adults with achondroplasia, 6 children with hypochondroplasia, 2 children with thanatophoric dysplasia, and 4 children and 1 adult with AMDM.

Results: Plasma levels of CNP and NTproCNP were higher in children with achondroplasia with CNP SD scores (SDSs) of 1.0 (0.3–1.4) (median [interquartile range]) and NTproCNP SDSs of 1.4 (0.4–1.8; $P < .0005$). NTproCNP levels correlated with height velocity. Levels were also elevated in adults with achondroplasia (CNP SDSs of 1.5 [0.7–2.1] and NTproCNP SDSs of 0.5 [0.1–1.0], $P < .005$). In children with hypochondroplasia, CNP SDSs were 1.3 (0.7–1.5) ($P = .08$) and NTproCNP SDSs were 1.9 (1.8–2.3) ($P < .05$). In children with AMDM, CNP SDSs were 1.6 (1.4–3.3) and NTproCNP SDSs were 4.2 (2.7–6.2) ($P < .01$).

Conclusions: In these skeletal dysplasias, elevated plasma levels of proCNP products suggest the presence of tissue resistance to CNP. **(*J Clin Endocrinol Metab* 100: E355–E359, 2015)**

C-type natriuretic peptide (CNP) is a member of the natriuretic peptide family that includes atrial natriuretic peptide and B-type natriuretic peptide. The cognate receptor for CNP is natriuretic peptide receptor-B (NPR-B, gene *NPR2*), a membrane receptor that generates cGMP as the second messenger. CNP is produced in the

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Abbreviations: AMDM, acromesomelic dysplasia, Maroteaux type; CNP, C-type natriuretic peptide; FGFR-3, fibroblast growth factor receptor-3; MEK, MAPK kinase; NPR, natriuretic peptide receptor; NTproCNP, amino-terminal propeptide of C-type natriuretic peptide; SDS, SD score.

growth plate and is a potent positive regulator of linear growth (reviewed in Ref. 1). Homozygous or biallelic inactivating mutations of *NPR2* cause acromesomelic dysplasia, Maroteaux type (AMDM) (MIM 602875), a form of short-limbed dwarfism (2).

CNP levels can be measured in plasma, although specific clearance pathways result in low levels. Biosynthetic processing of CNP generates an amino-terminal propeptide (NTproCNP) that is released from the cell in a ratio equimolar to that of CNP. This propeptide is not subject to specific clearance pathways. As a result, plasma NTproCNP levels reflect CNP production more accurately than levels of the active peptide (3). In a previous report, we documented greatly elevated plasma concentrations of CNP and NTproCNP in a child with AMDM (1), suggesting that reduced intracellular CNP pathway activity may increase CNP production.

Achondroplasia (MIM 100800) is the most common skeletal dysplasia with incidence estimates ranging from 1 in 15 000 to 1 in 26 000 births (4). Achondroplasia is caused by a mutation in the fibroblast growth factor receptor-3 (FGFR-3) gene (*FGFR3*) (5). A single mutation (G380R) accounts for greater than 98% of all reported cases of achondroplasia and is a gain-of-function mutation. Hypochondroplasia (MIM 146000) is a related, but milder, skeletal dysplasia. Thanatophoric dysplasia (MIM 187600) is a rarer syndrome of skeletal dysplasia, with phenotypic features more severe than those in achondroplasia and is often lethal in the neonatal period. Both hypochondroplasia and thanatophoric dysplasia are also caused by gain-of-function mutations in *FGFR3* (6, 7).

In the growth plate, FGFR-3 activates a number of signaling cascades, the most important of which appear to be the signal transducers and activators of transcription (STAT1) pathway, which inhibits chondrocyte proliferation, and the MAPK kinase (MEK)/ERK MAPK pathway, which inhibits chondrocytic differentiation and increases matrix degradation. The net result is poor bone growth (reviewed in Ref. 8). The MEK/ERK MAPK pathway and the CNP intracellular signaling pathway interact and are mutually inhibitory (9). Evidence of functional inhibition of NPR-B by FGFR-3 overactivity and our finding of raised plasma CNP peptides in a patient with a homozygous loss-of-function mutation in NPR2 led us to postulate that plasma levels will also be raised in disorders associated with constitutive activation of FGFR-3.

Subjects and Methods

Subjects

Subjects were healthy individuals with the clinical diagnosis of achondroplasia (63 children and 20 adults), hypochondroplasia (6 children), thanatophoric dysplasia (2 children), or AMDM (4 children and 1 adult). This study was approved by the Nemours Florida Institutional Review Board. All children had written parental permission obtained. All adult subjects had written informed consent obtained.

Study procedures

With the exception of AMDM, this was a prospective study. All subjects with achondroplasia, hypochondroplasia, or thanatophoric dysplasia were seen in the Skeletal Dysplasia Clinic at Nemours/Alfred I. duPont Hospital for Children in Wilmington, Delaware. Anthropometric measurements were obtained, including standing height by wall-mounted tape measure or recumbent length by measuring table and weight by electronic scale. If the subject was an established patient, heights from previous visits were obtained from the medical record for determination of annualized height velocity.

Subjects with AMDM were seen by a variety of geneticists around the world. Blood was drawn locally, and plasma was frozen and shipped for analysis.

Assays

Blood was drawn into EDTA tubes and stored at 4°C until processed. Blood was centrifuged at 4°C, and plasma was aliquoted and frozen at −80°C until assayed.

The radioimmunoassays used for CNP and NTproCNP were as described previously (10, 11).

Statistical analysis

Standard deviation scores (SDSs) were calculated using the LMS method (12). Height SDSs were calculated using Centers for Disease Control and Prevention 2000 data (13). For the subjects with AMDM residing outside the United States, country-specific height data were used. SDSs for CNP, NTproCNP, and the CNP/NTproCNP ratio were calculated using reference data from our previous studies of healthy children (10) and adults (11). Achondroplasia-specific height SDSs were calculated using estimates of age-specific means and SDs from height charts reported by Horton et al (14).

Data are summarized as medians and interquartile ranges (25th–75th percentiles). For height SDS data, one-sample Student *t* tests were used to compare groups with the general population. For the peptide assay data, because of the widely differing ranges of variance in the sample groups, nonparametric tests were used. For the children, comparisons between the reference population and subjects with achondroplasia, hypochondroplasia, or AMDM were made using Kruskal-Wallis tests, with Holm-adjusted Mann-Whitney rank sum tests for post hoc pairwise comparisons. For the adults, comparisons of SDS data were made using Mann-Whitney rank sum tests. Correlations between the NTproCNP level and height velocity were done by fitting a line by least squares and performing linear regression analysis. Pearson product-moment correlation coefficients (r) are reported. Statistics were calculated using Primer of Biostatistics software (version 7; The McGraw-Hill Companies, Inc). Significance was assumed for P values <0.05.

Results

Achondroplasia

The characteristics of the subjects with achondroplasia are shown in Table 1. In children with achondroplasia,

Table 1. Characteristics of Subjects With Achondroplasia or Hypochondroplasia

	Achondroplasia		
	Children	**Adults**	**Hypochondroplasia: Children**
No.	63	20	6
Sex (female/male)	31/32	11/9	3/3
Age, y	4.7 (2.9 to 7.5)	41 (36 to 45)	8.6 (6.6 to 10.9)
Height SDS[a]	− 4.8 (− 5.6 to − 4.2)[b]	ND	− 3.1 (− 3.7 to − 2.2)[c,d]
Height SDS[e]	−0.1 (−0.8 to 0.5)	ND	1.9 (1.3 to 3.0)[d]
CNP, pM	2.1 (1.7 to 2.4)	0.9 (0.7 to 1.1)	2.3 (1.9 to 2.5)
CNP SDS	1.0 (0.3 to 1.4)[b]	1.5 (0.7 to 2.1)[c]	1.3 (0.7 to 1.5)[c]
NTproCNP, pM	53.0 (47.3 to 63.0)	17.0 (16.0 to 19.3)	55.2 (52.1 to 58.7)
NTproCNP SDS	1.4 (0.4 to 1.8)[b]	0.5 (0.1 to 1.0)[c]	1.9 (1.8 to 2.3)*
NTproCNP/CNP ratio	26 (31 to 22)	21 (16 to 36)	23 (25 to 22)
NTproCNP/CNP SDS	−0.1 (−0.7 to 0.4)	−0.9 (−1.6 to 0.4)	0.2 (−0.4 to 0.2)

Abbreviation: ND, not determined. Data are median (interquartile range).

[a] Using general population reference standards.

[b] $P < .0005$ compared with the reference population.

[c] $P < .01$ compared with the reference population.

[d] $P < .01$, compared with subjects with achondroplasia.

[e] Using achondroplasia-specific reference standards.

plasma concentrations of both CNP and NTproCNP (Figure 1) were higher than those in the reference population ($P < .0005$ for both), despite markedly reduced height. Similarly, adults with achondroplasia also had higher levels of CNP and NTproCNP ($P < .005$ for both) (Table 1). The NTproCNP/CNP ratio is a measure of CNP clearance and did not differ from that of the reference population (Table 1).

Linear regression analysis showed that in children with achondroplasia, the NTproCNP level had a significant positive correlation with height velocity (n = 62, r^2 = 0.42, $P < .0005$) (Figure 1C). A similar relationship was found in the reference population (n = 139, r^2 =0.51, $P <$ .0005) (10). The regression line for children with achondroplasia differed from that of the reference population both for slope (1.76 ± 0.27 vs. 2.41 ± 0.20 pM/cm/y, respectively [mean ± SE], $P < .05$) and for intercept (46.7 ± 2.3 vs. 24.1 ± 1.3 pM, $P < .0005$).

Hypochondroplasia

Table 1 shows the characteristics of the subjects with hypochondroplasia, all of whom were children. Compared with the reference population, these subjects had elevated plasma CNP and NTproCNP levels (Figure 1) ($P < .05$ for both). Compared with subjects with achondroplasia, the CNP and NTproCNP SDSs were not different (Figure 1).

Thanatophoric dysplasia

We studied 2 young children with thanatophoric dysplasia. One subject was a 2.3-year-old boy with a height SDS of −11.5. His plasma CNP level was 3.0 pM (SDS of 3.0) and his NTproCNP level was 67.3 (SDS of 1.1). The second subject was a 2.7-year-old boy with a height SDS of −11.1. His plasma CNP level was 1.0 pM (SDS of 0.0), and his NTproCNP level was 72.2 (SDS of 1.8).

AMDM

Table 2 shows the characteristics of subjects with AMDM. In the children, CNP SDSs (n = 3, $P < .01$) and NTproCNP SDSs (n = 4, $P < .005$) were significantly higher than the values in the reference population and were also higher than the values in those with achondroplasia (CNP SDSs, $P < .05$; NTproCNP SDSs, $P < .005$, Figure 1). In the adult with AMDM, both plasma CNP and NTproCNP were markedly elevated.

Discussion

The finding that CNP products in plasma were greatly elevated in a subject with profound short stature due to a disruption of the CNP receptor (NPR-B) and reports from others that activation of the MEK/ERK MAPK pathway inhibits NPR-B signaling, led us to postulate that plasma levels would also be elevated in people with FGFR-3–related skeletal dysplasias such as achondroplasia. The current findings clearly show that circulating products of proCNP are raised not only in children and adults with achondroplasia but also in children with related conditions of FGFR-3 overactivity.

People with AMDM have absent or disrupted CNP receptors. Because CNP is a growth-promoting factor and people with AMDM have profound growth failure, this is

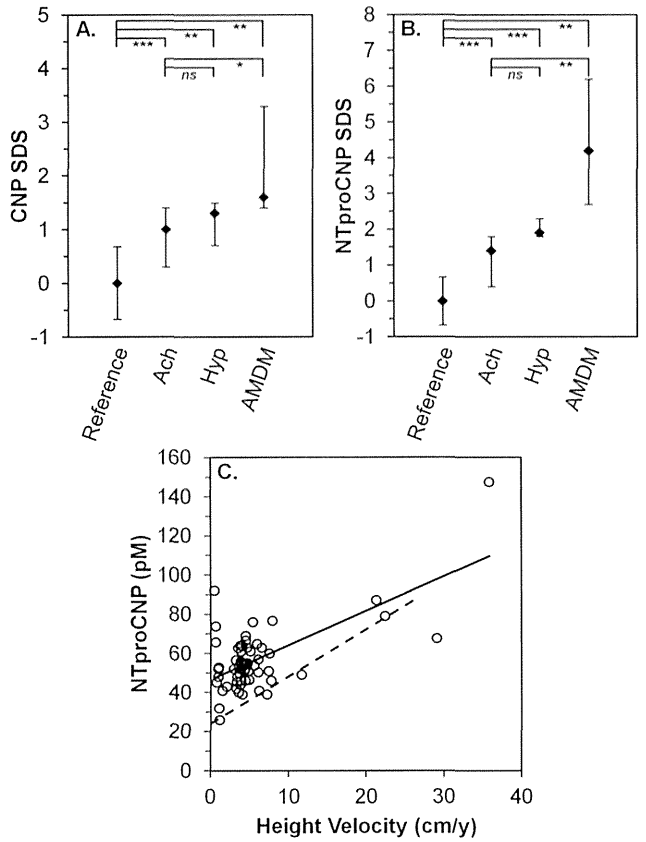

Figure 1. CNP and NTproCNP levels in children. A and B, Comparison between different skeletal dysplasias. SDSs are shown for CNP (A) and NTproCNP (B) for children from the reference population (n = 318), children with achondroplasia (Ach, n = 63), hypochondroplasia (Hyp, n = 6), and AMDM (n = 4). Diamonds show the median for each group and error bars the 25th and 75th percentile. ns, difference is not significant. *, $P < .05$; **, $P < .01$; ***, $P < .0005$. C, Correlation between height velocity and NTproCNP levels in children with achondroplasia. The annualized height velocity was determined using the height at a previous clinic visit and the height from the study visit. Solid line, least mean squares linear regression line. The correlation is significant (n = 62, r^2 =0.416, $P < .0005$). Dashed line, previously published regression line from children from the general population (n = 139, r^2 = 0.711, $P < .0005$) (10). The two regression lines differ both in intercept ($P < .05$) and in slope ($P < .0005$).

a classic instance of hormone resistance. We have shown here that CNP and NTproCNP levels are markedly elevated in people with AMDM, suggesting that CNP, as in virtually all other hormone axes, is regulated by a negative feedback loop. Supporting this conclusion are two reports of subjects with activating mutations of NPR-B causing skeletal overgrowth (15, 16), in whom plasma NT-proCNP concentrations were profoundly reduced. Little is known about the factors that regulate CNP expression and translation; the details of this feedback loop require further study.

The interaction between the MEK/ERK MAPK and CNP/cGMP pathways has been defined in vitro in chondrogenic cell systems and in organ culture. Phosphorylated MEK1/2 and/or ERK1/2 directly or indirectly inhibit cGMP generation by NPR-B (9). Meanwhile, NPR-B-generated cGMP, in a pathway that involves cGMP-dependent protein kinase II (PRKG2) and the MKK/p38 MAPK pathway, inhibits MEK/ERK activation by inhibiting RAF1 (9, 17–19). Hence, in vitro data describe a potential mechanism in which overactivation of the MEK/ERK MAPK pathway can result in resistance to CNP.

In this study, we observed a clear increase in CNP and NTproCNP levels in subjects with achondroplasia and hypochondroplasia. We also provide evidence for increased levels in 2 children with thanatophoric dysplasia, although the sample size was too small for statistical confirmation. Assuming the presence of the CNP regulatory feedback loop as suggested by the data from subjects with AMDM, the finding of elevated CNP levels in a population with severe short stature suggests that these individuals may also have resistance to CNP. This is further demonstrated by Figure 1C, which shows that the slope of the regression line linking NTproCNP and height velocity are significantly reduced in children with achondroplasia compared with those in the reference population.

There are other potential explanations for our findings. It may be that another branch of the FGFR-3 signaling cascade up-regulates CNP expression and that the MAPK inhibition of NPR-B signaling is not occurring or is not relevant in vivo. Another possibility is that the elevated blood levels of CNP are arising from other tissues and not the growth plate and hence not relevant to the growth failure. Now that the observation has been made, further definition is needed to provide clarity. Of interest, products of proCNP in plasma are also elevated in adults with achondroplasia or AMDM. The tissues that contribute to

Table 2. Characteristics of Subjects With AMDM

Age	Genotype	Sex	Height SDS	CNP, pM	CNP SDS	NTproCNP, pM	NTproCNP SDS	NT/CNP	NT/CNP SDS
2.5 y	G413E/G413E	M	−5.1	2.7	1.6	86.3	2.8	32.0	−0.5
4.9 y	del/del	M	−5.3	ND	ND	110.2	5.6		
7.5 y	R668stop/R218C	F	−2.3	2.1	1.1	58.0	2.4	27.6	−0.6
7.9 y	I364fs/I364fs	F	−8.5	7.6	5.0	172.0	7.9	22.6	0.1
30 y	Q853stop/R989L	M	−8.6	7.8	46.6	144.0	8.0	18.5	−1.7

Abbreviations: F, female; M, male; ND, not determined.

the plasma levels of CNP and NTproCNP after growth plates have closed have not been clearly defined but are likely to include skeletal, vascular, and cardiac (11, 20) tissue. CNP, NPR-B, and FGFR-3 are all expressed in these tissues. The finding of elevated plasma levels of CNP in adults with achondroplasia suggests that alteration of the CNP pathway by activating *FGFR3* mutations is not limited to the growth plate.

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