



Review

Molecular Mechanism of Induction of Bone Growth by the C-Type Natriuretic Peptide

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Abstract: The skeletal development process in the body occurs through sequential cellular and molecular processes called endochondral ossification. Endochondral ossification occurs in the growth plate where chondrocytes differentiate from resting, proliferative, hypertrophic to calcified zones. Natriuretic peptides (NPTs) are peptide hormones with multiple functions, including regulation of blood pressure, water-mineral balance, and many metabolic processes. NPTs secreted from the heart activate different tissues and organs, working in a paracrine or autocrine manner. One of the natriuretic peptides, C-type natriuretic peptide-, induces bone growth through several mechanisms. This review will summarize the knowledge, including the newest discoveries, of the mechanism of CNP activation in bone growth.

Keywords: natriuretic peptide; growth plate; chondrocyte cell; bone growth; molecular mechanism



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1. Introduction

The skeletal development process in the body occurs through sequential cellular and molecular processes called endochondral ossification. Only craniofacial bones and clavicles are not developed through endochondral ossification [1]. Endochondral ossification occurs in the growth plate where chondrocytes differentiate from resting (reserve), proliferative, hypertrophic, to calcified zones. Differentiated chondrocytes construct a cartilage extracellular matrix (ECM) through the upregulation of proteolytic enzyme production (matrix metalloproteinases). Together with the vascular endothelial growth factor, this process induces degradation of cartilage matrix components inducing vascularization. Eventually, cartilage will be replaced with mature bone tissue. Differentiation of growth plate cartilage is regulated by several factors, including hormones, growth factors, and components of the cartilage ECM [2].

Natriuretic peptides (NPTs) are peptide hormones with multiple functions, including the regulation of blood pressure, water-mineral balance, and many metabolic processes [3]. NPTs secreted from the heart (the cardiac atria and ventricles-ANP and BNP; vascular endothelium-CNP) activate different tissues and organs, working in a paracrine or autocrine manner. The first NPT was discovered as atrial NPT (ANP) in 1981, secreted from the cardiac atria and ventricles [4]. From that time, eight NPTs have been described: ANP, B-type natriuretic peptide (BNP), C-type NPT (CNP), D-type NPT (DNP), urodilatin, urogualin, osteocrin, myocardin, and musclin [3]. The functions of three main peptides (ANP, BNP, and CNP) were investigated in detail.

The peptides mentioned above can be used to diagnose heart or pulmonary diseases (Table 1). Various cellular factors stimulate the expression of genes coding for ANP, BNP, and CNP, and the secretion of these peptides. In the case of patients with heart failure,

levels of particular forms of ANP (proANP/ γ -ANP, β -ANP) or BNP (uncleaved proBNP, mature BNP, and N-terminal proBNP) increased compared to healthy controls. Precursors of the peptides are proteolytically degraded to their active forms, acting in tissues and organs [5]. Moreover, a decrease in the levels of NPTs and an increase in the receptor level that degrades the peptides (NPR-C) have been associated with diet and physical activity [6].

Table 1. Natriuretic peptides used in diagnosis and treatment.

NTPs	NTPs Used for Diagnosis/Evaluation		NTPs Tested for Diseases as a Treatment
	Final Peptide	Pro-form of the Peptide	
ANP	Heart failure [7–10] Cancer [11] Type-2 diabetes [12] Chronic renal failure [13]	Heart failure [10,14] Insulin resistance [15,16] Obesity [17]	Heart failure [18–22] Cardioprotective effects during surgery [23–30] Renal failure [31–36] Colorectal cancer [37] Forearm vasculature [37] Acute kidney injury [38] Lipid mobilization [39] Pulmonary vasorelaxant [40]
BNP	Heart failure [10,41–53] Acute dyspnea [54] Asthma [55] Type 2 diabetes [56] Cancer [11] Chronic renal failure [13] Obesity [17,57] Chronic kidney disease [58,59] SARS-CoV-2 [60] Atrial fibrillation [61] Hemodialysis patients [62] Diabetic nephropathy [63]	Heart failure [48,64–69] Type 2 diabetes [70,71] Obesity [17,72] Chronic kidney disease [58] Cardiac function [73] Stroke [73]	Forearm vasculature [37] Pulmonary vasorelaxant [40] Myocardial infarction [74,75]
CNP	Heart failure [47,76]	Heart failure [64] Growth failure [77]	Heart failure (CNP fused to DNP) [78] Renal failure [79,80] Achondroplasia [81,82] Anxiety [83]

There are three known NPT receptors: NPT receptor-A (NPR-A; also called NPR1 or GC-A), NPT receptor-B (NPR -B; another way NPR2 or GC-B), and NPT receptor-C (NPR-C; also called NPR3 or GC-C). ANP and BNP are ligands for NPR-A, while CNP activates NPR-B. NPR-C receptor is responsible for the degradation and internalization of circulating NPTs within the cell [84]. NPRs catalyze the synthesis of cyclic guanosine-3',5'-monophosphate (cyclic GMP; cGMP), known as the internal mediator for most effects of NPTs.

CNP structure differs from ANP and BNP in the second cysteine, lacking further C-terminal extension [85]. CNP is released by endothelial cells, cardiomyocytes, and fibroblasts and regulates the function of the cardiovascular system together with ANP and BNP [86]. However, CNP function not only affects the cardiovascular system but also governs bone growth [87–91]. Interestingly, the mechanism of CNP action can be different depending on the cell type. CNP activates ossification and calcification in bone cells, while in the valvular interstitial cells, those processes are inhibited by the CNP peptide [92]. The same pattern is observed with osteoblast and myofibroblast differentiation, where those processed are activated in bone cells and inhibited in the valvular interstitial cells with CNP treatment [92].

Some previous reviews explained CNP peptide and its bone mechanism, but still questions remain unanswered. This review will summarize our knowledge, including the newest discoveries, on the mechanism of CNP activation in bone growth. MeSH (Medical Subject Headings) was applied to select articles in this review process. In PubMed search,

we have used the keywords of “C-Type Natriuretic Peptide bone growth”, “C-Type Natriuretic Peptide”, “CNP bone”, and “C-Type Natriuretic Peptide mechanism bone”. A total of approximately 40 articles were found under the CNP activating bone mechanism. We have also searched the clinical trials via <https://clinicaltrials.gov/> (accessed on 4 April 2022) to prepare Table 1.

2. C-Type Natriuretic Peptide and Nitric Oxide as Bone Growth Regulators

CNP as a bone growth regulator was first mentioned in 1995 [93], where CNP was found to induce bone resorption. The production of CNP can be induced by cytokines (such as IL-1 α , IL-1 β , and TNF α), thus affecting osteoclast resorption. It is unclear if CNP acts directly on osteoclasts or not. When CNP was added to the culture, cGMP production increased (as well as bone resorption), meaning that the receptor for CNP (NPR-B) is present in the bone marrow culture cells. Adding the CNP inhibitor to the culture decreased the efficiency of bone marrow resorption in CNP-treated and untreated cells, indicating the bone marrow released CNP. Nevertheless, it is unclear what kind of cells secreted CNP peptide, as bone marrow is a heterogeneous mixture of the cells [93]. Not only resorption of osteoclasts but also proliferation and differentiation of osteoblasts are regulated by CNP peptides. Production of cGMP, induced by CNP, leads to bone formation in osteoblast-like cells. CNP activation increased the activities of alkaline phosphatase (ALP) and osteocalcin and the mineralization of nodules in the cell culture [94]. Similarly, CNP upregulated bone turnover biomarkers, both osteoblastic (osteocalcin, procollagen type I, total ALP, and bone-specific alkaline phosphatase) and osteolytic (tartrate-resistant acid phosphatase-TRAP and cross-linked C-terminal telopeptide of type I collagen- CTX-I) in osteoblast-like cells [91].

Another molecule in bone growth regulation is nitric oxide (NO). Mice deficient in the eNOS show bone abnormalities, while inducible NOS (iNOS) deficient mice show imbalances in bone osteogenesis and abnormalities in bone healing [95–97]. iNOS is synthesized in response to inflammatory stimulation, producing NO, thereby inducing bone loss. As NO is a highly reactive molecule, molecular bone targets are not fully understood [98]. Nevertheless, NO inhibits bone resorption by inducing cGMP synthesis (the same as CNP) [99]. Thus, CNP can affect bone remodeling [93].

Research on the interactions of NO and NPTs has shown that only ANP can inhibit NO through the NPR-A receptor in macrophages [100]. Even though all natriuretic receptors (with two types of NPR-B receptor) are produced in macrophages, CNP still does not inhibit NO. Receptor for CNP exists in two NPR-B receptors that differ in structure [101], while CNP is a ligand for both. However, one of the forms is shorter, without the ability to induce cGMP production by CNP binding [100]. NO is synthesized from L-arginine by inducible nitric-oxide synthase (iNOS) [102]. CNP neither inhibits the induction of iNOS nor induces L-arginine transport, which is critical for NO production [103].

Both NO and CNP stimulate the production of cGMP in cells. An additional product of the cGMP forming reaction could be 8-nitro-cGMP (Figure 1). The level of 8-nitro-cGMP increases in the presence of CNP in bone culture in vitro. Like cGMP, 8-nitro-cGMP stimulates the proliferation of cells in the growth plate as the bone grows. These results show that NO and CNP activation mechanisms are more complicated, activating cGMP and other molecules, which may work synergistically in the bone growth effect [101].

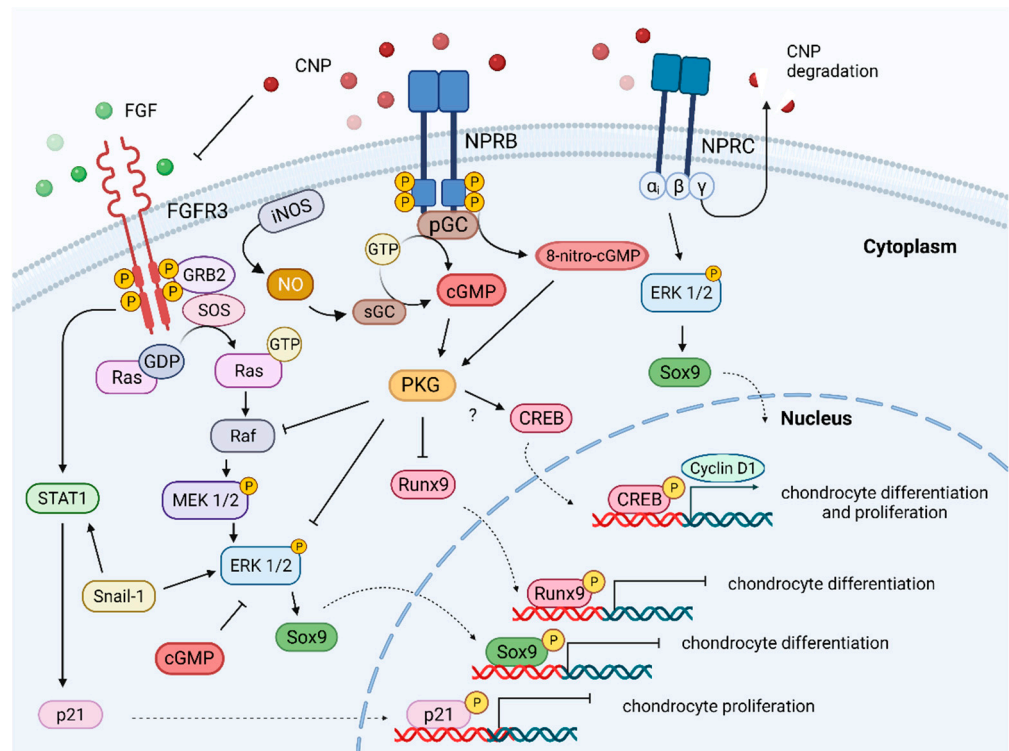


Figure 1. Signaling pathways activated or inhibited by CNP in the bone growth plate region. The cellular effects of CNP action are mediated through the NPR-B receptor, stimulating intracellular molecule cGMP production, which further results in the protein kinase G (PKG) activation. PKG activation by CNP results in the MAPK signaling pathway, which results in chondrocyte proliferation and differentiation. NPR-C receptor degrades CNP; additionally, Gi protein of the receptor modulates the level of ERK1/2 inside the cell. ?- it is not confirmed if CREB is activated by PKG.

3. CNP/NPR-B/cGMP Pathway

The signaling pathway of the chondroprotective effects with CNP is mediated by the NPR-B receptor, enhancing the activation and synthesis of cGMP. As a result, PKGII is activated, leading to the elevation of ECM (collagen II, aggrecan) and bone mineralization (ALP), finally inducing bone growth. Additionally, the activation of the NPR-B receptor inhibits the MEK/ERK signaling pathway, stopping cartilage degradation (Figure 1). However, this pathway can be stimulated by other factors (not only CNP) that either enhance or reduce bone growth. Aging and inflammation are some processes affecting reduced bone growth [104]. Thus, skeletal dysplasia should be treated at an early age of patients. There are also different CNP/NPR-B/cGMP signaling pathway inhibitors. One of the first described inhibitors was lysophosphatidic acid (LPA), which causes a decrease in cGMP concentration that was not correlated with the receptor loss. While previous research showed that PKC could be involved in the dephosphorylation of the NPR-B receptor at the Ser-523 residue [105], in this case, the activity of PKC was not changed. According to the authors, this response increases the response of fibroblasts to the wound-healing process [106]. Those investigations showed that CNP activation and different agents could regulate the NPR-B receptor. cGMP is converted from guanosine triphosphate in reactions catalyzed by various enzymes (guanyl, guanylyl, or guanylate cyclases). Guanylate cyclases are activated by different factors such as guanylin, uroguanylin, guanylyl cyclase-activating proteins, NO, as well as NPTs. Activation of the second intracellular messenger (cGMP) stimulates or modulates different pathways within the cell, depending on what receptor was activated (NPR-A, -B, or C). Processes that are activated by cGMP include platelet aggregation, blood pressure neurotransmission, sexual arousal, gut peristalsis, long bone

growth, intestinal fluid secretion, lipolysis, phototransduction, cardiac hypertrophy, and even oocyte maturation [107].

Guanylyl cyclases are ubiquitous enzymes with two classes occurring in mammals: soluble cytoplasmic guanylyl cyclase and single membrane-spanning forms (NPR-/GC-A, -B, -C, -D, -E, -F, and -G) that can be produced in different tissues. The localization within the body of these two guanylyl cyclases classes is different, as well as their ligands. ANP and BNP ligands activate NPR-A, produced in lung, kidney, adrenal, vascular smooth muscle, endothelium, heart, and adipose. Inactivation of NPR-A leads to heart-related problems (hypertension, cardiac fibrosis, and hypertrophy). The NPR-B, activated by CNP, is produced in bones, vascular smooth muscle, lung, brain, heart, liver, uterus, and follicles [108]. ATP increases the enzyme activity of NPR-B [109]. The degradation receptor for the NPTs is synthesized in the intestinal epithelium, stimulated by bacterial heat-stable enterotoxin, guanylin, and uroguanylin. Impaired production of GC-C leads to increased proliferation of colonic epithelial cells without a change in blood pressure [107].

4. CNP/NPR-B/cGMP/MAPK Pathway

The CNP/NPR-B/cGMP pathway activates endochondral ossification and induces bone growth in different models. FGFs are soluble or cell surface proteoglycans (family of 22 members [110] that activate FGFR-3 tyrosine receptors on the subphase of chondrocytes [111]. Activating mutations of FGFR3 are associated with skeletal diseases like achondroplasia, hypochondroplasia, or thanatophoric dysplasia [112]. Signal transduction of the FGFR-3 phosphorylates ERK1/2, a protein kinase of the MAPK signaling pathway [113]. Constantly phosphorylated ERK1/2 is associated with achondroplasia [114]. On the other side, the FGFs family is associated with slowing long bone growth. Intracellular interactions of FGFs/FGFR-3/MAPK and CNP/CG B/cGMP pathways were studied in mouse chondrogenic cell lines [115]. CNP signaling activation of cGMP was reduced by FGF-2 and FGF-18, while expression of the cGMP itself was not FGFs-dependent (Figure 1). CNP and cGMP inhibited the activation of pERK1/2 by FGFs/FGFR-3. CNP did not affect STAT-1 phosphorylation by FGFs/FGFR-3, another signaling pathway that could be activated (Figure 1). CNP treatment of fetal mouse tibias induced longitudinal bone growth, increasing in size and the number of chondrocytes. This effect was attenuated when FGF18 was added to the culture [115]. Crosstalk was visible between those two pathways, with evidence of the CNP influence on bone growth.

Further research confirmed the interaction of CNP signaling pathway inhibition of FGF-2 by both direct and indirect mechanisms [116]. CNP inhibited phosphorylation and activation of ERK protein kinase induced by the FGF-2 pathway in the rat chondrocyte model. CNP blocks the ERK mitogen pathway at the level of Raf-1, but not Ras or FRS2. To inhibit Raf-1, protein kinase G (PKG) is required because the inhibited CNP pathway was not able to block the ERK/MAPK pathway. The FGF-2 pathway without CNP induces degradation of chondrocyte ECM in a rat cell model of chondrosarcoma. With CNP, FGF-2 was partially inhibited, leading to the expression, activation, and release of ECM molecules and proteins (including matrix metalloproteinase 2 (MMP2), -3, -9, -10, and MMP13). Moreover, CNP induced matrix-mediated production independently of FGF-2 [116] (Figure 2).

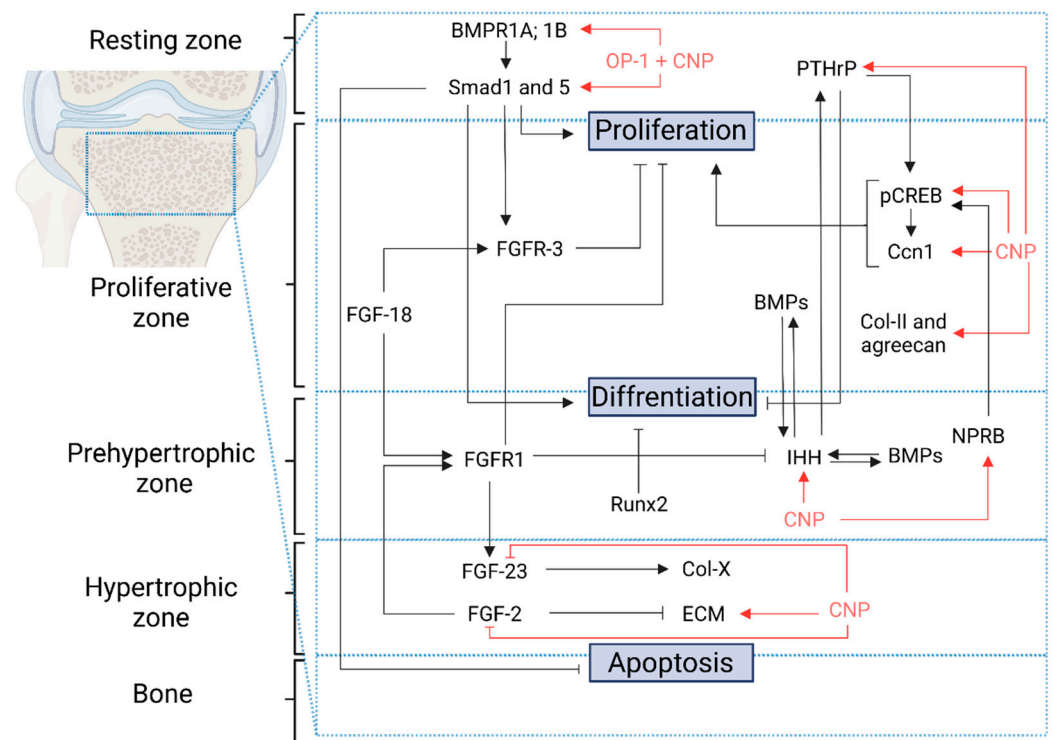


Figure 2. CNP and related bone growth involved protein expression patterns in the growth plate. BMPR1A; 1B: Bone morphogenetic protein receptors; Smad1; 5: Suppressor of Mothers against Decapentaplegic; PTHrP: Parathyroid hormone-related peptide; pCREB: phosphorylated cAMP-response element-binding protein; Ccn1: Cyclin 1 gene; Col-II: Collagen type 2; BMPs: Bone morphogenetic proteins; IHH: Indian Hedgehog; NPRB: NTP Receptor Type B; FGF: Fibroblast growth factor; FGFR: Fibroblast growth factor receptor; Runx2: Runt-related transcription factor 2; Col-X: Collagen type X; ECM: Extracellular Matrix Proteins; CNP: C-type natriuretic peptide. Red arrows and lines means accordingly activation or inhibition by CNP.

None of the previous studies show the direct influence of CNP on the FGF-23 expression. FGF-23 negatively regulates the expression of a gene coding for collagen-X [117] and disturbs collagen X metabolism [118]. Collagen X is produced primarily on the hypertrophic zone in the growth plate of cartilage, although it is involved in calcifying the cartilage [119]. Collagen II and aggrecan are primarily produced in the proliferative zone. One of the most recent reports describing studies on the primary rat osteoblast-like cells showed that CNP stimulates osteoblast proliferation with increased collagen-X levels. While CNP downregulated FGF-23 production, its receptor FGFR-1/Klotho was not changed. These results suggest that CNP can stimulate collagen X production through FGF-23 inhibition [91].

5. CNP/NPR-B/cGMP/pCREB Pathway

Mutations in either the NPPC gene encoding CNP [88] or the NPR2 gene coding for the CNP receptor called NPR-B [120] result in marked dwarfism with skeletal defects. Further studies confirmed acromesomelic dysplasia Maroteaux-type syndrome (MIM #602875) with a mutation in NPR2 [121]. Very recent studies on the Maroteaux syndrome revealed a different mechanism caused by the mutations in the NPR2 gene. Three different mutations p.Leu314Arg), p.Arg371*, and p.Arg1032* lead to variable phenotypes in the patients. These mutations affect the activation and function of the guanylate cyclase: binding of the CNP (ligand) to the NPR-B homodimer; depletion of the three domains in the receptor (transmembrane, protein kinase, and guanylate cyclase); and the deterioration of the guanylate cyclase domain. Those results indicated that the critical function of the NPR-B receptor is the activation of the cGMP in the cell [122].

Interestingly, in epiphyseal chondrodysplasia, Miura type (ECDM; OMIM #615923) disease with the heterozygous NPR2 pathogenic variant caused opposite symptoms to those in the homozygous mutation. So far, only four families with this disease have been reported. These activating mutations in NPR2 cause tall stature, long bones, scoliosis, and elongated toes and fingers in patients. The heterozygous variant (c.2647G>A (p.Val883Met)) is characterized by amino acid alternation localized in one of the domains in the NPR-B receptor-carboxyl-terminal guanylyl cyclase domain [123].

A mouse model with a gain of function mutation in the NPR2 gene exhibited skeletal overgrowth with a thick hypertrophic zone in the cartilage growth plate (higher level of the collagen type X than WT) [124]. The same pattern was not visible in the proliferating zone (similar collagen type II and aggrecan levels to WT mice). Moreover, there was no difference in apoptotic cells in the growth plate when comparing WT with *Npr2* mice. Those results suggest that the NPRB receptor regulates chondrocyte maturation at later stages of development in the hypertrophic layer. It was possible to screen for molecular pathway activation using a cell model for endochondral ossification (ATDC5). Cells were cultured for several weeks (0–8) and treated with CNP to activate the NPRB receptor. Several genes were checked to determine at which stage the chondrocytes are. The main transcription factors critical for chondrocyte gene expression are Runx2, Sox9, and MEF2C [125]. Sox9 is a transcription factor involved in chondrocyte differentiation [126], Sox9 was increased after two weeks of culturing, then declined in untreated cells once cells reached the mature stage (hypertrophic cells) [124]. On the other hand, Runx2 is expressed during chondrocyte hypertrophy [127], did not decline after two weeks, and stayed at the same level. CNP treatment did not affect the expression of both genes, Sox9 and Runx2 [124], while genes encoding CNP and its receptor NPRB peaked at two weeks. The expression level of the cell proliferation gene *Ccnd1*, encoding Cyclin D1 responsible for the progression of the cell cycle in the G1-phase [128], was the highest at four weeks of chondrogenic induction and decreased after that time [124]. When treated with CNP, a similar effect was obtained even if CNP was added at a different time (six or eight weeks), upregulating the expression of *Ccnd1*. Bone growth is regulated by the parathyroid hormone that is present at the end of long bones and delays chondrocytes hypotrophy [129], thus the expression of the genes coding for a parathyroid hormone-related protein (*Pthrp*) and its receptor parathyroid hormone 1 receptor (*Pth1r*) were measured in cell culture. Expression of both *Col2a1* and *Pthrp* was upregulated after CNP treatment, as was *Pth1r* even six weeks after treatment with CNP. Moreover, NPR-B was active when CNP was added to the ATDC5 cells due to the markedly increased production of cGMP [124].

Treatment of hypertrophic chondrocytes with cell-permeable cGMP resulted in increased phosphorylation of CREB, the transcription factor stimulating the production of the Cyclin D1, thus, showing chondrocyte activation through the NPRB-mediated molecular pathway [124]. Those experiments were also confirmed in cartilage isolated from WT neonates and treated with CNP (increased phosphorylation of CREB and Cyclin D1 expression). Contrary to previous research [130], ERK1/2 was not phosphorylated in the primary chondrocytes [124]. In the FGFR-MAPK pathway, FGFR3 is produced in the proliferating and pre-hypertrophic zone of the growth plate, not in the hypertrophic zone [131]. Thus, the CNP pathway can activate and regulate chondrocytes in different ways.

The expression of the *Npr3* gene, encoding the NPR-C receptor, was elevated after CNP treatment, which is consistent with previous research with the organ culture [132]. In the primary chondrocyte cell culture, Sox9, *Prhrp*, and *Ihh* (Indian hedgehog) expressions were increased after CNP treatment, suggesting CNP involvement in both proliferation and differentiation of the cells. Moreover, when cells were treated with CNP, cell numbers increased. With the addition of the inhibitor for either CREB or cyclin D1, CNP-induced NPRB activation was abolished, suggesting the apparent involvement of CREB and cyclin D1 in skeletal overgrowth [124]. The action of parathyroid hormone and its receptor is mediated through cAMP/protein kinase A (PKA)/CREB, regulating chondrocytes by keeping them in the proliferation stage [129]. Nevertheless, CNP treatment increased

cGMP production in the primary chondrocytes, with no cAMP effect. CNP induced phosphorylation of CREB even with PKA inhibitor treatment while it suppressed the phosphorylation of CREB by PTHrP [129]. The same pattern of CREB phosphorylation through NPRB independent of PKA was demonstrated recently [124].

Similarly, when protein kinase-G II (PKGII), downregulated by CNP, is removed from cartilage, cartilage matrix production and chondrocyte proliferation were disturbed in mice [133]. The overexpression of CNP and activating mutations in the *Npr2* gene positively affects cartilage matrix production and chondrocyte proliferation. In a previous experiment, a systemic knockout of the CNP gene was made. The generation of cartilage-specific knockout (using Cre recombinase (Cre)-loxP) of the genes revealed differences in endochondral bone growth in the growth plate between CNP and its receptor [88]. Knockout in the NPR-B receptor resulted in shorter mice than CNP knockout mice. There are several explanations for why it happened. First, and most likely, secretion of CNP from other than cartilage tissues (like blood vessels) causes its binding to the receptor, resulting in lower impairment of growth. Second, there is a higher ligand (CNP) amount than its receptor (NPR-B). When the receptor is missing and the ligand is still present, the general effect on endochondral growth could be more substantial if there is a knockout in the receptor rather than the ligand itself. Furthermore, the sensitivity of the NPR-B receptor could increase due to the absence of CNP. Additionally, the clearance system of the peptides could not work correctly because of the downregulation of the NPR-C receptor [134] or an increased level of osteocrin, a natural ligand for the NPR-C receptor [135]. Due to osteocrin's attachment to the NPR-C receptor, the clearance system of the NPTs is not working. If NPTs cannot be cleared, they will be ligands to receptors that they generally do not activate. The NPR-B receptor may be activated by other ligands, such as ANP or BNP (normally reacting with the NPR-A receptor) [88]. In both models, hypertrophic chondrocytes in the growth plate were drastically reduced with chondrocytes proliferation [88].

While inhibition of the MEK1/2-ERK1/2 pathway stimulates bone growth, p38 MAPK is one of the mediators in the CNP signaling pathway [136]. p38 inhibits stimulation of endochondral bone growth and delays mineralization in tibia organ culture by CNP. These studies confirmed that CNP targets hypertrophic chondrocytes in the growth plate rather than other zones. Even though the CNP receptor is present in all three zones at a similar level, CNP signaling pathway kinases (PKG-I and -II) are primarily produced in the hypertrophic cells. This can potentially explain the spatial distribution of CNP effects. Moreover, CNP induces the expression of NPR2 and NPR3, suggesting the existence of a feedback loop to limit CNP signaling. CNP also regulates bone morphogenic proteins (BMP) signaling and cell adhesion processes [136].

6. C-Type Natriuretic Peptide as Extracellular Matrix Regulator

CNP is engaged in the intracellular processes required for bone growth and the extracellular matrix (ECM) [137]. CNP increases the number of chondrogenic condensations in the micro mass culture of mouse embryonic limb cells. Production of N-cadherin, a cell adhesion molecule, increased after treatment with CNP peptide. Glycosaminoglycan synthesis (chondroitin sulfate) was also stimulated, probably through increased levels of the enzyme (xylosyltransferases I) necessary to produce cartilage glycosaminoglycans. On the other hand, chondrogenic transcription factors (Sox9, -5, and -6), modulators of ECM, as well as ECM building proteins (collagen II and aggrecan) were not changed by CNP [137]. Another study investigated CNP and the formation of the nodules with biglycan (BGN), a small proteoglycan involved with bone formation in osteoblasts. BGN mRNA expression increased with CNP, which shows CNP's ability to regulate gene expression to be closely correlated with bone mineralization [138].

7. Synergic Effect of OP-1 and CNP

Mutation in the gene coding for the growth hormone-insulin like growth factor-I (IGF-1) leads to prenatal and postnatal growth failure. Interaction of growth hormone with

IGF-1 regulates endochondral bone growth. IGF-1 modulates bone growth through the paracrine mechanism rather than systematically. The synergic mechanism of osteoblast differentiation and proliferation by IGF-1 and osteogenic protein-1 (OP-1) was observed when both agents were added to the rat osteoblastic cell culture [139].

Bone morphogenetic proteins (BMPs) are growth factors that induce bone growth, and the main downstream molecules for BMPs are Smads. Activated Smad5 plays an important role in BMP target gene activation. After phosphorylation, Smad5 interacts with Smad4, making a complex translocated to the nucleus, activating BMP target genes [140]. OP-1 activated Smad5 through phosphorylation of the C-terminal region.

Similarly, the synergic mechanism of OP-1 and CNP was observed in the phosphorylation of the Smad5 transcription factor responsible for bone morphogenetic protein gene activation [141]. While CNP has been added alone to the primarily osteoblastic cells, activation of Smad5 was not observed. Alkaline phosphatase (ALP) induction by OP-1 was enhanced by CNP, leading to the mineralization of bone nodules. CNP+OP-1 also enhanced the protein expression level of bone morphogenetic protein receptors IB and II (BMPR-IB; -II), compared to OP-1 alone. CNP induces the expression of genes involved in the bone mineralization process. Nevertheless, the Runx2 protein level was not enhanced by CNP but was elevated after treatment with OP-1 alone, and it was slightly reduced by the combination treatment [141].

8. Survival of Animals with C-Type Natriuretic Peptide

The survival rate of the mice with total CNP knockout was around 40% [142], which was due to severe malocclusion [88]. Mice with the CNP knockout could not eat by themselves; however, when food was pulverized, the survival rate increased to ~70% [88]. The cartilage specific CNP knockout mice survival rate was almost the same as total CNP knockout mice (75.0%). The survival rate of about ~70% is caused by their impaired skeletal growth [88]. As the mice could have higher mortality, it was impossible to analyze them in adult age. For this reason, a generation of a rat model with CNP knockout was performed. The phenotype of the rats was similar to that of the mouse model, manifesting short stature with endochondral bone growth retardation. Compared to the mice model, rats survived for over one year without malocclusions visible in the mouse model. Interestingly, only bones were affected by CNP deficiency in histological analysis, and no other tissues studied were changed compared to WT littermates. Growth plate width in the bone of CNP knockout rats (mainly hypertrophic chondrocyte layer) was lower together with reduced proliferation of growth plate chondrocytes [143]. On the other hand, crossing animals with circulating CNP peptide (SAP-Nppc-Th mice) with chondrodysplastic CNP-depleted mice leads to increased survival. Moreover, circulation of CNP recovers chondrodysplastic CNP-depleted animals from impaired endochondral bone growth, together with increased body weight [87].

9. C-Type Natriuretic Peptide as a Treatment

Achondroplasia is one of the most common diseases leading to dwarfism (1:25,000) by the mutation in a single gene FGFR3 coding for fibroblast growth factor receptor 3). In 80% of the cases, de novo dominant mutation occurs in the egg or sperm that forms the embryo [144]. Parents with new mutations usually do not have a growth deficit. The gene mutation probability increases if the father is more than 35 years old. It is the most common disease in the group of growth defects diseases. Symptoms include dwarfism, midfacial hypoplasia, incorrect torso length, excessive lordosis of the lumbar spine, narrowing of the spinal stricture, small cubic vertebral bodies, prominent forehead, collapsed nose bridge, micromelia (small hands), with normal intellectual development [112]. FGFR3 inhibits long bone growth through different pathways. Activation of phosphorylation of STAT-1 regulated by FGFR3 inhibited chondrocyte proliferation. Moreover, in the MAPK pathway regulated by FGFR3, phosphorylation of ERK1/2 inhibited chondrocyte differen-

tiation. Overall, activation of FGFR3 seems to inhibit the proliferation and differentiation of chondrocytes [130].

The mechanism of CNP in achondroplasia shows that CNP inhibits the MAPK pathway, through decreasing FGF-2 activated level of pERK1/2, what results in animals' normal length [145]. However, CNP did not affect the level of phosphoSTAT-1 in the cartilage of neonatal mice [145]. The achondroplasia mouse model, with FGFR3 mutation leading to jaw deformities, was crossed with mouse overexpressing CNP [146]. Fgfr3ach/SAP-Nppc-Tg mice had significantly improved the craniofacial region comparing symptoms to the Fgfr3ach mouse model. The thickness of synchondrosis was noticed, with increased chondrocytes proliferation within the craniofacial cartilage. Moreover, enhanced endochondral bone growth in the combined mouse model ameliorated the foramen magnum stenosis compared to Fgfr3ach only mice [146]. CNP induces endochondral ossification in the long bones and helps restore craniofacial bones in the achondroplasia mouse model.

Achondroplasia is the most common growth retardation disorder. However, growth retardation can also be a side effect of glucocorticoid-based drug administration [147]. Recent studies showed that high doses of glucocorticoid inhaled by children with asthma correlate with growth retardation [148]. The mechanism of glucocorticoids directly act on chondrocytes within the growth plate, including proliferation and differentiation, together inhibiting bone growth [147]. Growth hormone therapy to restore growth retardation connected to glucocorticoid-based drugs is ineffective [149]. Exogenous CNP-53 is a stable molecular form of intrinsic CNP peptide. It was administered to mice with induced glucocorticoid drug growth retardation [147]. Glucocorticoid-based drug (dexamethasone; DEX) and CNP-53 (dose 0.5 mg/kg/day) were injected into four- to eight-week-old mice by subcutaneous injection. The effect of CNP depended on age, as it was more effective in four-week-old mice. The molecular mechanism of this effect is not based on the action of DEX on cGMP level induction by CNP but instead on the reduction of Erk1/2 phosphorylation by combined treatment [147].

In another study, four-week-old CNP-knockout (CNP-KO) and WT rats were subcutaneously infused with 0.15 or 0.5 mg/kg CNP-53 peptide daily for four weeks. There was no significant difference in plasma bone turnover markers between WT and CNP-KO rats. The short stature of the CNP-knockout mice was restored with circulating CNP peptide administration, where CNP-KO rats were more sensitive to the treatment than WT rats [150]. In acceptance with previous research [101], gene enrichment analysis using fetal rat tibia confirmed that CNP negatively influences the MAPK pathway in chondrocytes [150].

An essential part of mechanism regulation is the feedback loop. In the case of CNP, it remains unclear how it is regulated in the systemic feedback regulation. Several factors indicate that CNP can be regulated in a systemic feedback manner.

Administration of exogenous CNP (CNP-53) to four-week-old rats (0.5 mg/kg/day) for three consecutive days did not alter the blood level of CNP [151]. However, this administration decreased mRNA expression in cartilage tissues. Interestingly, female rats were more sensitive to CNP administration, with a greater decrease in mRNA level. The influence of exogenous administration on endogenous CNP levels in tibia culture and chondrogenic cell lines did not show direct autoregulation. Thus, cartilage tissue is not directly regulated by CNP administration [151].

10. Discussion

CNP is a paracrine growth factor widely expressed in various tissues [152]. Diverse functions include the regulation of endochondral bone growth. The most critical role in humans is recognized as the hormone in skeletal growth related to growth plate proliferation. Studies in mice show that the local production of CNP within the growth plate determines physiological endochondral bone growth [88]. The dynamic role of CNP in bone growth remains a challenge due to the rapid clearance of CNP and low concentrations in the blood. An inactive portion of the synthesized product in the growth plate (amino-terminal propeptide CNP; NTproCNP) is not subject to clearance or rapid degradation. NTproCNP

is an equimolar product of CNP biosynthesis, which is easily measured in plasma. Its level in blood correlates to linear growth velocity throughout growth in both children and mouse models [153]. CNP and NTproCNP levels for both sexes were high in infancy, lower in early childhood, rising during puberty, and then falling to low adult levels. Peak levels of NTproCNP are coincident with the age of peak height velocity. Thus, CNP synthesis (as measured by NTproCNP levels in plasma) is closely related to linear growth in healthy children at all ages, resulting in an excellent biomarker of linear growth [154].

CNP products in plasma were elevated in skeletal dysplasia with profound short stature due to a disruption of the CNP receptor (NPR-B) (acromesomelic dysplasia type Maroteux), activation of the MEK/ERK MAPK pathway inhibiting NPR-B signaling, activating mutations of *FGFR3* (thanatophoric dysplasia, achondroplasia, and hypochondroplasia) [1]. Two reports support those activating mutations of NPR-B cause skeletal overgrowth [155,156], reducing plasma NTproCNP. Little is known about the factors that regulate CNP expression and translation; the details of this feedback loop require further study.

The relation between the MEK/ERK MAPK and CNP/cGMP pathways was investigated in chondrogenic cells and organ culture. Phosphorylated MEK1/2 and/or ERK1/2 inhibit cGMP generation by NPR-B. Meanwhile, NPR-B-generated cGMP inhibits MEK/ERK activation by inhibiting RAF1. Thus, overactivation of the MEK/ERK MAPK pathway leads to resistance to CNP [115]. The finding of elevated CNP levels in a population with severe short stature suggests that these individuals may also have resistance to CNP.

It is noteworthy that patients with achondroplasia affecting the CNP pathway activity have elevated plasma NTproCNP concentrations, while intracellular CNP activity is reduced [116,145,157]. In achondroplasia, the normal reciprocal antagonism between *FGFR3* (inhibiting endochondral bone growth) and CNP signaling (stimulating bone growth) is disregarded by a gain of function mutation in *FGR3* [115]. CNP production is subject to feedback regulation. Endogenous CNP remains subject to regulation during the administration of growth-promoting doses of the CNP analog in achondroplasia. With the restraining impact of the genetic mutation on CNP signaling, baseline levels of CNP products in plasma are elevated. During periods of accelerating long bone growth induced by the CNP analog, endogenous CNP is reduced in keeping with indirect negative feedback. Direct inhibition of CNP by exogenous CNP occurs in proportion to the overall level of hormone resistance. Unusual high values of plasma NTproCNP in adolescent years with achondroplasia patients receiving the CNP analog are unexplained and require future study [115].

Take-home message;

1. CNP produced in the growth plate is a potent positive regulator of linear growth.
2. Reduced intracellular CNP pathway activity may increase CNP production; a negative feedback loop regulates CNP
3. In a group of skeletal dysplasias, elevated plasma levels of NTproCNP indicate the presence of tissue resistance to CNP.
4. Long-term CNP therapy for achondroplasia and other skeletal disorders remains unknown.
5. The interaction between the CNP/NPR-B pathway and other pathways should be explored to elucidate the bone growth.

11. Conclusions

When organs were cultured, several mechanisms were found to be stimulated by CNP, including chondrocyte proliferation, cell hypertrophy, cartilage matrix production, and ECM activation. When the only CNP stimulation (cGMP) product was added to the culture, the same stimulation of longitudinal growth and glycosaminoglycan synthesis was observed. Nevertheless, cGMP only could not induce the proliferation of chondrocytes without an effect on cell hypertrophy. The second messenger for CNP (cGMP) could reproduce some of the CNP effects but not all, indicating that CNP activation is a more complex mechanism [158]. The signaling pathway of the CNP peptide has not been fully

understood. Thus, this review has discussed known mechanisms at present, as summarized in Figures 1 and 2.

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References

1. Long, F.; Ornitz, D.M. Development of the Endochondral Skeleton. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a008334. [[CrossRef](#)] [[PubMed](#)]
2. Salhotra, A.; Shah, H.N.; Levi, B.; Longaker, M.T. Mechanisms of Bone Development and Repair. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 696–711. [[CrossRef](#)] [[PubMed](#)]
3. Santhekadur, P.K.; Kumar, D.P.; Seneshaw, M.; Mirshahi, F.; Sanyal, A.J. The Multifaceted Role of Natriuretic Peptides in Metabolic Syndrome. *Biomed. Pharmacother.* **2017**, *92*, 826–835. [[CrossRef](#)] [[PubMed](#)]
4. Sonnenberg, H.; Chong, C.K.; Veress, A.T. Cardiac Atrial Factor—an Endogenous Diuretic? *Can. J. Physiol. Pharmacol.* **1981**, *59*, 1278–1279. [[CrossRef](#)]
5. Nakagawa, Y.; Nishikimi, T.; Kuwahara, K. Atrial and Brain Natriuretic Peptides: Hormones Secreted from the Heart. *Peptides* **2019**, *111*, 18–25. [[CrossRef](#)]
6. Collins, S. A Heart–Adipose Tissue Connection in the Regulation of Energy Metabolism. *Nat. Rev. Endocrinol.* **2014**, *10*, 157–163. [[CrossRef](#)]
7. Berglund, H.; Nyquist, O.; Beermann, B.; Jensen-Urstad, M.; Theodorsson, E. Influence of Angiotensin Converting Enzyme Inhibition on Relation of Atrial Natriuretic Peptide Concentration to Atrial Pressure in Heart Failure. *Heart* **1994**, *72*, 521–527. [[CrossRef](#)]
8. Kotby, A.A.; Taman, K.H.; Sedky, H.T.A.; Habeeb, N.M.; El-Hadidi, E.S.; Yosseif, H.S. Atrial Natriuretic Peptide as a Marker of Heart Failure in Children with Left Ventricular Volume Overload. *J. Paediatr. Child Health* **2013**, *49*, 43–47. [[CrossRef](#)]
9. Asakura, M.; Jiyoong, K.; Minamino, T.; Shintani, Y.; Asanuma, H.; Kitakaze, M.; The J-WIND Investigators. Rationale and Design of a Large-Scale Trial Using Atrial Natriuretic Peptide (ANP) as an Adjunct to Percutaneous Coronary Intervention for ST-Segment Elevation Acute Myocardial Infarction—Japan-Working Groups of Acute Myocardial Infarction for the Reduction of Necrotic Damage by ANP (J-WIND-ANP). *Circ. J.* **2004**, *68*, 95–100. [[CrossRef](#)]
10. Nishikimi, T.; Mori, Y.; Ishimura, K.; Tadokoro, K.; Yagi, H.; Yabe, A.; Horinaka, S.; Matsuoka, H. Association of Plasma Atrial Natriuretic Peptide, n-Terminal Proatrial Natriuretic Peptide, and Brain Natriuretic Peptide Levels with Coronary Artery Stenosis in Patients with Normal Left Ventricular Systolic Function. *Am. J. Med.* **2004**, *116*, 517–523. [[CrossRef](#)]
11. Hui, D.; Naberhuis, J.; Dibaj, S.; Naqvi, M.; Liu, D.; Bruera, E. Association Between Plasma Brain Natriuretic Peptide and Overall Survival in Patients With Advanced Cancer: Preliminary Findings. *J. Pain Symptom Manag.* **2019**, *58*, 465–471. [[CrossRef](#)] [[PubMed](#)]
12. Moore, K.B.; McKenna, K.; Osman, M.; Tormey, W.P.; McDonald, D.; Thompson, C.J. Atrial Natriuretic Peptide Increases Urinary Albumin Excretion in People with Normoalbuminuric Type-2 Diabetes. *Ir. J. Med. Sci.* **2007**, *176*, 67–73. [[CrossRef](#)] [[PubMed](#)]
13. Pedersen, E.B.; Bacevicius, E.; Bech, J.N.; Solling, K.; Pedersen, H.B. Abnormal Rhythmic Oscillations of Atrial Natriuretic Peptide and Brain Natriuretic Peptide in Chronic Renal Failure. *Clin. Sci.* **2006**, *110*, 491–501. [[CrossRef](#)] [[PubMed](#)]
14. Katan, M.; Fluri, F.; Schuetz, P.; Morgenthaler, N.G.; Zweifel, C.; Bingisser, R.; Kappos, L.; Steck, A.; Engelter, S.T.; Müller, B.; et al. Midregional Pro-Atrial Natriuretic Peptide and Outcome in Patients With Acute Ischemic Stroke. *J. Am. Coll. Cardiol.* **2010**, *56*, 1045–1053. [[CrossRef](#)]
15. Zois, N.E.; Terzic, D.; Færch, K.; Plomgaard, P.; Hansen, J.S.; Rossing, P.; Goetze, J.P. Effect of Pancreatic Hormones on Pro-Atrial Natriuretic Peptide in Humans. *EBioMedicine* **2017**, *17*, 88–94. [[CrossRef](#)]
16. Jujić, A.; Nilsson, P.M.; Persson, M.; Holst, J.J.; Torekov, S.S.; Lyssenko, V.; Groop, L.; Melander, O.; Magnusson, M. Atrial Natriuretic Peptide in the High Normal Range Is Associated With Lower Prevalence of Insulin Resistance. *J. Clin. Endocrinol. Metab.* **2016**, *101*, 1372–1380. [[CrossRef](#)]

17. Parcha, V.; Patel, N.; Gutierrez, O.M.; Li, P.; Gamble, K.L.; Musunuru, K.; Margulies, K.B.; Cappola, T.P.; Wang, T.J.; Arora, G.; et al. Chronobiology of Natriuretic Peptides and Blood Pressure in Lean and Obese Individuals. *J. Am. Coll. Cardiol.* **2021**, *77*, 2291–2303. [[CrossRef](#)]
18. Molina, C.R.; Fowler, M.B.; Mccrory, S.; Peterson, C.; Myers, B.D.; Schroeder, J.S.; Murad, F. Hemodynamic, Renal and Endocrine Effects of Atrial Natriuretic Peptide Infusion in Severe Heart Failure. *J. Am. Coll. Cardiol.* **1988**, *12*, 175–186. [[CrossRef](#)]
19. Tanaka, T.; Tsutamoto, T.; Sakai, H.; Nishiyama, K.; Fujii, M.; Yamamoto, T.; Horie, M. Effect of Atrial Natriuretic Peptide on Adiponectin in Patients with Heart Failure. *Eur. J. Heart Fail.* **2008**, *10*, 360–366. [[CrossRef](#)]
20. Valsson, F.; Lundin, S.; Kirno, K.; Hedner, T.; Houltz, E.; Saito, Y.; Ricksten, S.-E. Atrial Natriuretic Peptide Attenuates Pacing-Induced Myocardial Ischemia During General Anesthesia in Patients with Coronary Artery Disease. *Anesth. Analg.* **1999**, *88*, 279–285. [[CrossRef](#)]
21. Nojiri, T.; Yamamoto, K.; Maeda, H.; Takeuchi, Y.; Funakoshi, Y.; Inoue, M.; Okumura, M. Effect of Low-Dose Human Atrial Natriuretic Peptide on Postoperative Atrial Fibrillation in Patients Undergoing Pulmonary Resection for Lung Cancer: A Double-Blind, Placebo-Controlled Study. *J. Thorac. Cardiovasc. Surg.* **2012**, *143*, 488–494. [[CrossRef](#)] [[PubMed](#)]
22. Kitakaze, M.; Asakura, M.; Kim, J.; Shintani, Y.; Asanuma, H.; Hamasaki, T.; Seguchi, O.; Myoishi, M.; Minamino, T.; Ohara, T.; et al. Human Atrial Natriuretic Peptide and Nicorandil as Adjuncts to Reperfusion Treatment for Acute Myocardial Infarction (J-WIND): Two Randomised Trials. *Lancet* **2007**, *370*, 1483–1493. [[CrossRef](#)]
23. Sezai, A.; Wakui, S.; Akiyama, K.; Hata, M.; Yoshitake, I.; Unosawa, S.; Shiono, M.; Hirayama, A. Myocardial Protective Effect of Human Atrial Natriuretic Peptide in Cardiac Surgery—“HANP Shot” in Clinical Safety Trial. *Circ. J.* **2011**, *75*, 2144–2150. [[CrossRef](#)] [[PubMed](#)]
24. Nojiri, T.; Yamamoto, H.; Hamasaki, T.; Onda, K.; Ohshima, K.; Shintani, Y.; Okumura, M.; Kangawa, K. A Multicenter Randomized Controlled Trial of Surgery Alone or Surgery with Atrial Natriuretic Peptide in Lung Cancer Surgery: Study Protocol for a Randomized Controlled Trial. *Trials* **2017**, *18*, 183. [[CrossRef](#)]
25. Hayashida, N.; Chihara, S.; Kashikie, H.; Tayama, E.; Yokose, S.; Akasu, K.; Aoyagi, S. Effects of Intraoperative Administration of Atrial Natriuretic Peptide. *Ann. Thorac. Surg.* **2000**, *70*, 1319–1326. [[CrossRef](#)]
26. Park, K.; Itoh, H.; Yamahara, K.; Sone, M.; Miyashita, K.; Oyamada, N.; Sawada, N.; Taura, D.; Inuzuka, M.; Sonoyama, T.; et al. Therapeutic Potential of Atrial Natriuretic Peptide Administration on Peripheral Arterial Diseases. *Endocrinology* **2008**, *149*, 483–491. [[CrossRef](#)]
27. Groban, L.; Cowley, A.W.; Ebert, T.J. Atrial Natriuretic Peptide Augments Forearm Capillary Filtration in Humans. *Am. J. Physiol.-Heart Circ. Physiol.* **1990**, *259*, H258–H263. [[CrossRef](#)]
28. Kasama, S.; Toyama, T.; Hatori, T.; Sumino, H.; Kumakura, H.; Takayama, Y.; Ichikawa, S.; Suzuki, T.; Kurabayashi, M. Effects of Intravenous Atrial Natriuretic Peptide on Cardiac Sympathetic Nerve Activity and Left Ventricular Remodeling in Patients With First Anterior Acute Myocardial Infarction. *J. Am. Coll. Cardiol.* **2007**, *49*, 667–674. [[CrossRef](#)]
29. Moriyama, T.; Hagihara, S.; Shiramomo, T.; Nagaoka, M.; Iwakawa, S.; Kanmura, Y. The Protective Effect of Human Atrial Natriuretic Peptide on Renal Damage during Cardiac Surgery. *J. Anesth.* **2017**, *31*, 163–169. [[CrossRef](#)]
30. Mitaka, C.; Ohnuma, T.; Murayama, T.; Kunimoto, F.; Nagashima, M.; Takei, T.; Iguchi, N.; Tomita, M. Effects of Low-Dose Atrial Natriuretic Peptide Infusion on Cardiac Surgery–Associated Acute Kidney Injury: A Multicenter Randomized Controlled Trial. *J. Crit. Care* **2017**, *38*, 253–258. [[CrossRef](#)]
31. Rahman, S.N.; Kim, G.E.; Mathew, A.S.; Goldberg, C.A.; Allgren, R.; Schrier, R.W.; Conger, J.D. Effects of Atrial Natriuretic Peptide in Clinical Acute Renal Failure. *Kidney Int.* **1994**, *45*, 1731–1738. [[CrossRef](#)] [[PubMed](#)]
32. Swärd, K.; Valsson, F.; Odencrants, P.; Samuelsson, O.; Ricksten, S.-E. Recombinant Human Atrial Natriuretic Peptide in Ischemic Acute Renal Failure: A Randomized Placebo-Controlled Trial*. *Crit. Care Med.* **2004**, *32*, 1310–1315. [[CrossRef](#)] [[PubMed](#)]
33. Mitaka, C.; Kudo, T.; Jibiki, M.; Sugano, N.; Inoue, Y.; Makita, K.; Imai, T. Effects of Human Atrial Natriuretic Peptide on Renal Function in Patients Undergoing Abdominal Aortic Aneurysm Repair*. *Crit. Care Med.* **2008**, *36*, 745–751. [[CrossRef](#)]
34. Koda, M.; Sakamoto, A.; Ogawa, R. Effects of Atrial Natriuretic Peptide at a Low Dose on Water and Electrolyte Metabolism during General Anesthesia. *J. Clin. Anesth.* **2005**, *17*, 3–7. [[CrossRef](#)]
35. Swärd, K.; Valson, F.; Ricksten, S.-E. Long-Term Infusion of Atrial Natriuretic Peptide (ANP) Improves Renal Blood Flow and Glomerular Filtration Rate in Clinical Acute Renal Failure. *Acta Anaesthesiol. Scand.* **2001**, *45*, 536–542. [[CrossRef](#)] [[PubMed](#)]
36. Takahashi, H.; Takeda, T.; Nishizawa, Y.; Ogino, T.; Miyoshi, N.; Matsuda, C.; Yamamoto, H.; Mizushima, T.; Doki, Y.; Eguchi, H. Phase I Study of the Administration of Low-Dose Perioperative Human Atrial Natriuretic Peptide in Patients With Resectable Colorectal Cancer. *Anticancer Res.* **2020**, *40*, 5301–5307. [[CrossRef](#)]
37. van der Zander, K. Effects of Brain Natriuretic Peptide on Forearm Vasculature: Comparison with Atrial Natriuretic Peptide. *Cardiovasc. Res.* **1999**, *44*, 595–600. [[CrossRef](#)]
38. Mori, Y.; Kamada, T.; Ochiai, R. Reduction in the Incidence of Acute Kidney Injury after Aortic Arch Surgery with Low-Dose Atrial Natriuretic Peptide. *Eur. J. Anaesthesiol.* **2014**, *31*, 381–387. [[CrossRef](#)]
39. Moro, C.; Polak, J.; Hejnova, J.; Klimcakova, E.; Crampes, F.; Stich, V.; Lafontan, M.; Berlan, M. Atrial Natriuretic Peptide Stimulates Lipid Mobilization during Repeated Bouts of Endurance Exercise. *Am. J. Physiol.-Endocrinol. Metab.* **2006**, *290*, E864–E869. [[CrossRef](#)]
40. Cargill, R.I.; Lipworth, B.J. Pulmonary Vasorelaxant Activity of Atrial Natriuretic Peptide and Brain Natriuretic Peptide in Humans. *Thorax* **1995**, *50*, 183–185. [[CrossRef](#)]

41. Mukoyama, M.; Nakao, K.; Saito, Y.; Ogawa, Y.; Hosoda, K.; Suga, K.; Shirakami, G.; Jougasaki, M.; Imura, H. Increased Human Brain Natriuretic Peptide in Congestive Heart Failure. *N. Engl. J. Med.* **1990**, *323*, 757–758. [[CrossRef](#)] [[PubMed](#)]
42. Mukoyama, M.; Nakao, K.; Hosoda, K.; Suga, S.; Saito, Y.; Ogawa, Y.; Shirakami, G.; Jougasaki, M.; Obata, K.; Yasue, H. Brain Natriuretic Peptide as a Novel Cardiac Hormone in Humans. Evidence for an Exquisite Dual Natriuretic Peptide System, Atrial Natriuretic Peptide and Brain Natriuretic Peptide. *J. Clin. Investig.* **1991**, *87*, 1402–1412. [[CrossRef](#)] [[PubMed](#)]
43. Maisel, A.S.; Krishnaswamy, P.; Nowak, R.M.; McCord, J.; Hollander, J.E.; Duc, P.; Omland, T.; Storrow, A.B.; Abraham, W.T.; Wu, A.H.B.; et al. Rapid Measurement of B-Type Natriuretic Peptide in the Emergency Diagnosis of Heart Failure. *N. Engl. J. Med.* **2002**, *347*, 161–167. [[CrossRef](#)] [[PubMed](#)]
44. Maisel, A.; Barnard, D.; Jaski, B.; Frivold, G.; Marais, J.; Azer, M.; Miyamoto, M.I.; Lombardo, D.; Kelsay, D.; Borden, K.; et al. Primary Results of the HABIT Trial (Heart Failure Assessment With BNP in the Home). *J. Am. Coll. Cardiol.* **2013**, *61*, 1726–1735. [[CrossRef](#)]
45. Maisel, A.S.; Shah, K.S.; Barnard, D.; Jaski, B.; Frivold, G.; Marais, J.; Azer, M.; Miyamoto, M.I.; Lombardo, D.; Kelsay, D.; et al. How B-Type Natriuretic Peptide (BNP) and Body Weight Changes Vary in Heart Failure With Preserved Ejection Fraction Compared With Reduced Ejection Fraction: Secondary Results of the HABIT (HF Assessment With BNP in the Home) Trial. *J. Card. Fail.* **2016**, *22*, 283–293. [[CrossRef](#)]
46. Liu, Q.; Yang, J.; Bolun, Z.; Pei, M.; Ma, B.; Tong, Q.; Yin, H.; Zhang, Y.; You, L.; Xie, R. Comparison of Cardiac Function between Left Bundle Branch Pacing and Right Ventricular Outflow Tract Septal Pacing in the Short-Term: A Registered Controlled Clinical Trial. *Int. J. Cardiol.* **2021**, *322*, 70–76. [[CrossRef](#)]
47. Passino, C.; del Ry, S.; Severino, S.; Gabutti, A.; Prontera, C.; Clerico, A.; Giannessi, D.; Emdin, M. C-Type Natriuretic Peptide Expression in Patients with Chronic Heart Failure: Effects of Aerobic Training. *Eur. J. Cardiovasc. Prev. Rehabil.* **2008**, *15*, 168–172. [[CrossRef](#)]
48. Mann, D.L.; Greene, S.J.; Givertz, M.M.; Vader, J.M.; Starling, R.C.; Ambrosy, A.P.; Shah, P.; McNulty, S.E.; Mahr, C.; Gupta, D.; et al. Sacubitril/Valsartan in Advanced Heart Failure With Reduced Ejection Fraction. *JACC Heart Fail.* **2020**, *8*, 789–799. [[CrossRef](#)]
49. Zhang, W.; Wang, X.; Li, J.; Xu, M.; Ren, X.; Liu, H.; Xu, L.; Shao, J. Astragaloside IV Reduces OxLDL-Induced BNP Overexpression by Regulating HDAC. *J. Healthc. Eng.* **2021**, *2021*, 3433615. [[CrossRef](#)]
50. Morbach, C.; Buck, T.; Rost, C.; Peter, S.; Günther, S.; Störk, S.; Prettin, C.; Erbel, R.; Ertl, G.; Angermann, C.E. Point-of-Care B-Type Natriuretic Peptide and Portable Echocardiography for Assessment of Patients with Suspected Heart Failure in Primary Care: Rationale and Design of the Three-Part Handheld-BNP Program and Results of the Training Study. *Clin. Res. Cardiol.* **2018**, *107*, 95–107. [[CrossRef](#)]
51. Hu, J.; Wan, Q.; Zhang, Y.; Zhou, J.; Li, M.; Jiang, L.; Yuan, F. Efficacy and Safety of Early Ultrafiltration in Patients with Acute Decompensated Heart Failure with Volume Overload: A Prospective, Randomized, Controlled Clinical Trial. *BMC Cardiovasc. Disord.* **2020**, *20*, 447. [[CrossRef](#)] [[PubMed](#)]
52. Bristow, M.R.; Silva Enciso, J.; Gersh, B.J.; Grady, C.; Rice, M.M.; Singh, S.; Sopko, G.; Boineau, R.; Rosenberg, Y.; Greenberg, B.H. Detection and Management of Geographic Disparities in the TOPCAT Trial. *JACC Basic Transl. Sci.* **2016**, *1*, 180–189. [[CrossRef](#)] [[PubMed](#)]
53. Karlström, P.; Dahlström, U.; Boman, K.; Alehagen, U. Responder to BNP-Guided Treatment in Heart Failure. The Process of Defining a Responder. *Scand. Cardiovasc. J.* **2015**, *49*, 316–324. [[CrossRef](#)] [[PubMed](#)]
54. Mueller, C.; Scholer, A.; Laule-Kilian, K.; Martina, B.; Schindler, C.; Buser, P.; Pfisterer, M.; Perruchoud, A.P. Use of B-Type Natriuretic Peptide in the Evaluation and Management of Acute Dyspnea. *N. Engl. J. Med.* **2004**, *350*, 647–654. [[CrossRef](#)]
55. Motamed, H.; Forouzan, A.; Heybar, H.; Khorasani, M.J.; Hesam, S. Bronchodilatory Effects of B-Type Natriuretic Peptide in Acute Asthma Attacks: A Randomized Controlled Clinical Trial. *Adv. Respir. Med.* **2020**, *88*, 531–538. [[CrossRef](#)]
56. Ogawa, S.; Takeuchi, K.; Ito, S. Plasma BNP Levels in the Treatment of Type 2 Diabetes with Pioglitazone. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 3993–3996. [[CrossRef](#)]
57. Stavrakis, S.; Pakala, A.; Thadani, U.; Thomas, J.; Chaudhry, M.A. Obesity, Brain Natriuretic Peptide Levels and Mortality in Patients Hospitalized With Heart Failure and Preserved Left Ventricular Systolic Function. *Am. J. Med. Sci.* **2013**, *345*, 211–217. [[CrossRef](#)]
58. Pitt, B.; Kober, L.; Ponikowski, P.; Gheorghide, M.; Filippatos, G.; Krum, H.; Nowack, C.; Kolkhof, P.; Kim, S.-Y.; Zannad, F. Safety and Tolerability of the Novel Non-Steroidal Mineralocorticoid Receptor Antagonist BAY 94-8862 in Patients with Chronic Heart Failure and Mild or Moderate Chronic Kidney Disease: A Randomized, Double-Blind Trial. *Eur. Heart J.* **2013**, *34*, 2453–2463. [[CrossRef](#)]
59. Koomen, J.; Stevens, J.; Bakris, G.; Correa-Rotter, R.; Hou, F.F.; Kitzman, D.W.; Kohan, D.; Makino, H.; McMurray, J.J.; Parving, H.; et al. Inter-individual Variability in Atrasentan Exposure Partly Explains Variability in Kidney Protection and Fluid Retention Responses: A Post Hoc Analysis of the SONAR Trial. *Diabetes Obes. Metab.* **2021**, *23*, 561–568. [[CrossRef](#)]
60. Qin, W.; Bai, W.; Liu, K.; Liu, Y.; Meng, X.; Zhang, K.; Zhang, M. Clinical Course and Risk Factors of Disease Deterioration in Critically Ill Patients with COVID-19. *Hum. Gene Ther.* **2021**, *32*, 310–315. [[CrossRef](#)]
61. Inohara, T.; Kim, S.; Pieper, K.; Blanco, R.G.; Allen, L.A.; Fonarow, G.C.; Gersh, B.J.; Ezekowitz, M.D.; Kowey, P.R.; Reiffel, J.A.; et al. B-Type Natriuretic Peptide, Disease Progression and Clinical Outcomes in Atrial Fibrillation. *Heart* **2018**, *105*, 370–377. [[CrossRef](#)] [[PubMed](#)]

62. Sugiyama, M.; Hazama, T.; Nakano, K.; Urae, K.; Moriyama, T.; Ariyoshi, T.; Kurokawa, Y.; Kodama, G.; Wada, Y.; Yano, J.; et al. Effects of Reducing L-Carnitine Supplementation on Carnitine Kinetics and Cardiac Function in Hemodialysis Patients: A Multicenter, Single-Blind, Placebo-Controlled, Randomized Clinical Trial. *Nutrients* **2021**, *13*, 1900. [[CrossRef](#)] [[PubMed](#)]
63. Seki, N.; Matsumoto, T.; Fukazawa, M. Relationship Between the Brain Natriuretic Peptide (BNP) Level and Prognosis of Diabetic Nephropathy with Microalbuminuria: A 7-Year Follow-Up Study. *Horm. Metab. Res.* **2018**, *50*, 389–396. [[CrossRef](#)] [[PubMed](#)]
64. Lok, D.J.; Klip, I.T.; Voors, A.A.; Lok, S.I.; Bruggink-André de la Porte, P.W.; Hillege, H.L.; Jaarsma, T.; van Veldhuisen, D.J.; van der Meer, P. Prognostic Value of N-Terminal pro C-Type Natriuretic Peptide in Heart Failure Patients with Preserved and Reduced Ejection Fraction. *Eur. J. Heart Fail.* **2014**, *16*, 958–966. [[CrossRef](#)]
65. Fudim, M.; Sayeed, S.; Xu, H.; Matsouaka, R.A.; Heidenreich, P.A.; Velazquez, E.J.; Yancy, C.W.; Fonarow, G.C.; Hernandez, A.F.; DeVore, A.D. Representativeness of the PIONEER-HF Clinical Trial Population in Patients Hospitalized With Heart Failure and Reduced Ejection Fraction. *Circ. Heart Fail.* **2020**, *13*, e006645. [[CrossRef](#)]
66. Mortensen, S.A.; Rosenfeldt, F.; Kumar, A.; Dolliner, P.; Filipiak, K.J.; Pella, D.; Alehagen, U.; Steurer, G.; Littarru, G.P. The Effect of Coenzyme Q 10 on Morbidity and Mortality in Chronic Heart Failure. *JACC Heart Fail.* **2014**, *2*, 641–649. [[CrossRef](#)]
67. Pellicori, P.; Ferreira, J.P.; Mariottoni, B.; Brunner-La Rocca, H.; Ahmed, F.Z.; Verdonschot, J.; Collier, T.; Cuthbert, J.J.; Petutschnigg, J.; Mujaj, B.; et al. Effects of Spironolactone on Serum Markers of Fibrosis in People at High Risk of Developing Heart Failure: Rationale, Design and Baseline Characteristics of a Proof-of-concept, Randomised, Precision-medicine, Prevention Trial. The Heart OMics in AGING (HOMAGE) Trial. *Eur. J. Heart Fail.* **2020**, *22*, 1711–1723. [[CrossRef](#)]
68. Pfisterer, M.; Buser, P.; Rickli, H.; Gutmann, M.; Erne, P.; Rickenbacher, P.; Vuillomenet, A.; Jeker, U.; Dubach, P.; Beer, H.; et al. BNP-Guided vs Symptom-Guided Heart Failure Therapy. *JAMA* **2009**, *301*, 383. [[CrossRef](#)]
69. Khan, A.; Johnson, D.K.; Carlson, S.; Hocum-Stone, L.; Kelly, R.F.; Gravely, A.A.; Mbai, M.; Green, D.L.; Santilli, S.; Garcia, S.; et al. NT-Pro BNP Predicts Myocardial Injury Post-Vascular Surgery and Is Reduced with CoQ10: A Randomized Double-Blind Trial. *Ann. Vasc. Surg.* **2020**, *64*, 292–302. [[CrossRef](#)]
70. Walford, G.A.; Ma, Y.; Christophi, C.A.; Goldberg, R.B.; Jarolim, P.; Horton, E.; Mather, K.J.; Barrett-Connor, E.; Davis, J.; Florez, J.C.; et al. Circulating Natriuretic Peptide Concentrations Reflect Changes in Insulin Sensitivity over Time in the Diabetes Prevention Program. *Diabetologia* **2014**, *57*, 935–939. [[CrossRef](#)]
71. Wolsk, E.; Claggett, B.; Pfeiffer, M.A.; Diaz, R.; Dickstein, K.; Gerstein, H.C.; Lawson, F.C.; Lewis, E.F.; Maggioni, A.P.; McMurray, J.J.; et al. Role of B-Type Natriuretic Peptide and N-Terminal Prohormone BNP as Predictors of Cardiovascular Morbidity and Mortality in Patients With a Recent Coronary Event and Type 2 Diabetes Mellitus. *J. Am. Heart Assoc.* **2017**, *6*, e004743. [[CrossRef](#)] [[PubMed](#)]
72. Buckley, L.F.; Canada, J.M.; del Buono, M.G.; Carbone, S.; Trankle, C.R.; Billingsley, H.; Kadariya, D.; Arena, R.; van Tassel, B.W.; Abbate, A. Low NT-ProBNP Levels in Overweight and Obese Patients Do Not Rule out a Diagnosis of Heart Failure with Preserved Ejection Fraction. *ESC Heart Fail.* **2018**, *5*, 372–378. [[CrossRef](#)] [[PubMed](#)]
73. Fontes-Carvalho, R.; Sampaio, F.; Teixeira, M.; Gama, V.; Leite-Moreira, A.F. The Role of a Structured Exercise Training Program on Cardiac Structure and Function after Acute Myocardial Infarction: Study Protocol for a Randomized Controlled Trial. *Trials* **2015**, *16*, 90. [[CrossRef](#)]
74. Hillock, R.J.; Frampton, C.M.; Yandle, T.G.; Troughton, R.W.; Lainchbury, J.G.; Richards, A.M. B-Type Natriuretic Peptide Infusions in Acute Myocardial Infarction. *Heart* **2008**, *94*, 617–622. [[CrossRef](#)] [[PubMed](#)]
75. Hubers, S.A.; Schirger, J.A.; Sangaralingham, S.J.; Chen, Y.; Burnett, J.C., Jr.; Hodge, D.; Chen, H.H. B-Type Natriuretic Peptide and Cardiac Remodelling after Myocardial Infarction: A Randomised Trial. *Heart* **2021**, *107*, 396–402. [[CrossRef](#)] [[PubMed](#)]
76. Kalra, P.R.; Clague, J.R.; Bolger, A.P.; Anker, S.D.; Poole-Wilson, P.A.; Struthers, A.D.; Coats, A.J. Myocardial Production of C-Type Natriuretic Peptide in Chronic Heart Failure. *Circulation* **2003**, *107*, 571–573. [[CrossRef](#)]
77. Yuan, X.; Zhiya, D.; Wenli, L.; Xiuming, W.; Wenxin, S.; Defen, W.; Jihong, N.; Fengsheng, C.; Junqi, W.; Wei, W. Measurement of Amino-Terminal Propeptide of C-Type Natriuretic Peptide in Patients with Idiopathic Short Stature or Isolated Growth Hormone Deficiency. *J. Pediatr. Endocrinol. Metab.* **2011**, *24*, 989–994. [[CrossRef](#)]
78. Kawakami, R.; Lee, C.Y.W.; Scott, C.; Bailey, K.R.; Schirger, J.A.; Chen, H.H.; Benike, S.L.; Cannone, V.; Martin, F.L.; Sangaralingham, S.J.; et al. A Human Study to Evaluate Safety, Tolerability, and Cyclic GMP Activating Properties of Cenderitide in Subjects With Stable Chronic Heart Failure. *Clin. Pharmacol. Ther.* **2018**, *104*, 546–552. [[CrossRef](#)]
79. Igaki, T.; Itoh, H.; Suga, S.; Hama, N.; Ogawa, Y.; Komatsu, Y.; Mukoyama, M.; Sugawara, A.; Yoshimasa, T.; Tanaka, I.; et al. C-Type Natriuretic Peptide in Chronic Renal Failure and Its Action in Humans. *Kidney Int. Suppl.* **1996**, *55*, S144–S147.
80. Barletta, G.; Lazzeri, C.; Vecchiarino, S.; del Bene, R.; Messeri, G.; dello Sbarba, A.; Mannelli, M.; la Villa, G. Low-Dose C-Type Natriuretic Peptide Does Not Affect Cardiac and Renal Function in Humans. *Hypertension* **1998**, *31*, 802–808. [[CrossRef](#)]
81. Savarirayan, R.; Irving, M.; Bacino, C.A.; Bostwick, B.; Charrow, J.; Cormier-Daire, V.; le Quan Sang, K.-H.; Dickson, P.; Harmatz, P.; Phillips, J.; et al. C-Type Natriuretic Peptide Analogue Therapy in Children with Achondroplasia. *N. Engl. J. Med.* **2019**, *381*, 25–35. [[CrossRef](#)] [[PubMed](#)]
82. Savarirayan, R.; Tofts, L.; Irving, M.; Wilcox, W.R.; Bacino, C.A.; Hoover-Fong, J.; Font, R.U.; Harmatz, P.; Rutsch, F.; Bober, M.B.; et al. Safe and Persistent Growth-Promoting Effects of Vosoritide in Children with Achondroplasia: 2-Year Results from an Open-Label, Phase 3 Extension Study. *Genet. Med.* **2021**, *23*, 2443–2447. [[CrossRef](#)] [[PubMed](#)]
83. Kellner, M.; Yassouridis, A.; Hua, Y.; Wendrich, M.; Jahn, H.; Wiedemann, K. Intravenous C-Type Natriuretic Peptide Augments Behavioral and Endocrine Effects of Cholecystokinin Tetrapeptide in Healthy Men. *J. Psychiatr. Res.* **2002**, *36*, 1–6. [[CrossRef](#)]

84. Potter, L.R.; Yoder, A.R.; Flora, D.R.; Antos, L.K.; Dickey, D.M. Natriuretic Peptides: Their Structures, Receptors, Physiologic Functions and Therapeutic Applications. In *cGMP: Generators, Effectors and Therapeutic Implications*; Springer: Berlin/Heidelberg, Germany, 2009; pp. 341–366.
85. Sudoh, T.; Minamino, N.; Kangawa, K.; Matsuo, H. C-Type Natriuretic Peptide (CNP): A New Member of Natriuretic Peptide Family Identified in Porcine Brain. *Biochem. Biophys. Res. Commun.* **1990**, *168*, 863–870. [[CrossRef](#)]
86. Moyes, A.J.; Hobbs, A.J. C-Type Natriuretic Peptide: A Multifaceted Paracrine Regulator in the Heart and Vasculature. *Int. J. Mol. Sci.* **2019**, *20*, 2281. [[CrossRef](#)]
87. Fujii, T.; Komatsu, Y.; Yasoda, A.; Kondo, E.; Yoshioka, T.; Nambu, T.; Kanamoto, N.; Miura, M.; Tamura, N.; Arai, H.; et al. Circulating C-Type Natriuretic Peptide (CNP) Rescues Chondrodysplastic CNP Knockout Mice from Their Impaired Skeletal Growth and Early Death. *Endocrinology* **2010**, *151*, 4381–4388. [[CrossRef](#)]
88. Nakao, K.; Osawa, K.; Yasoda, A.; Yamanaka, S.; Fujii, T.; Kondo, E.; Koyama, N.; Kanamoto, N.; Miura, M.; Kuwahara, K.; et al. The Local CNP/GC-B System in Growth Plate Is Responsible for Physiological Endochondral Bone Growth. *Sci. Rep.* **2015**, *5*, 10554. [[CrossRef](#)]
89. Breinholt, V.M.; Rasmussen, C.E.; Mygind, P.H.; Kjølgaard-Hansen, M.; Faltinger, F.; Bernhard, A.; Zettler, J.; Hersel, U. TransCon CNP, a Sustained-Release C-Type Natriuretic Peptide Prodrug, a Potentially Safe and Efficacious New Therapeutic Modality for the Treatment of Comorbidities Associated with Fibroblast Growth Factor Receptor 3–Related Skeletal Dysplasias. *J. Pharmacol. Exp. Ther.* **2019**, *370*, 459–471. [[CrossRef](#)]
90. Yamashita, T.; Fujii, T.; Yamauchi, I.; Ueda, Y.; Hirota, K.; Kanai, Y.; Yasoda, A.; Inagaki, N. C-Type Natriuretic Peptide Restores Growth Impairment Under Enzyme Replacement in Mice With Mucopolysaccharidosis VII. *Endocrinology* **2020**, *161*, bqaa008. [[CrossRef](#)]
91. Chen, W.X.; Liu, H.H.; Li, R.X.; Mammadov, G.; Wang, J.J.; Liu, F.F.; Samadli, S.; Wu, Y.F.; Zhang, D.D.; Luo, H.H.; et al. C-Type Natriuretic Peptide Stimulates Osteoblastic Proliferation and Collagen-X Expression but Suppresses Fibroblast Growth Factor-23 Expression in Vitro. *Pediatr. Rheumatol.* **2020**, *18*, 46. [[CrossRef](#)] [[PubMed](#)]
92. Yip, C.Y.Y.; Blaser, M.C.; Mirzaei, Z.; Zhong, X.; Simmons, C.A. Inhibition of Pathological Differentiation of Valvular Interstitial Cells by C-Type Natriuretic Peptide. *Arterioscler. Thromb. Vasc. Biol.* **2011**, *31*, 1881–1889. [[CrossRef](#)]
93. Holliday, L.S.; Dean, A.D.; Greenwald, J.E.; Gluck, S.L. C-Type Natriuretic Peptide Increases Bone Resorption in 1,25-Dihydroxyvitamin D₃-Stimulated Mouse Bone Marrow Cultures. *J. Biol. Chem.* **1995**, *270*, 18983–18989. [[CrossRef](#)] [[PubMed](#)]
94. Hagiwara, H.; Inoue, A.; Yamaguchi, A.; Yokose, S.; Furuya, M.; Tanaka, S.; Hirose, S. CGMP Produced in Response to ANP and CNP Regulates Proliferation and Differentiation of Osteoblastic Cells. *Am. J. Physiol.-Cell Physiol.* **1996**, *270*, C1311–C1318. [[CrossRef](#)] [[PubMed](#)]
95. Teixeira, C.C.; Agoston, H.; Beier, F. Nitric Oxide, C-Type Natriuretic Peptide and CGMP as Regulators of Endochondral Ossification. *Dev. Biol.* **2008**, *319*, 171–178. [[CrossRef](#)]
96. Armour, K.E.; Armour, K.J.; Gallagher, M.E.; Gödecke, A.; Helfrich, M.H.; Reid, D.M.; Ralston, S.H. Defective Bone Formation and Anabolic Response to Exogenous Estrogen in Mice with Targeted Disruption of Endothelial Nitric Oxide Synthase**This Study Was Supported by Grants from the Arthritis Research Campaign (UK) and the Medical Research Council (UK). *Endocrinology* **2001**, *142*, 760–766. [[CrossRef](#)] [[PubMed](#)]
97. Aguirre, J.; Buttery, L.; O’Shaughnessy, M.; Afzal, F.; Fernandez de Marticorena, I.; Hukkanen, M.; Huang, P.; MacIntyre, I.; Polak, J. Endothelial Nitric Oxide Synthase Gene-Deficient Mice Demonstrate Marked Retardation in Postnatal Bone Formation, Reduced Bone Volume, and Defects in Osteoblast Maturation and Activity. *Am. J. Pathol.* **2001**, *158*, 247–257. [[CrossRef](#)]
98. van’T Hof, R.J.; Ralston, S.H. Nitric Oxide and Bone. *Immunology* **2001**, *103*, 255–261. [[CrossRef](#)]
99. Mancini, L.; Moradi-Bidhendi, N.; Becherini, L.; Martinetti, V.; MacIntyre, I. The Biphasic Effects of Nitric Oxide in Primary Rat Osteoblasts Are CGMP Dependent. *Biochem. Biophys. Res. Commun.* **2000**, *274*, 477–481. [[CrossRef](#)]
100. Kiemer, A.K.; Vollmar, A.M. Effects of Different Natriuretic Peptides on Nitric Oxide Synthesis in Macrophages¹. *Endocrinology* **1997**, *138*, 4282–4290. [[CrossRef](#)]
101. Hoshino, M.; Kaneko, K.; Miyamoto, Y.; Yoshimura, K.; Suzuki, D.; Akaike, T.; Sawa, T.; Ida, T.; Fujii, S.; Ihara, H.; et al. 8-Nitro-CGMP Promotes Bone Growth through Expansion of Growth Plate Cartilage. *Free Radic. Biol. Med.* **2017**, *110*, 63–71. [[CrossRef](#)] [[PubMed](#)]
102. Meyerhoff, M.E.; Howland, J.L. Methods in Nitric Oxide Research. *Biochem. Educ.* **1997**, *25*, 184–185. [[CrossRef](#)]
103. Kiemer, A.K.; Angelika, M. Vollmar Induction of L-Arginine Transport Is Inhibited by Atrial Natriuretic Peptide: A Peptide Hormone as a Novel Regulator of Inducible Nitric-Oxide Synthase Substrate Availability. *Mol. Pharmacol.* **2001**, *60*, 421–426. [[PubMed](#)]
104. Peake, N.J.; Hobbs, A.J.; Pinguan-Murphy, B.; Salter, D.M.; Berenbaum, F.; Chowdhury, T.T. Role of C-Type Natriuretic Peptide Signalling in Maintaining Cartilage and Bone Function. *Osteoarthr. Cartil.* **2014**, *22*, 1800–1807. [[CrossRef](#)] [[PubMed](#)]
105. Potter, L.R.; Hunter, T. Activation of Protein Kinase C Stimulates the Dephosphorylation of Natriuretic Peptide Receptor-B at a Single Serine Residue. *J. Biol. Chem.* **2000**, *275*, 31099–31106. [[CrossRef](#)]
106. Abbey, S.E.; Potter, L.R. Lysophosphatidic Acid Inhibits C-Type Natriuretic Peptide Activation of Guanylyl Cyclase-B. *Endocrinology* **2003**, *144*, 240–246. [[CrossRef](#)]
107. Potter, L.R. Guanylyl Cyclase Structure, Function and Regulation. *Cell. Signal.* **2011**, *23*, 1921–1926. [[CrossRef](#)]

108. Schulz, S.; Singh, S.; Bellet, R.A.; Singh, G.; Tubb, D.J.; Chin, H.; Garbers, D.L. The Primary Structure of a Plasma Membrane Guanylate Cyclase Demonstrates Diversity within This New Receptor Family. *Cell* **1989**, *58*, 1155–1162. [[CrossRef](#)]
109. Antos, L.K.; Abbey-Hosch, S.E.; Flora, D.R.; Potter, L.R. ATP-Independent Activation of Natriuretic Peptide Receptors. *J. Biol. Chem.* **2005**, *280*, 26928–26932. [[CrossRef](#)]
110. Ornitz, D.M.; Itoh, N. Fibroblast Growth Factors. *Genome Biol.* **2001**, *2*, reviews3005.1. [[CrossRef](#)]
111. Wilke, T.A.; Gubbels, S.; Schwartz, J.; Richman, J.M. Expression of Fibroblast Growth Factor Receptors (FGFR1, FGFR2, FGFR3) in the Developing Head and Face. *Dev. Dyn.* **1997**, *210*, 41–52. [[CrossRef](#)]
112. Ornitz, D.M.; Legeai-Mallet, L. Achondroplasia: Development, Pathogenesis, and Therapy. *Dev. Dyn.* **2017**, *246*, 291–309. [[CrossRef](#)] [[PubMed](#)]
113. Kanai, M.; Göke, M.; Tsunekawa, S.; Podolsky, D.K. Signal Transduction Pathway of Human Fibroblast Growth Factor Receptor 3. *J. Biol. Chem.* **1997**, *272*, 6621–6628. [[CrossRef](#)]
114. Su, N.; Sun, Q.; Li, C.; Lu, X.; Qi, H.; Chen, S.; Yang, J.; Du, X.; Zhao, L.; He, Q.; et al. Gain-of-Function Mutation in FGFR3 in Mice Leads to Decreased Bone Mass by Affecting Both Osteoblastogenesis and Osteoclastogenesis. *Hum. Mol. Genet.* **2010**, *19*, 1199–1210. [[CrossRef](#)]
115. Ozasa, A.; Komatsu, Y.; Yasoda, A.; Miura, M.; Sakuma, Y.; Nakatsuru, Y.; Arai, H.; Itoh, N.; Nakao, K. Complementary Antagonistic Actions between C-Type Natriuretic Peptide and the MAPK Pathway through FGFR-3 in ATDC5 Cells. *Bone* **2005**, *36*, 1056–1064. [[CrossRef](#)] [[PubMed](#)]
116. Krejci, P.; Masri, B.; Fontaine, V.; Mekikian, P.B.; Weis, M.; Prats, H.; Wilcox, W.R. Interaction of Fibroblast Growth Factor and C-Natriuretic Peptide Signaling in Regulation of Chondrocyte Proliferation and Extracellular Matrix Homeostasis. *J. Cell Sci.* **2005**, *118*, 5089–5100. [[CrossRef](#)]
117. Liu, S.; Zhang, D.; Wu, Y.; Luo, H.; Jiang, G.; Xu, Y.; Wu, Y.; Xia, X.; Wei, W.; Hu, B.; et al. Fibroblast Growth Factor-23 May Serve as a Novel Biomarker for Renal Osteodystrophy Progression. *Int. J. Mol. Med.* **2018**, *43*, 535–546. [[CrossRef](#)]
118. Wu, Y.; Zhang, J.; Chen, F. The Effects of Elevated Fibroblast Growth Factor 23 on Mandibular Growth in Rats. *Arch. Oral Biol.* **2018**, *95*, 156–164. [[CrossRef](#)]
119. Zheng, Q.; Zhou, G.; Morello, R.; Chen, Y.; Garcia-Rojas, X.; Lee, B. Type X Collagen Gene Regulation by Runx2 Contributes Directly to Its Hypertrophic Chondrocyte-Specific Expression in Vivo. *J. Cell Biol.* **2003**, *162*, 833–842. [[CrossRef](#)]
120. Tsuji, T.; Kunieda, T. A Loss-of-Function Mutation in Natriuretic Peptide Receptor 2 (Npr2) Gene Is Responsible for Disproportionate Dwarfism in Cn/Cn Mouse. *J. Biol. Chem.* **2005**, *280*, 14288–14292. [[CrossRef](#)]
121. Pejchalova, K.; Krejci, P.; Wilcox, W.R. C-Natriuretic Peptide: An Important Regulator of Cartilage. *Mol. Genet. Metab.* **2007**, *92*, 210–215. [[CrossRef](#)] [[PubMed](#)]
122. Zeb, A.; Shinwari, N.; Shah, K.; Gilani, S.Z.T.; Khan, S.; Lee, K.W.; Raza, S.I.; Hussain, S.; Liaqat, K.; Ahmad, W.; et al. Molecular and in Silico Analyses Validates Pathogenicity of Homozygous Mutations in the NPR2 Gene Underlying Variable Phenotypes of Acromesomelic Dysplasia, Type Maroteaux. *Int. J. Biochem. Cell Biol.* **2018**, *102*, 76–86. [[CrossRef](#)]
123. Kenis, V.; Melchenko, E.; Mazunin, I.; Pekkinen, M.; Mäkitie, O. A New Family with Epiphyseal Chondrodysplasia Type Miura. *Am. J. Med. Genet. Part A* **2021**, *185*, 112–118. [[CrossRef](#)] [[PubMed](#)]
124. Yamamoto, K.; Kawai, M.; Yamazaki, M.; Tachikawa, K.; Kubota, T.; Ozono, K.; Michigami, T. CREB Activation in Hypertrophic Chondrocytes Is Involved in the Skeletal Overgrowth in Epiphyseal Chondrodysplasia Miura Type Caused by Activating Mutations of Natriuretic Peptide Receptor B. *Hum. Mol. Genet.* **2019**, *28*, 1183–1198. [[CrossRef](#)] [[PubMed](#)]
125. Mackie, E.J.; Ahmed, Y.A.; Tatarczuch, L.; Chen, K.-S.; Mirams, M. Endochondral Ossification: How Cartilage Is Converted into Bone in the Developing Skeleton. *Int. J. Biochem. Cell Biol.* **2008**, *40*, 46–62. [[CrossRef](#)]
126. Akiyama, H.; Chaboissier, M.-C.; Martin, J.F.; Schedl, A.; de Crombrughe, B. The Transcription Factor Sox9 Has Essential Roles in Successive Steps of the Chondrocyte Differentiation Pathway and Is Required for Expression of Sox5 and Sox6. *Genes Dev.* **2002**, *16*, 2813–2828. [[CrossRef](#)]
127. Yoshida, C.A.; Komori, T. Role of Runx Proteins in Chondrogenesis. *Crit. Rev. TM Eukaryot. Gene Expr.* **2005**, *15*, 243–254. [[CrossRef](#)]
128. Fu, M.; Wang, C.; Li, Z.; Sakamaki, T.; Pestell, R.G. Minireview: Cyclin D1: Normal and Abnormal Functions. *Endocrinology* **2004**, *145*, 5439–5447. [[CrossRef](#)]
129. Beier, F.; Ali, Z.; Mok, D.; Taylor, A.C.; Leask, T.; Albanese, C.; Pestell, R.G.; LuValle, P. TGF β and PTHrP Control Chondrocyte Proliferation by Activating Cyclin D1 Expression. *Mol. Biol. Cell* **2001**, *12*, 3852–3863. [[CrossRef](#)]
130. L'Hôte, C.G.M.; Knowles, M.A. Cell Responses to FGFR3 Signalling: Growth, Differentiation and Apoptosis. *Exp. Cell Res.* **2005**, *304*, 417–431. [[CrossRef](#)]
131. Ornitz, D.M.; Marie, P.J. Fibroblast Growth Factor Signaling in Skeletal Development and Disease. *Genes Dev.* **2015**, *29*, 1463–1486. [[CrossRef](#)] [[PubMed](#)]
132. Kuehl, A.; Pelisek, J.; Pongratz, J.; Eckstein, H.-H. C-Type Natriuretic Peptide and Its Receptors in Atherosclerotic Plaques of the Carotid Artery of Clinically Asymptomatic Patients. *Eur. J. Vasc. Endovasc. Surg.* **2012**, *43*, 649–654. [[CrossRef](#)] [[PubMed](#)]
133. Miyazawa, T.; Ogawa, Y.; Chusho, H.; Yasoda, A.; Tamura, N.; Komatsu, Y.; Pfeifer, A.; Hofmann, F.; Nakao, K. Cyclic GMP-Dependent Protein Kinase II Plays a Critical Role in C-Type Natriuretic Peptide-Mediated Endochondral Ossification. *Endocrinology* **2002**, *143*, 3604–3610. [[CrossRef](#)]

134. Kake, T.; Kitamura, H.; Adachi, Y.; Yoshioka, T.; Watanabe, T.; Matsushita, H.; Fujii, T.; Kondo, E.; Tachibe, T.; Kawase, Y.; et al. Chronically Elevated Plasma C-Type Natriuretic Peptide Level Stimulates Skeletal Growth in Transgenic Mice. *Am. J. Physiol.-Endocrinol. Metab.* **2009**, *297*, E1339–E1348. [[CrossRef](#)] [[PubMed](#)]
135. Moffatt, P.; Thomas, G.; Sellin, K.; Bessette, M.-C.; Lafrenière, F.; Akhouayri, O.; St-Arnaud, R.; Lanctôt, C. Osteocrin Is a Specific Ligand of the Natriuretic Peptide Clearance Receptor That Modulates Bone Growth. *J. Biol. Chem.* **2007**, *282*, 36454–36462. [[CrossRef](#)]
136. Agoston, H.; Khan, S.; James, C.G.; Gillespie, J.R.; Serra, R.; Stanton, L.-A.; Beier, F. C-Type Natriuretic Peptide Regulates Endochondral Bone Growth through P38 MAP Kinase-Dependent and—Independent Pathways. *BMC Dev. Biol.* **2007**, *7*, 18. [[CrossRef](#)]
137. Woods, A.; Khan, S.; Beier, F. C-Type Natriuretic Peptide Regulates Cellular Condensation and Glycosaminoglycan Synthesis during Chondrogenesis. *Endocrinology* **2007**, *148*, 5030–5041. [[CrossRef](#)]
138. Inoue, A.; Hayakawa, T.; Otsuka, E.; Kamiya, A.; Suzuki, Y.; Hirose, S.; Hagiwara, H. Correlation between Induction of Expression of Biglycan and Mineralization by C-Type Natriuretic Peptide in Osteoblastic Cells. *J. Biochem.* **1999**, *125*, 103–108. [[CrossRef](#)]
139. Yeh, L.-C.C.; Adamo, M.L.; Olson, M.S.; Lee, J.C. Osteogenic Protein-1 and Insulin-Like Growth Factor I Synergistically Stimulate Rat Osteoblastic Cell Differentiation and Proliferation¹. *Endocrinology* **1997**, *138*, 4181–4190. [[CrossRef](#)]
140. Chen, D.; Zhao, M.; Mundy, G.R. Bone Morphogenetic Proteins. *Growth Factors* **2004**, *22*, 233–241. [[CrossRef](#)]
141. Yeh, L.-C.C.; Zavala, M.C.; Lee, J.C. C-Type Natriuretic Peptide Enhances Osteogenic Protein-1-Induced Osteoblastic Cell Differentiation via Smad5 Phosphorylation. *J. Cell. Biochem.* **2006**, *97*, 494–500. [[CrossRef](#)] [[PubMed](#)]
142. Chusho, H.; Tamura, N.; Ogawa, Y.; Yasoda, A.; Suda, M.; Miyazawa, T.; Nakamura, K.; Nakao, K.; Kurihara, T.; Komatsu, Y.; et al. Dwarfism and Early Death in Mice Lacking C-Type Natriuretic Peptide. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 4016–4021. [[CrossRef](#)] [[PubMed](#)]
143. Fujii, T.; Hirota, K.; Yasoda, A.; Takizawa, A.; Morozumi, N.; Nakamura, R.; Yotsumoto, T.; Kondo, E.; Yamashita, Y.; Sakane, Y.; et al. Rats Deficient C-Type Natriuretic Peptide Suffer from Impaired Skeletal Growth without Early Death. *PLoS ONE* **2018**, *13*, e0194812. [[CrossRef](#)] [[PubMed](#)]
144. Trotter, T.L.; Hall, J.G. Health Supervision for Children With Achondroplasia. *Pediatrics* **2005**, *116*, 771–783. [[CrossRef](#)] [[PubMed](#)]
145. Yasoda, A.; Komatsu, Y.; Chusho, H.; Miyazawa, T.; Ozasa, A.; Miura, M.; Kurihara, T.; Rogi, T.; Tanaka, S.; Suda, M.; et al. Overexpression of CNP in Chondrocytes Rescues Achondroplasia through a MAPK-Dependent Pathway. *Nat. Med.* **2004**, *10*, 80–86. [[CrossRef](#)]
146. Yamanaka, S.; Nakao, K.; Koyama, N.; Isobe, Y.; Ueda, Y.; Kanai, Y.; Kondo, E.; Fujii, T.; Miura, M.; Yasoda, A.; et al. Circulatory CNP Rescues Craniofacial Hypoplasia in Achondroplasia. *J. Dent. Res.* **2017**, *96*, 1526–1534. [[CrossRef](#)]
147. Ueda, Y.; Yasoda, A.; Hirota, K.; Yamauchi, I.; Yamashita, T.; Kanai, Y.; Sakane, Y.; Fujii, T.; Inagaki, N. Exogenous C-Type Natriuretic Peptide Therapy for Impaired Skeletal Growth in a Murine Model of Glucocorticoid Treatment. *Sci. Rep.* **2019**, *9*, 8547. [[CrossRef](#)]
148. Jackson, D.J.; Bacharier, L.B.; Mauger, D.T.; Boehmer, S.; Beigelman, A.; Chmiel, J.F.; Fitzpatrick, A.M.; Gaffin, J.M.; Morgan, W.J.; Peters, S.P.; et al. Quintupling Inhaled Glucocorticoids to Prevent Childhood Asthma Exacerbations. *N. Engl. J. Med.* **2018**, *378*, 891–901. [[CrossRef](#)]
149. Rivkees, S.A.; Danon, M.; Herrin, J. Prednisone Dose Limitation of Growth Hormone Treatment of Steroid-Induced Growth Failure. *J. Pediatr.* **1994**, *125*, 322–325. [[CrossRef](#)]
150. Hirota, K.; Furuya, M.; Morozumi, N.; Yoshikiyo, K.; Yotsumoto, T.; Jindo, T.; Nakamura, R.; Murakami, K.; Ueda, Y.; Hanada, T.; et al. Exogenous C-Type Natriuretic Peptide Restores Normal Growth and Prevents Early Growth Plate Closure in Its Deficient Rats. *PLoS ONE* **2018**, *13*, e0204172. [[CrossRef](#)]
151. Ueda, Y.; Hirota, K.; Yamauchi, I.; Hakata, T.; Yamashita, T.; Fujii, T.; Yasoda, A.; Inagaki, N. Is C-Type Natriuretic Peptide Regulated by a Feedback Loop? A Study on Systemic and Local Autoregulatory Effect. *PLoS ONE* **2020**, *15*, e0240023. [[CrossRef](#)] [[PubMed](#)]
152. Prickett, T.C.; & Espiner, E.A. Circulating products of C-type natriuretic peptide and links with organ function in health and disease. *Peptides* **2020**, *132*, 170363. [[CrossRef](#)] [[PubMed](#)]
153. Espiner, E.; Prickett, T.; Olney, R. Plasma C-Type Natriuretic Peptide: Emerging Applications in Disorders of Skeletal Growth. *Horm. Res. Paediatr.* **2018**, *90*, 345–357. [[CrossRef](#)]
154. Olney, R.C.; Permuy, J.W.; Prickett, T.C.; Han, J.C.; Espiner, E.A. Amino-terminal propeptide of C-type natriuretic peptide (NTproCNP) predicts height velocity in healthy children. *Clin. Endocrinol.* **2012**, *77*, 416–422. [[CrossRef](#)] [[PubMed](#)]
155. Miura, K.; Namba, N.; Fujiwara, M.; Ohata, Y.; Ishida, H.; Kitaoka, T.; Kubota, T.; Hirai, H.; Higuchi, C.; Tsumaki, N.; et al. An overgrowth disorder associated with excessive production of cGMP due to a gain-of-function mutation of the natriuretic peptide receptor 2 gene. *PLoS ONE* **2012**, *7*, e42180. [[CrossRef](#)] [[PubMed](#)]
156. Hannema, S.E.; van Duyvenvoorde, H.A.; Premisler, T.; Yang, R.B.; Mueller, T.D.; Gassner, B.; Oberwinkler, H.; Roelfsema, F.; Santen, G.W.; Prickett, T.; et al. An activating mutation in the kinase homology domain of the natriuretic peptide receptor-2 causes extremely tall stature without skeletal deformities. *J. Clin. Endocrinol. Metab.* **2013**, *98*, E1988–98. [[CrossRef](#)] [[PubMed](#)]

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157. Hutchison, M.R. BDNF Alters ERK/p38 MAPK Activity Ratios to Promote Differentiation in Growth Plate Chondrocytes. *Mol. Endocrinol.* **2012**, *26*, 1406–1416. [[CrossRef](#)]
 158. Mericq, V.; Uyeda, J.A.; Barnes, K.M.; de Luca, F.; Baron, J. Regulation of Fetal Rat Bone Growth by C-Type Natriuretic Peptide and CGMP. *Pediatr. Res.* **2000**, *47*, 189. [[CrossRef](#)]