CNP function, and for those which occur more commonly, could benefit diagnosis and clinical trial enrollment for eligible patients having short stature.

[008] In one aspect, the present disclosure provides a use of a CNP variant in the preparation of a medicament for treating a CNP-responsive bone-related disorder, skeletal dysplasia or short stature disorder in a subject, wherein the CNP variant is to be administered according to a weight-band dosing regimen, wherein

i) a subject between 10–11 kg receives between about 22–24 µg/kg CNP variant;

ii) a subject between 12–16 kg receives between about 18–23 µg/kg CNP variant;

iii) a subject between 17–21 kg receives between about 15–19 µg/kg CNP variant;

iv) a subject between 22–32 kg receives between about 13–18 µg/kg CNP variant;

v) a subject between 33–43 kg receives between about 12–15 µg/kg CNP variant;

vi) a subject between 44–59 kg receives between about 10–14 µg/kg CNP variant;

vii) a subject between 60–89 kg receives between about 8–12 µg/kg CNP variant; or

viii) a subject of weight of ≥ 90 kg receives about ≤ 9 µg/kg CNP variant.

[008a] In another aspect, the present disclosure provides a method of identifying a variant gene associated with short stature that is a gain of function (GoF) or loss of function (LoF) variant comprising:

-contacting cells comprising a cGMP-reporter expression construct and a lentiviral vector expressing a polynucleotide encoding a variant protein associated with short stature operably linked to one or more unique barcode sequences in culture with c-type natriuretic peptide (CNP) or a variant thereof having CNP activity;

-sorting the cells from the culture based on the level of expression of reporter produced by the cell; and

-identifying the variant protein associated with short stature as a GoF variant or a LoF variant, wherein a GoF variant has a higher level of cGMP production compared to a control, and wherein a LoF variant has a lower level of cGMP production compared to a control.

[008b] The disclosure provides a method of identifying a variant gene associated with short stature that is a gain of function (GoF) or loss of function (LoF) variant comprising:

-transfecting cells comprising a cGMP-GFP reporter expression construct with a lentiviral vector expressing a polynucleotide encoding a variant protein associated with short stature operably linked to one or more unique barcode sequences;

-contacting the cells in culture with c-type natriuretic peptide (CNP) or a variant thereof having CNP activity;

-sorting the cells from the culture based on the level of expression of GFP produced by the cell; and

-identifying the variant protein associated with short stature as a GoF variant or a LoF variant, wherein a GoF variant has a higher level of cGMP production compared to a control, and wherein a LoF variant has a lower level of cGMP production compared to a control.

[009] In various examples, the variant gene associated with short stature is collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), indian hedgehog (IHH), PTPN11, NPR2, NPPC, FGFR3, insulin growth factor 1 receptor (IGF1R), DTL, PAPPA2, or combinations thereof. In various examples, the variant gene associated with short stature is selected from the group consisting of natriuretic peptide receptor 2 (NPR2), Natriuretic peptide precursor C (NPPC), fibroblast growth factor receptor 3 (FGFR3) or combinations thereof. In various examples, the variant gene associated with short stature is NPR2.

[0010] Also contemplated herein is a method of identifying a variant of NPR2 as a gain of function (GoF) or loss of function (LoF) variant comprising:

-transfecting cells comprising a cGMP-GFP reporter expression construct with a lentiviral vector expressing a polynucleotide encoding a NPR2 variant protein operably linked to one or more unique barcode sequences;

-contacting the cells in culture with c-type natriuretic peptide (CNP) or a variant thereof having CNP activity;

-sorting the cells from the culture based on the level of expression of GFP produced by the cell; and

-identifying the NPR2 variant as a GoF variant or a LoF variant, wherein a GoF variant has a higher level of cGMP production compared to a control, and wherein a LoF variant has a lower level of cGMP production compared to a control.

[0011] In various examples, the cells are a mammalian cell line. In certain examples, the cells are HEK293 cells.

[0012] In various examples, the cells are sorted by flow cytometry.

[0013] In various examples, the lentiviral vector comprises a polynucleotide encoding a variant gene associated with short stature, a polynucleotide encoding a puromycin resistance gene and a polynucleotide encoding T2A-BFP promoter.

[0014] In various examples, the lentiviral vector further comprises between 20 and 60 barcode sequences. In various examples, the barcode sequences are from 15 to 30 basepairs.

[0015] In various examples, the barcode sequences are 3' to the variant gene polynucleotide.

[0016] In various examples, the expression construct comprises a polynucleotide encoding a GFP protein operably linked to a cGMP binding domain. In various examples, the cGMP binding domain is from mouse or human phosphodiesterase.

[0017] In various examples, the expression construct further comprises a CMV promoter operably linked to cGull and a PGK promoter operably linked to a blastocidin resistance gene.

[0018] In various examples, the CNP variant is selected from the group consisting of:

PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37) (SEQ ID NO: 1);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP-37) (SEQ ID NO: 2);
GDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP53) (SEQ ID NO: 3);
PDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP53) (SEQ ID NO: 4);
MDLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP53) (SEQ ID NO: 5);
DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-53(M48N)] (SEQ ID NO: 6);
LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-52) (SEQ ID NO: 7);

RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-51) (SEQ ID NO: 8);
VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP- 50) (SEQ ID NO: 9);
DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-49) (SEQ ID NO: 10);
TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-48) (SEQ ID NO: 11);
KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-47) (SEQ ID NO: 12);
SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-46) (SEQ ID NO: 13);
RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-45) (SEQ ID NO: 14);
AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-44) (SEQ ID NO: 15);
AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-43) (SEQ ID NO: 16); WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-42) (SEQ ID NO: 17); ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-41) (SEQ ID NO: 18); RLLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-40) (SEQ ID NO: 19); LLQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-39) (SEQ ID NO: 20); LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 21);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-37) (SEQ ID NO: 22);
EHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-36) (SEQ ID NO: 23);
HPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-35) (SEQ ID NO: 24);
PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-34) (SEQ ID NO: 25);
NARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-33) (SEQ ID NO: 26);
ARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-32) (SEQ ID NO: 27);
RKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-31) (SEQ ID NO: 28);
KYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-30) (SEQ ID NO: 29);
YKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-29) (SEQ ID NO: 30);
KGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-28) (SEQ ID NO: 31);
GANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-27) (SEQ ID NO: 32);
ANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-26) (SEQ ID NO: 33);
NKKGLSKGCFGLKLDRIGSMSGLGC (CNP-25) (SEQ ID NO: 34);
KKGLSKGCFGLKLDRIGSMSGLGC (CNP-24) (SEQ ID NO: 35);
KGLSKGCFGLKLDRIGSMSGLGC (CNP-23) (SEQ ID NO: 36);

LSKGCFGLKLDRIGSMSGLGC (CNP-21) (SEQ ID NO: 37);
SKGCFGLKLDRIGSMSGLGC (CNP-20) (SEQ ID NO: 38);
KGCFGLKLDRIGSMSGLGC (CNP-19) (SEQ ID NO: 39);
GCFGLKLDRIGSMSGLGC (CNP-18) (SEQ ID NO: 40);
QEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [CNP-37(M32N)] (SEQ ID NO: 41);
PQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-CNP-37) (SEQ ID NO: 42);
MQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-CNP-37) (SEQ ID NO: 43);
GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSNSGLGC [Gly-CNP-37(M32N)] (SEQ ID NO: 44);
MGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Met-Gly-CNP-37) (SEQ ID NO: 45);
PGQEHPQARRYRGAQRRGLSRGCFGLKLDRIGSMSGLGC (SEQ ID NO: 46);
PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 47);
PGQEHPNARRYRGANRRGLSRGCFGLKLDRIGSMSGLGC (SEQ ID NO: 48);
PGQEHPQARKYKGAQKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 49);
GQEHPNARKYKGANPKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 50);
GQEHPNARKYKGANQKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 51);
GQEHPNARKYKGANQQGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 52); and
GQEHPNARKYKGANKPGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 53).

[0019] In various examples, the CNP is contacted with the cells at a dose between about 1 to about 100 nM. In various examples, the CNP is contacted with the cells at a dose of about 1, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95 or about 100 nM.

[0020] In various examples, the lentivirus is transfected into the cells at a multiplicity of infection of MOI between about 0.1 and about 0.5. In various examples, the lentivirus is transfected into the cells at a multiplicity of infection of MOI about 0.1, about 0.2, about 0.3, about 0.4 or about 0.5.

[0021] In another aspect, the present disclosure provides a lentiviral vector comprising

i) a polynucleotide encoding a variant gene associated with short stature, a polynucleotide encoding a puromycin resistance gene and a polynucleotide encoding T2A-BFP promoter; optionally wherein the variant gene associated with short stature is selected from the group consisting of natriuretic peptide receptor 2 (NPR2), Natriuretic peptide

precursor C (NPPC), fibroblast growth factor receptor 3 (FGFR3) or combinations thereof; or

ii) a NPR2 variant, a polynucleotide encoding a puromycin resistance gene and a polynucleotide encoding T2A-BFP promoter.

[0021a] In various examples, the disclosure provides a lentiviral vector comprising a polynucleotide encoding a variant gene associated with short stature, a polynucleotide encoding a puromycin resistance gene and a polynucleotide encoding T2A-BFP promoter.

[0022] In various examples, the variant gene associated with short stature is selected from the group consisting of natriuretic peptide receptor 2 (NPR2), natriuretic peptide precursor C (NPPC), fibroblast growth factor receptor 3 (FGFR3) or combinations thereof. In various examples, the variant gene associated with short stature is NPR2.

[0023] Further contemplated is a lentiviral vector comprising a polynucleotide encoding a NPR2 variant, a polynucleotide encoding a puromycin resistance gene and a polynucleotide encoding T2A-BFP promoter. In various examples, the lentiviral vector further comprises between 20 and 60 barcode sequences. In various examples, the barcode sequences are from 15 to 30 basepairs. In various examples, the barcode sequences are 3' to the variant gene polynucleotide.

[0024] In various embodiments, the disclosure provides a method of making a lentiviral library comprising a variant gene associated with short stature, the method comprising:

-amplifying variant genes associated with short stature from a mammalian genome or genomic DNA database;

-cloning the amplified variants into a lentivial vector, wherein the lentiviral vector comprises between 20-60 unique barcodes per vector;

-sequencing the variant associated with the barcodes in the vector;

-aligning the variant sequences with a control gene sequence to generate a read structure;

-extracting the barcodes from the variant read structure;

-identifying the barcodes for each variant; and

-isolating the lentiviral vectors expressing variant genes.

[0025] In various embodiments, the variant gene associated with short stature is selected from the group consisting of NPR2, NPPC, FGFR3 or combinations thereof.

[0026] Also provided is a method of making an NPR2 variant lentiviral library comprising

-amplifying NPR2 variants from a mammalian genome or genomic DNA database;

-cloning the amplified NPR2 variants into a lentivial vector, wherein the lentiviral vector comprises between 20-60 unique barcodes per vector;

-sequencing the NPR2 variant associated with the barcodes in the vector;

-aligning the NPR2 variant sequences with a control NPR2 gene sequence to generate a read structure;

-extracting the barcodes from the NPR2 variant read structure;

-identifying the barcodes for each NPR2 variant; and

-isolating the lentiviral vectors expressing NPR2 variants.

[0027] The disclosure also contemplates a method for treating a subject with a short stature disorder comprising administering a CNP variant to a subject identified as having a loss of

function variant of a gene associated with short stature identified using a method of described herein.

[0028] In various examples, the disclosure provides a method of improving and/or maintaining bone strength in a subject in need thereof comprising administering a C-type natriuretic peptide (CNP) to the subject. In various examples, the subject has a short stature disorder.

[0029] In another aspect, the present disclosure provides a use of a CNP variant in the preparation of a medicament for treating a subject with a short stature disorder and identified as having a loss of function variant of a gene associated with short stature identified using a method disclosed herein.

[0029a] In various embodiments, the short stature disorder is selected from the group consisting of achondroplasia, hypochondroplasia, short stature, idiopathic short stature, dwarfism, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis congenita, achondrogenesis, homozygous achondroplasia, chondrodysplasia congenita, campomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis congenita, short-rib polydactyly syndromes, rhizomelic type of chondrodysplasia congenita, Jansen-type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenital, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer-type mesomelic dysplasia, Nievergelt-type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia, disorders related to NPR2 mutation, SHOX mutations, Turner's syndrome/Leri Weill, PTPN11 mutations, Noonan's syndrome, and IGF1R mutation.

[0030] The disclosure also contemplates a population PK model for dosing of CNP variant to a subject. In various examples, the disclosure provides a method for treating a CNP-responsive bone-related disorder, skeletal dysplasia or short stature disorder comprising administering to a subject in need thereof a CNP variant, wherein the CNP variant is administered according to a weight-band dosing regimen, wherein

i) a subject between 10–11 kg receives between about 22–24 µg/kg CNP variant;

ii) a subject between 12–16 kg receives between about 18–23 µg/kg CNP variant;

iii) a subject between 17–21 kg receives between about 15–19 µg/kg CNP variant;

iv) a subject between 22–32 kg receives between about 13–18 µg/kg CNP variant;

v) a subject between 33–43 kg receives between about 12–15 µg/kg CNP variant;

vi) a subject between 44–59 kg receives between about 10–14 µg/kg CNP variant;

vii) a subject between 60–89 kg receives between about 8–12 µg/kg CNP variant; or

viii) a subject of weight of ≥ 90 kg receives about ≤ 9 µg/kg CNP variant.

[0031] In various examples,

i) a subject between 10–11 kg receives about 0.24 mg CNP variant;

ii) a subject between 12–16 kg receives about 0.28 mg CNP variant;

iii) a subject between 17–21 kg receives about 0.32 mg CNP variant;

iv) a subject between 22–32 kg receives about 0.40 mg CNP variant;

v) a subject between 33–43 kg receives about 0.50 mg CNP variant;

vi) a subject between 44–59 kg receives about 0.60 mg CNP variant;

vii) a subject between 60–89 kg receives about 0.7 mg CNP variant; or

viii) a subject having a weight of ≥ 90 kg receives about 0.80 mg CNP variant.

[0032] In various examples, the CNP-responsive bone-related disorder, skeletal dysplasia or short stature disorder is selected from the group consisting of achondroplasia, hypochondroplasia, short stature, idiopathic short stature, dwarfism, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis congenita, achondrogenesis, homozygous achondroplasia, chondrodysplasia congenita, campomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis congenita, short-rib polydactyly syndromes, rhizomelic type of chondrodysplasia congenita, Jansen-type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenital, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer-type mesomelic dysplasia, Nievergelt-type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia, disorders related to NPR2 mutation, SHOX mutations, Turner's syndrome/Leri Weill, PTPN11 mutations, Noonan's syndrome, and IGF1R mutation.

[0033] In various examples, the CNP variant is set out in SEQ ID NOs: 1-53. In various examples, the CNP variant is PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP37) (SEQ ID NO: 1); GQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Gly-CNP-37) (SEQ ID NO: 2); or LQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (CNP-38) (SEQ ID NO: 21).

[0034] In various examples, the CNP variant further comprises a hydrophilic moiety. In various examples, the hydrophilic moiety is PEG.

[0035] Also contemplated is an expression construct comprising a polynucleotide encoding a GFP protein operably linked to a cGMP binding domain as described herein useful in a

reporter assay as described herein. The cGMP binding domain is from mouse or human phosphodiesterase. In various examples, the expression construct further comprises a CMV promoter operably linked to cGull and PGK promoter operably linked to a blastocidin resistance gene.

[0036] Further examples and advantages will be apparent to those of ordinary skill in the art from a review of the following detailed description, taken in conjunction with the drawings. While the compositions, articles, and methods are susceptible of examples in various forms, the description hereafter includes specific examples with the understanding that the disclosure is illustrative, and is not intended to limit the invention to the specific examples described herein. For the compositions, articles, and methods described herein, optional features, including but not limited to components, compositional ranges thereof, substituents, conditions, and steps, are contemplated to be selected from the various examples, examples, and examples provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] Figures 1A-1D depict a schematic of a high throughput screening process using an NPR2 expressing lentivirus and a cGMP reporter construct. Figure 1A. Construct of bidirectional lentivirus that expresses NPR2 and puro-T2A-BFP. Figure 1B. cGMP-responsive GFP-fluorescent reporter reconstituted into HEK293s cells. Figure 1C. Barcoding strategy and PacBio (long-read) and Illumina (short-read) based methods for associating barcode to variant. Figure 1D. GFP-sorting-based screening strategy.

[0038] Figure 2 illustrates a method of generating a NPR2 variant lentiviral library.

[0039] Figures 3A-3B show levels of cGMP detected by catchpoint assay (Figure 3A) or cGMP-GFP reporter construct of the disclosure (Figure 3B).

[0040] Figures 4A-4C show the process of screening samples using PCR generation of NPR2 variants (Figure 4A), screening where LoF or GoF functions will have different levels of cGMP production (Figure 4B), and results of a cGMP-GFP reporter screen on a GoF or LoF NPR2 variant (Figure 4C).

[0041] Figure 5 shows a representative sample sort from flow cytometric analysis of a GFP high or GFP low screen, and demonstrates the screen is highly correlative with the function of the NPR2 variant and cGMP-GFP expression.

[0042] Figures 6A-6B show that the high throughput screen using the cGMP-GFP construct (Figure 6A) correlates with previous published results of NPR2 variant function (Figure 6B).

[0043] Figure 7A-7C show variant functional activity by genetic consequences and phenotypic effects. Figure 7A. Measured cGMP levels by variant functional consequence.