Methods Of Treating Decreased Bone Mineral Density With Kringle Containing Transmembrane Protein 1 (KREMEN1) Inhibitors

Reference To Sequence Listing

This application includes a Sequence Listing filed electronically as a text file named 18923808102SEQ, created on June 29, 2022, with a size of 196 kilobytes. The Sequence Listing is incorporated herein by reference.

Field

The present disclosure relates generally to the treatment of subjects having decreased bone mineral density or at risk of developing decreased bone mineral density with Kringle Containing Transmembrane Protein 1 (KREMEN1) inhibitors, and methods of identifying subjects having an increased risk of developing decreased bone mineral density.

Background

Degenerative conditions of the bone can make individuals susceptible to bone fractures, bone pain, and other complications. Two significant degenerative conditions of the bone are osteopenia and osteoporosis. Decreased bone mineral density (osteopenia) is a condition of the bone that is a precursor to osteoporosis and is characterized by a reduction in bone mass due to the loss of bone at a rate greater than new bone growth. Osteopenia manifests in bone having a mineral density lower than normal peak bone mineral density, but not as low as found in osteoporosis. Osteopenia can arise from a decrease in muscle activity, which may occur as the result of a bone fracture, bed rest, fracture immobilization, joint reconstruction, arthritis, and the like. Osteoporosis is a progressive disease characterized by a gradual bone weakening due to demineralization of the bone. Osteoporosis manifests in bones that are thin and brittle making them more susceptible to breaking. Hormone deficiencies related to menopause in women, and hormone deficiencies due to aging in both sexes contribute to degenerative conditions of the bone. In addition, insufficient dietary uptake of minerals essential to bone growth and maintenance are significant causes of bone loss.

The effects of osteopenia can be slowed, stopped, and even reversed by reproducing some of the effects of muscle use on the bone. This typically involves some application or simulation of the effects of mechanical stress on the bone. Compounds for the treatment of

osteopenia or osteoporosis include pharmaceutical preparations that induce bone growth or retard bone demineralization, or mineral complexes that supplement the diet in an effort to replenish lost bone minerals. Low levels of estrogen in women, and low levels of androgen in men are the primary hormonal deficiencies that cause osteoporosis in the respective sexes. Other hormones such as the thyroid hormones, progesterone, and testosterone contribute to bone health. As such, the aforementioned hormonal compounds have been developed synthetically, or extracted from non-mammalian sources, and compounded into therapies for treating osteoporosis. Mineral supplement preparations containing iodine, zinc, manganese, boron, strontium, vitamin D3, calcium, magnesium, vitamin K, phosphorous, and copper have also been used to supplement insufficient dietary uptake of such minerals. However, long-term hormonal therapies have undesirable side effects such as increased cancer risk. Moreover, therapies using many synthetic or non-mammalian hormones have additional undesirable side effects, such as an increased risk of cardiovascular disorders, neurological disorders, or the exacerbation of pre-existing conditions.

Kringle Containing Transmembrane Protein 1 (KREMEN1) is a cell surface molecule that regulates WNT signaling by binding to DKK and LRP5/6, thereby promoting uptake of this complex through clathrin-mediated endocytosis (Mao et al., Nature, 2002, 417, 664-667).

Summary

The present disclosure provides methods of treating a subject having decreased bone mineral density or at risk of developing decreased bone mineral density, the methods comprising administering a KREMEN1 inhibitor to the subject.

Disclosed herein is the use of a KREMEN1 inhibitor in the preparation of a medicament for treating decreased bone mineral density or risk of development of decreased bone mineral density in a subject, wherein the KREMEN1 inhibitor comprises an inhibitory nucleic acid molecule that hybridizes to a KREMEN1 nucleic acid molecule, and wherein the inhibitory nucleic acid molecule comprises an antisense nucleic acid molecule, a small interfering RNA (siRNA), or a short hairpin RNA (shRNA).

The present disclosure also provides methods of treating a subject having osteopenia or at risk of developing osteopenia, the methods comprising administering a KREMEN1 inhibitor to the subject.

The present disclosure also provides methods of treating a subject having Type I osteoporosis or at risk of developing Type I osteoporosis, the methods comprising administering a KREMEN1 inhibitor to the subject.

The present disclosure also provides methods of treating a subject having Type II osteoporosis or at risk of developing Type II osteoporosis, the methods comprising administering a KREMEN1 to the subject.

The present disclosure also provides methods of treating a subject having secondary osteoporosis or at risk of developing secondary osteoporosis, the methods comprising administering a KREMEN1 inhibitor to the subject.

The present disclosure also provides methods of treating a subject with a therapeutic agent that treats or prevents decreased bone mineral density, wherein the subject has decreased bone mineral density or is at risk of developing decreased bone mineral density, the methods comprising the steps of: determining whether the subject has a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide by: obtaining or having obtained a biological sample from the subject; and performing or having performed a sequence analysis on the biological sample to determine if the subject has a genotype comprising the KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide; and: i) administering or continuing to administer the therapeutic agent that treats or prevents decreased bone mineral density in a standard dosage amount to a subject that is KREMEN1 reference, and/or administering a KREMEN1 inhibitor to the subject; ii) administering or continuing to administer the therapeutic agent that treats or prevents decreased bone mineral density in an amount that is the same as or less than a standard dosage amount to a subject that is heterozygous for the KREMEN1 variant nucleic acid molecule, and/or administering a KREMEN1 inhibitor to the subject; or iii) administering or continuing to administer the therapeutic agent that treats or prevents decreased bone mineral density in an amount that is the same as or less than a standard dosage amount to a subject that is homozygous for the KREMEN1 variant nucleic acid molecule; wherein the presence of a genotype having the KREMEN1 variant nucleic acid molecule encoding the KREMEN1 predicted loss-of-function polypeptide indicates the subject has a decreased risk of developing decreased bone mineral density.

Disclosed herein is the use of a therapeutic agent that treats decreased bone mineral density in the preparation of a medicament for treating a subject that has decreased bone

mineral density or is at risk of developing decreased bone mineral density, wherein the subject has been determined to have a Kringle Containing Transmembrane Protein 1 (KREMEN1) variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide by: performing or having performed a sequence analysis on a biological sample obtained or having been obtained from the subject to determine if the subject has a genotype comprising the KREMEN1 variant nucleic acid molecule; and wherein the therapeutic agent that treats decreased bone mineral density is to be administered or continued to be administered in a standard dosage amount to a subject that is KREMEN1 reference, and/or a KREMEN1 inhibitor is to be administered to the subject; wherein the therapeutic agent that treats decreased bone mineral density is to be administered or continued to be administered in an amount that is the same as or less than a standard dosage amount to a subject that is heterozygous for the KREMEN1 variant nucleic acid molecule, and/or a KREMEN1 inhibitor is to be administered to the subject; or wherein the therapeutic agent that treats decreased bone mineral density is to be administered or continued to be administered in an amount that is the same as or less than a standard dosage amount to a subject that is homozygous for the KREMEN1 variant nucleic acid molecule; wherein the presence of a genotype having the KREMEN1 variant nucleic acid molecule encoding the KREMEN1 predicted loss-of-function polypeptide indicates the subject has a decreased risk of developing decreased bone mineral density, wherein the therapeutic agent is chosen from alendronate, ibandronate, zoledronate, risedronate, calcitonin, teriparatide, denosumab, estrogen and progesterone, raloxifene, or any combination thereof; and wherein the KREMEN1 inhibitor comprises an inhibitory nucleic acid molecule that hybridizes to a KREMEN1 nucleic acid molecule, and wherein the inhibitory nucleic acid molecule comprises an antisense nucleic acid molecule, a small interfering RNA (siRNA), or a short hairpin RNA (shRNA).

Disclosed herein is a method of identifying a subject having an increased risk of developing decreased bone mineral density, the method comprising: determining or having determined the presence or absence of a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide in a biological sample obtained from the subject; when the subject is KREMEN1 reference, then the subject has an increased risk of developing decreased bone mineral density; and when the subject is heterozygous or homozygous for the KREMEN1 variant nucleic acid molecule encoding the KREMEN1 predicted

loss-of-function polypeptide, then the subject has a decreased risk of developing decreased bone mineral density.

The present disclosure also provides therapeutic agents that treat or prevent decreased bone mineral density for use in the treatment or prevention of decreased bone mineral density in a subject having: a KREMEN1 variant genomic nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide; a KREMEN1 variant mRNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; or a KREMEN1 variant cDNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide.

Disclosed herein is the use of a therapeutic agent that treats decreased bone mineral density in the preparation of a medicament for use in treating decreased bone mineral density in a subject having: a KREMEN1 variant genomic nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide; a KREMEN1 variant mRNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; or a KREMEN1 variant cDNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; and wherein the therapeutic agent is chosen from alendronate, ibandronate, zoledronate, risedronate, calcitonin, teriparatide, denosumab, estrogen and progesterone, raloxifene, or any combination thereof.

The present disclosure also provides KREMEN1 inhibitors for use in the treatment or prevention of decreased bone mineral density in a subject that: a) is reference for a KREMEN1 genomic nucleic acid molecule, a KREMEN1 mRNA molecule, or a KREMEN1 cDNA molecule; or b) is heterozygous for: i) a KREMEN1 variant genomic nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide; ii) a KREMEN1 variant mRNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; or iii) a KREMEN1 variant cDNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide.

Disclosed herein is the use of a KREMEN1 inhibitor in the preparation of a medicament for treating decreased bone mineral density in a subject that: a) is reference for a KREMEN1 genomic nucleic acid molecule, a KREMEN1 mRNA molecule, or a KREMEN1 cDNA molecule; or b) is heterozygous for: i) a KREMEN1 variant genomic nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide; ii) a KREMEN1 variant mRNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; or iii) a KREMEN1 variant cDNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; and wherein the KREMEN1 inhibitor comprises an inhibitory nucleic acid molecule that hybridizes to a KREMEN1

nucleic acid molecule, and wherein the inhibitory nucleic acid molecule comprises an antisense nucleic acid molecule, a small interfering RNA (siRNA), or a short hairpin RNA (shRNA).

Brief Description Of The Drawings

The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several features of the present disclosure.

Figure 1 shows an association of rare predicted loss-of-function (pLoF) and predicted deleterious missense variants in KREMEN1 with higher estimated bone mineral density (eBMD). Estimates of association for the burden of KREMEN1 pLoF or predicted deleterious missense variants with AAF < 1%, as derived in United Kingdom Biobank (UKB). Missense variants were predicted to be deleterious by five out five in silico algorithms (see Genotype Data for description of *in silico* algorithms used to characterize variant deleteriousness). Genotype counts indicates the number of individuals in each of three genotype categories: RR indicates individuals carrying no rare pLoF or predicted deleterious missense variants in KREMEN1; RA indicates individuals carrying a rare pLoF or predicted deleterious missense variant in a single KREMEN1 allele; AA indicates individuals carrying rare pLoF or predicted deleterious missense variants in both KREMEN1 alleles. AAF indicates the alternative allele frequency of variants included in this analysis. g/cm^2, grams per centimeter squared; SD, standard deviation; CI, confidence interval.

Figure 2 shows an association of rare pLoF variants in KREMEN1 with higher eBMD. Estimates of association pertain to the burden of KREMEN1 pLoF variants with AAF <1% and were derived in UKB. Genotype counts indicates the number of individuals in each of three genotype categories: RR indicates individuals carrying no rare pLoF variants in KREMEN1; RA indicates individuals carrying at least one rare pLoF in a single KREMEN1 allele; AA indicates individuals carrying any rare pLoF variants in both KREMEN1 alleles. AAF, alternative allele frequency of variants included in this analysis. g/cm^2, grams per centimeter squared; SD, standard deviation; CI, confidence interval.

Figure 3 shows KREMEN1 pLoF or predicted deleterious missense variants identified by whole-exome sequencing (WES) and included in the gene burden association analysis. The genomic coordinates column indicates the chromosome, physical genomic position in base pairs, reference allele, and alternative allele for each variant, according to build 38 of the

Human Genome sequence by the Human Genome Reference Consortium. Coding DNA and protein changes are provided according to the Human Genome Variation Society nomenclature, and refer to the KREMEN1 transcripts shown in the "Transcript(s)" column. Transcripts were sourced from the Ensembl database (Howe et al., Nuc. Acids Res., 2020, 49(D1), D884-D891). AAF, alternative allele frequency of variants included in this analysis; pLoF, predicted loss-of-function.

Description

Various terms relating to aspects of the present disclosure are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art, unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

Unless otherwise expressly stated, it is in no way intended that any method or aspect set forth herein be construed as requiring that its steps be performed in a specific order. Accordingly, where a method claim does not specifically state in the claims or descriptions that the steps are to be limited to a specific order, it is in no way intended that an order be inferred, in any respect. This holds for any possible non-expressed basis for interpretation, including matters of logic with respect to arrangement of steps or operational flow, plain meaning derived from grammatical organization or punctuation, or the number or type of aspects described in the specification.

As used herein, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

As used herein, the term "about" means that the recited numerical value is approximate and small variations would not significantly affect the practice of the disclosed embodiments. Where a numerical value is used, unless indicated otherwise by the context, the term "about" means the numerical value can vary by ±10% and remain within the scope of the disclosed embodiments.

As used herein, the term "comprising" may be replaced with "consisting" or "consisting essentially of" in particular embodiments as desired.

As used herein, the term "isolated", in regard to a nucleic acid molecule or a polypeptide, means that the nucleic acid molecule or polypeptide is in a condition other than its native environment, such as apart from blood and/or animal tissue. In some embodiments, an

isolated nucleic acid molecule or polypeptide is substantially free of other nucleic acid molecules or other polypeptides, particularly other nucleic acid molecules or polypeptides of animal origin. In some embodiments, the nucleic acid molecule or polypeptide can be in a highly purified form, i.e., greater than 95% pure or greater than 99% pure. When used in this context, the term “isolated” does not exclude the presence of the same nucleic acid molecule or polypeptide in alternative physical forms, such as dimers or Alternately phosphorylated or derivatized forms.

As used herein, the terms “nucleic acid”, “nucleic acid molecule”, “nucleic acid sequence”, “polynucleotide”, or “oligonucleotide” can comprise a polymeric form of nucleotides of any length, can comprise DNA and/or RNA, and can be single-stranded, double-stranded, or multiple stranded. One strand of a nucleic acid also refers to its complement.

As used herein, the term “subject” includes any animal, including mammals. Mammals include, but are not limited to, farm animals (such as, for example, horse, cow, pig), companion animals (such as, for example, dog, cat), laboratory animals (such as, for example, mouse, rat, rabbits), and non-human primates. In some embodiments, the subject is a human. In some embodiments, the human is a patient under the care of a physician.

It has been observed in accordance with the present disclosure that KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide (whether these variations are homozygous or heterozygous in a particular subject) associate with a decreased risk of developing decreased bone mineral density. It is believed that KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide have not been associated with decreased bone mineral density in genome-wide or exome-wide association studies. Therefore, subjects that are KREMEN1 reference or heterozygous for KREMEN1 variant nucleic acid molecules encoding KREMEN1 predicted loss-of-function polypeptides may be treated with a KREMEN1 inhibitor such that decreased bone mineral density is inhibited, the symptoms thereof are reduced, and/or development of symptoms is repressed. It is also believed that such subjects having decreased bone mineral density may further be treated with therapeutic agents that treat or prevent decreased bone mineral density.

For purposes of the present disclosure, any particular subject, such as a human, can be categorized as having one of three KREMEN1 genotypes: i) KREMEN1 reference; ii) heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted

loss-of-function polypeptide; or iii) homozygous for a KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide. A subject is KREMEN1 reference when the subject does not have a copy of a KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide. A subject is heterozygous for a KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide when the subject has a single copy of a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide. A KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide is any nucleic acid molecule (such as, a genomic nucleic acid molecule, an mRNA molecule, or a cDNA molecule) encoding a variant KREMEN1 polypeptide having a partial loss-of-function, a complete loss-of-function, a predicted partial loss-of-function, or a predicted complete loss-of-function. A subject who has a KREMEN1 polypeptide having a partial loss-of-function (or predicted partial loss-of-function) is hypomorphic for KREMEN1. A subject is homozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide when the subject has two copies (same or different) of a KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide.

For subjects that are genotyped or determined to be KREMEN1 reference, such subjects have an increased risk of developing decreased bone mineral density, such as osteopenia, Type I osteoporosis, Type II osteoporosis, and/or secondary osteoporosis. For subjects that are genotyped or determined to be either KREMEN1 reference or heterozygous for a KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide, such subjects or subjects can be treated with a KREMEN1 inhibitor.

In any of the embodiments described herein, the KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide can be any nucleic acid molecule (such as, for example, genomic nucleic acid molecule, mRNA molecule, or cDNA molecule) encoding a KREMEN1 variant polypeptide having a partial loss-of-function, a complete loss-of-function, a predicted partial loss-of-function, or a predicted complete loss-of-function. In some embodiments, the KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide is associated with a reduced *in vitro* response to KREMEN1 ligands compared with reference KREMEN1. In some embodiments, the KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide is a KREMEN1 variant that results or is predicted to result in a premature truncation of a KREMEN1

polypeptide compared to the human reference genome sequence. In some embodiments, the KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide is a variant that is predicted to be damaging by *in vitro* prediction algorithms such as Polyphen, SIFT, or similar algorithms. In some embodiments, the KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide is a variant that causes or is predicted to cause a nonsynonymous amino-acid substitution in KREMEN1 and whose allele frequency is less than 1/100 alleles in the population from which the subject is selected. In some embodiments, the KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide is any rare variant (allele frequency < 0.1%; or 1 in 1,000 alleles), or any splice-site, stop-gain, start-loss, stop-loss, frameshift, or in-frame indel, or other frameshift KREMEN1 variant.

In any of the embodiments described herein, the KREMEN1 predicted loss-of-function polypeptide can be any KREMEN1 polypeptide having a partial loss-of-function, a complete loss-of-function, a predicted partial loss-of-function, or a predicted complete loss-of-function.

In any of the embodiments described herein, the KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide can include variations at positions of chromosome 22 using the nucleotide sequence of the KREMEN1 reference genomic nucleic acid molecule (SEQ ID NO:1; ENSG00000183762.13; ENST00000327813.9; chr22:29073118-29168333 in the GRCh38/hg38 human genome assembly; alternately, chr22:29073035-29168333 or chr22:29073077-29168333) as a reference sequence.

Numerous genetic variants in KREMEN1 exist which cause subsequent changes in the KREMEN1 polypeptide sequence including, but not limited to the variants listed in Figure 3, Table 2, or otherwise listed herein.

Any one or more (i.e., any combination) of the KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide can be used within any of the methods described herein to determine whether a subject has an increased risk of developing decreased bone mineral density. The combinations of particular variants can form a mask used for statistical analysis of the particular correlation of KREMEN1 and increased risk of developing decreased bone mineral density.

In any of the embodiments described herein, the decreased bone mineral density is osteopenia, Type I osteoporosis, Type II osteoporosis, and/or secondary osteoporosis. In some embodiments, the decreased bone mineral density is osteopenia. In some embodiments, the

decreased bone mineral density is Type I osteoporosis. In some embodiments, the decreased bone mineral density is Type II osteoporosis. In some embodiments, the decreased bone mineral density is secondary osteoporosis.

Symptoms of a decreased bone mineral density include, but are not limited to, increased bone fragility (manifesting as bone fracture as a result of a mild to moderate trauma), reduced bone density, localized bone pain and weakness in an area of a broken bone, loss of height or change in posture, such as stooping over, high levels of serum calcium or alkaline phosphatase on a blood test, vitamin D deficiency, and joint or muscle aches, or any combination thereof.

The present disclosure provides methods of treating a subject having decreased bone mineral density or at risk of developing decreased bone mineral density, the methods comprising administering a KREMEN1 inhibitor to the subject.

The present disclosure also provides methods of treating a subject having osteopenia or at risk of developing osteopenia, the methods comprising administering a KREMEN1 inhibitor to the subject.

The present disclosure also provides methods of treating a subject having Type I osteoporosis or at risk of developing Type I osteoporosis, the methods comprising administering a KREMEN1 inhibitor to the subject.

The present disclosure also provides methods of treating a subject having Type II osteoporosis or at risk of developing Type II osteoporosis, the methods comprising administering a KREMEN1 to the subject.

The present disclosure also provides methods of treating a subject having secondary osteoporosis or at risk of developing secondary osteoporosis, the methods comprising administering a KREMEN1 inhibitor to the subject.

In some embodiments, the KREMEN1 inhibitor comprises an inhibitory nucleic acid molecule. Examples of inhibitory nucleic acid molecules include, but are not limited to, antisense nucleic acid molecules, small interfering RNAs (siRNAs), and short hairpin RNAs (shRNAs). Such inhibitory nucleic acid molecules can be designed to target any region of a KREMEN1 nucleic acid molecule. In some embodiments, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within a KREMEN1 genomic nucleic acid molecule or mRNA molecule and decreases expression of the KREMEN1 polypeptide in a cell in the subject. In some embodiments, the KREMEN1 inhibitor comprises an antisense molecule that hybridizes to a

KREMEN1 genomic nucleic acid molecule or mRNA molecule and decreases expression of the KREMEN1 polypeptide in a cell in the subject. In some embodiments, the KREMEN1 inhibitor comprises an siRNA that hybridizes to a KREMEN1 genomic nucleic acid molecule or mRNA molecule and decreases expression of the KREMEN1 polypeptide in a cell in the subject. In some embodiments, the KREMEN1 inhibitor comprises an shRNA that hybridizes to a KREMEN1 genomic nucleic acid molecule or mRNA molecule and decreases expression of the KREMEN1 polypeptide in a cell in the subject.

The inhibitory nucleic acid molecules can comprise RNA, DNA, or both RNA and DNA. The inhibitory nucleic acid molecules can also be linked or fused to a heterologous nucleic acid sequence, such as in a vector, or a heterologous label. For example, the inhibitory nucleic acid molecules can be within a vector or as an exogenous donor sequence comprising the inhibitory nucleic acid molecule and a heterologous nucleic acid sequence. The inhibitory nucleic acid molecules can also be linked or fused to a heterologous label. The label can be directly detectable (such as, for example, fluorophore) or indirectly detectable (such as, for example, hapten, enzyme, or fluorophore quencher). Such labels can be detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. Such labels include, for example, radiolabels, pigments, dyes, chromogens, spin labels, and fluorescent labels. The label can also be, for example, a chemiluminescent substance; a metal-containing substance; or an enzyme, where there occurs an enzyme-dependent secondary generation of signal. The term "label" can also refer to a "tag" or hapten that can bind selectively to a conjugated molecule such that the conjugated molecule, when added subsequently along with a substrate, is used to generate a detectable signal. For example, biotin can be used as a tag along with an avidin or streptavidin conjugate of horseradish peroxidate (HRP) to bind to the tag, and examined using a calorimetric substrate (such as, for example, tetramethylbenzidine (TMB)) or a fluorogenic substrate to detect the presence of HRP. Exemplary labels that can be used as tags to facilitate purification include, but are not limited to, myc, HA, FLAG or 3XFLAG, 6XHis or polyhistidine, glutathione-S-transferase (GST), maltose binding protein, an epitope tag, or the Fc portion of immunoglobulin. Numerous labels include, for example, particles, fluorophores, haptens, enzymes and their calorimetric, fluorogenic and chemiluminescent substrates and other labels.

The inhibitory nucleic acid molecules can comprise, for example, nucleotides or non-natural or modified nucleotides, such as nucleotide analogs or nucleotide substitutes. Such nucleotides include a nucleotide that contains a modified base, sugar, or phosphate group, or

that incorporates a non-natural moiety in its structure. Examples of non-natural nucleotides include, but are not limited to, dideoxynucleotides, biotinylated, aminated, deaminated, alkylated, benzylated, and fluorophor-labeled nucleotides.

The inhibitory nucleic acid molecules can also comprise one or more nucleotide analogs or substitutions. A nucleotide analog is a nucleotide which contains a modification to either the base, sugar, or phosphate moieties. Modifications to the base moiety include, but are not limited to, natural and synthetic modifications of A, C, G, and T/U, as well as different purine or pyrimidine bases such as, for example, pseudouridine, uracil-5-yl, hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl. Modified bases include, but are not limited to, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo (such as, for example, 5-bromo), 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine, 7-methyladenine, 8-azaguanine, 8-azaadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, and 3-deazaadenine.

Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety include, but are not limited to, natural modifications of the ribose and deoxy ribose as well as synthetic modifications. Sugar modifications include, but are not limited to, the following modifications at the 2’ position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl, and alkynyl may be substituted or unsubstituted C_{1-10}alkyl or C_{2-10}alkenyl, and C_{2-10}alkynyl. Exemplary 2’ sugar modifications also include, but are not limited to, $-O[(CH_2)_nO]_mCH_3$, $-O(CH_2)_nOCH_3$, $-O(CH_2)_nNH_2$, $-O(CH_2)_nCH_3$, $-O(CH_2)_n$-ONH_2, and $-O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m, independently, are from 1 to about 10. Other modifications at the 2’ position include, but are not limited to, C_{1-10}alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar modifications may also be made at other

positions on the sugar, particularly the 3’ position of the sugar on the 3’ terminal nucleotide or in 2’-5’ linked oligonucleotides and the 5’ position of 5’ terminal nucleotide. Modified sugars can also include those that contain modifications at the bridging ring oxygen, such as CH_2 and S. Nucleotide sugar analogs can also have sugar mimetics, such as cyclobutyl moieties in place of the pentofuranosyl sugar.

Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include, but are not limited to, those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3’-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3’-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. These phosphate or modified phosphate linkage between two nucleotides can be through a 3’-5’ linkage or a 2’-5’ linkage, and the linkage can contain inverted polarity such as 3’-5’ to 5’-3’ or 2’-5’ to 5’-2’. Various salts, mixed salts, and free acid forms are also included. Nucleotide substitutes also include peptide nucleic acids (PNAs).

In some embodiments, the antisense nucleic acid molecules are gapmers, whereby the first one to seven nucleotides at the 5’ and 3’ ends each have 2’-methoxyethyl (2’-MOE) modifications. In some embodiments, the first five nucleotides at the 5’ and 3’ ends each have 2’-MOE modifications. In some embodiments, the first one to seven nucleotides at the 5’ and 3’ ends are RNA nucleotides. In some embodiments, the first five nucleotides at the 5’ and 3’ ends are RNA nucleotides. In some embodiments, each of the backbone linkages between the nucleotides is a phosphorothioate linkage.

In some embodiments, the siRNA molecules have termini modifications. In some embodiments, the 5’ end of the antisense strand is phosphorylated. In some embodiments, 5’-phosphate analogs that cannot be hydrolyzed, such as 5’-(E)-vinyl-phosphonate are used.

In some embodiments, the siRNA molecules have backbone modifications. In some embodiments, the modified phosphodiester groups that link consecutive ribose nucleosides have been shown to enhance the stability and *in vivo* bioavailability of siRNAs The non-ester groups (-OH, =O) of the phosphodiester linkage can be replaced with sulfur, boron, or acetate to give phosphorothioate, boranophosphate, and phosphonoacetate linkages. In addition, substituting the phosphodiester group with a phosphotriester can facilitate cellular uptake of

siRNAs and retention on serum components by eliminating their negative charge. In some embodiments, the siRNA molecules have sugar modifications. In some embodiments, the sugars are deprotonated (reaction catalyzed by exo- and endonucleases) whereby the 2'-hydroxyl can act as a nucleophile and attack the adjacent phosphorous in the phosphodiester bond. Such alternatives include 2'-O-methyl, 2'-O-methoxyethyl, and 2'-fluoro modifications.

In some embodiments, the siRNA molecules have base modifications. In some embodiments, the bases can be substituted with modified bases such as pseudouridine, 5'-methylcytidine, N6-methyladenosine, inosine, and N7-methylguanosine.

In some embodiments, the siRNA molecules are conjugated to lipids. Lipids can be conjugated to the 5' or 3' termini of siRNA to improve their *in vivo* bioavailability by allowing them to associate with serum lipoproteins. Representative lipids include, but are not limited to, cholesterol and vitamin E, and fatty acids, such as palmitate and tocopherol.

In some embodiments, a representative siRNA has the following formula:

Sense: mN*mN*/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/
i2FN/*mN*/32FN/

Antisense: /52FN/*/i2FN/*mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/
i2FN/mN/i2FN/mN*N*N

wherein: "N" is the base; "2F" is a 2'-F modification; "m" is a 2'-O-methyl modification, "I" is an internal base; and "*" is a phosphorothioate backbone linkage.

The present disclosure also provides vectors comprising any one or more of the inhibitory nucleic acid molecules. In some embodiments, the vectors comprise any one or more of the inhibitory nucleic acid molecules and a heterologous nucleic acid. The vectors can be viral or nonviral vectors capable of transporting a nucleic acid molecule. In some embodiments, the vector is a plasmid or cosmid (such as, for example, a circular double-stranded DNA into which additional DNA segments can be ligated). In some embodiments, the vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Expression vectors include, but are not limited to, plasmids, cosmids, retroviruses, adenoviruses, adeno-associated viruses (AAV), plant viruses such as cauliflower mosaic virus and tobacco mosaic virus, yeast artificial chromosomes (YACs), Epstein-Barr (EBV)-derived episomes, and other expression vectors known in the art.

The present disclosure also provides compositions comprising any one or more of the inhibitory nucleic acid molecules. In some embodiments, the composition is a pharmaceutical

composition. In some embodiments, the compositions comprise a carrier and/or excipient. Examples of carriers include, but are not limited to, poly(lactic acid) (PLA) microspheres, poly(D,L-lactic-coglycolic-acid) (PLGA) microspheres, liposomes, micelles, inverse micelles, lipid cochleates, and lipid microtubules. A carrier may comprise a buffered salt solution such as PBS, HBSS, etc.

Exemplary KREMEN1 inhibitors include, but are not limited to, KREMEN2 (Sumia et al., Cell Death Discovery, 2019, 5, 91) and its ligand Dickkopf-1 (DKK-1), a secreted glycoprotein, as well as R-Spondin1.

In some embodiments, the KREMEN1 inhibitor comprises a nuclease agent that induces one or more nicks or double-strand breaks at a recognition sequence(s) or a DNA-binding protein that binds to a recognition sequence within a KREMEN1 genomic nucleic acid molecule. The recognition sequence can be located within a coding region of the KREMEN1 gene, or within regulatory regions that influence the expression of the gene. A recognition sequence of the DNA-binding protein or nuclease agent can be located in an intron, an exon, a promoter, an enhancer, a regulatory region, or any non-protein coding region. The recognition sequence can include or be proximate to the start codon of the KREMEN1 gene. For example, the recognition sequence can be located about 10, about 20, about 30, about 40, about 50, about 100, about 200, about 300, about 400, about 500, or about 1,000 nucleotides from the start codon. As another example, two or more nuclease agents can be used, each targeting a nuclease recognition sequence including or proximate to the start codon. As another example, two nuclease agents can be used, one targeting a nuclease recognition sequence including or proximate to the start codon, and one targeting a nuclease recognition sequence including or proximate to the stop codon, wherein cleavage by the nuclease agents can result in deletion of the coding region between the two nuclease recognition sequences. Any nuclease agent that induces a nick or double-strand break into a desired recognition sequence can be used in the methods and compositions disclosed herein. Any DNA-binding protein that binds to a desired recognition sequence can be used in the methods and compositions disclosed herein.

Suitable nuclease agents and DNA-binding proteins for use herein include, but are not limited to, zinc finger protein or zinc finger nuclease (ZFN) pair, Transcription Activator-Like Effector (TALE) protein or Transcription Activator-Like Effector Nuclease (TALEN), or Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)/CRISPR-associated (Cas) systems. The length of the recognition sequence can vary, and includes, for example, recognition

sequences that are about 30-36 bp for a zinc finger protein or ZFN pair, about 15-18 bp for each ZFN, about 36 bp for a TALE protein or TALEN, and about 20 bp for a CRISPR/Cas guide RNA.

In some embodiments, CRISPR/Cas systems can be used to modify a KREMEN1 genomic nucleic acid molecule within a cell. The methods and compositions disclosed herein can employ CRISPR-Cas systems by utilizing CRISPR complexes (comprising a guide RNA (gRNA) complexed with a Cas protein) for site-directed cleavage of KREMEN1 nucleic acid molecules.

Cas proteins generally comprise at least one RNA recognition or binding domain that can interact with gRNAs. Cas proteins can also comprise nuclease domains (such as, for example, DNase or RNase domains), DNA binding domains, helicase domains, protein-protein interaction domains, dimerization domains, and other domains. Suitable Cas proteins include, for example, a wild type Cas9 protein and a wild type Cpf1 protein (such as, for example, FnCpf1). A Cas protein can have full cleavage activity to create a double-strand break in a KREMEN1 genomic nucleic acid molecule or it can be a nickase that creates a single-strand break in a KREMEN1 genomic nucleic acid molecule. Additional examples of Cas proteins include, but are not limited to, Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5e (CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9 (Csn1 or Csx12), Cas10, Cas10d, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (CasA), Cse2 (CasB), Cse3 (CasE), Cse4 (CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966, and homologs or modified versions thereof. Cas proteins can also be operably linked to heterologous polypeptides as fusion proteins. For example, a Cas protein can be fused to a cleavage domain, an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. Cas proteins can be provided in any form. For example, a Cas protein can be provided in the form of a protein, such as a Cas protein complexed with a gRNA. Alternately, a Cas protein can be provided in the form of a nucleic acid molecule encoding the Cas protein, such as an RNA or DNA.

In some embodiments, targeted genetic modifications of KREMEN1 genomic nucleic acid molecules can be generated by contacting a cell with a Cas protein and one or more gRNAs that hybridize to one or more gRNA recognition sequences within a target genomic locus in the KREMEN1 genomic nucleic acid molecule. For example, a gRNA recognition sequence can be located within a region of SEQ ID NO:1. The gRNA recognition sequence can include or be proximate to the start codon of a KREMEN1 genomic nucleic acid molecule or the stop codon of

a KREMEN1 genomic nucleic acid molecule. For example, the gRNA recognition sequence can be located from about 10, from about 20, from about 30, from about 40, from about 50, from about 100, from about 200, from about 300, from about 400, from about 500, or from about 1,000 nucleotides of the start codon or the stop codon.

The gRNA recognition sequences within a target genomic locus in a KREMEN1 genomic nucleic acid molecule are located near a Protospacer Adjacent Motif (PAM) sequence, which is a 2-6 base pair DNA sequence immediately following the DNA sequence targeted by the Cas9 nuclease. The canonical PAM is the sequence 5'-NGG-3' where “N” is any nucleobase followed by two guanine (“G”) nucleobases. gRNAs can transport Cas9 to anywhere in the genome for gene editing, but no editing can occur at any site other than one at which Cas9 recognizes PAM. In addition, 5'-NGA-3' can be a highly efficient non-canonical PAM for human cells. Generally, the PAM is about 2-6 nucleotides downstream of the DNA sequence targeted by the gRNA. The PAM can flank the gRNA recognition sequence. In some embodiments, the gRNA recognition sequence can be flanked on the 3’ end by the PAM. In some embodiments, the gRNA recognition sequence can be flanked on the 5’ end by the PAM. For example, the cleavage site of Cas proteins can be about 1 to about 10, about 2 to about 5 base pairs, or three base pairs upstream or downstream of the PAM sequence. In some embodiments (such as when Cas9 from *S. pyogenes* or a closely related Cas9 is used), the PAM sequence of the non-complementary strand can be 5'-NGG-3', where N is any DNA nucleotide and is immediately 3' of the gRNA recognition sequence of the non-complementary strand of the target DNA. As such, the PAM sequence of the complementary strand would be 5'-CCN-3', where N is any DNA nucleotide and is immediately 5' of the gRNA recognition sequence of the complementary strand of the target DNA.

A gRNA is an RNA molecule that binds to a Cas protein and targets the Cas protein to a specific location within a KREMEN1 genomic nucleic acid molecule. An exemplary gRNA is a gRNA effective to direct a Cas enzyme to bind to or cleave a KREMEN1 genomic nucleic acid molecule, wherein the gRNA comprises a DNA-targeting segment that hybridizes to a gRNA recognition sequence within the KREMEN1 genomic nucleic acid molecule. Exemplary gRNAs comprise a DNA-targeting segment that hybridizes to a gRNA recognition sequence present within a KREMEN1 genomic nucleic acid molecule that includes or is proximate to the start codon or the stop codon. For example, a gRNA can be selected such that it hybridizes to a gRNA recognition sequence that is located from about 5, from about 10, from about 15, from about

20, from about 25, from about 30, from about 35, from about 40, from about 45, from about 50, from about 100, from about 200, from about 300, from about 400, from about 500, or from about 1,000 nucleotides of the start codon or located from about 5, from about 10, from about 15, from about 20, from about 25, from about 30, from about 35, from about 40, from about 45, from about 50, from about 100, from about 200, from about 300, from about 400, from about 500, or from about 1,000 nucleotides of the stop codon. Suitable gRNAs can comprise from about 17 to about 25 nucleotides, from about 17 to about 23 nucleotides, from about 18 to about 22 nucleotides, or from about 19 to about 21 nucleotides. In some embodiments, the gRNAs can comprise 20 nucleotides.

Examples of suitable gRNA recognition sequences located within the human KREMEN1 reference gene are set forth in Table 1 as SEQ ID NOs:17-36.

Table 1: Guide RNA Recognition Sequences Near KREMEN1

Strand	gRNA Recognition Sequence	SEQ ID NO:
+	CACTCTGAAATACCCCAACG	17
+	TGATTACTGGAAGTACGGGG	18
+	TCCCCGACACCTATGCCACG	19
+	AACTGGACAGCACTACAAGG	20
-	GGAAGCGGGCTAGGACACGG	21
+	TCTGAAATACCCCAACGGGG	22
+	CTTCCCCGACACCTATGCCA	23
-	GTCTCGTTCCAAAACAGACA	24
-	GAAGCGGGCTAGGACACGGT	25
+	TCCTGATTACTGGAAGTACG	26
+	AACACTCTGAAATACCCCAA	27
+	CCGACACCTATGCCACGGGG	28
+	ACTGGACCATCCGGGTTCCG	29
+	GCCAATGGTGCGGATTATAG	30
-	GTATAAAACAGCAAATCCCT	31
+	ACTGGACAGCACTACAAGGC	32
+	CGTGAGCCCCTGGTGCTATG	33
-	GGGCATACTCACTATCAAAG	34

-	TTACTGGTGCCAGTTAGAGG	35
+	GTGCTATGTGGCAGAGCACG	36

The Cas protein and the gRNA form a complex, and the Cas protein cleaves the target KREMEN1 genomic nucleic acid molecule. The Cas protein can cleave the nucleic acid molecule at a site within or outside of the nucleic acid sequence present in the target KREMEN1 genomic nucleic acid molecule to which the DNA-targeting segment of a gRNA will bind. For example, formation of a CRISPR complex (comprising a gRNA hybridized to a gRNA recognition sequence and complexed with a Cas protein) can result in cleavage of one or both strands in or near (such as, for example, within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or more base pairs from) the nucleic acid sequence present in the KREMEN1 genomic nucleic acid molecule to which a DNA-targeting segment of a gRNA will bind.

Such methods can result, for example, in a KREMEN1 genomic nucleic acid molecule in which a region of SEQ ID NO:1 is disrupted, the start codon is disrupted, the stop codon is disrupted, or the coding sequence is disrupted or deleted. Optionally, the cell can be further contacted with one or more additional gRNAs that hybridize to additional gRNA recognition sequences within the target genomic locus in the KREMEN1 genomic nucleic acid molecule. By contacting the cell with one or more additional gRNAs (such as, for example, a second gRNA that hybridizes to a second gRNA recognition sequence), cleavage by the Cas protein can create two or more double-strand breaks or two or more single-strand breaks.

In some embodiments, the methods of treatment further comprise detecting the presence or absence of a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide in a biological sample from the subject. As used throughout the present disclosure, a “KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide” is any KREMEN1 nucleic acid molecule (such as, for example, genomic nucleic acid molecule, mRNA molecule, or cDNA molecule) encoding a KREMEN1 polypeptide having a partial loss-of-function, a complete loss-of-function, a predicted partial loss-of-function, or a predicted complete loss-of-function.

The present disclosure also provides methods of treating a subject with a therapeutic agent that treats or prevents decreased bone mineral density, wherein the subject has decreased bone mineral density or is at risk of developing decreased bone mineral density. In some embodiments, the subject has decreased bone mineral density. In some embodiments,

the subject is at risk of developing decreased bone mineral density. In some embodiments, the methods comprise determining whether the subject has a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide by obtaining or having obtained a biological sample from the subject, and performing or having performed a sequence analysis on the biological sample to determine if the subject has a genotype comprising the KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide. In some embodiments, the methods further comprise administering or continuing to administer the therapeutic agent that treats or prevents decreased bone mineral density in a standard dosage amount to a subject that is KREMEN1 reference, and/or administering a KREMEN1 inhibitor to the subject. In some embodiments, the methods further comprise administering or continuing to administer the therapeutic agent that treats or prevents decreased bone mineral density in an amount that is the same as or less than a standard dosage amount to a subject that is heterozygous for the KREMEN1 variant nucleic acid molecule, and/or administering a KREMEN1 inhibitor to the subject. In some embodiments, the methods further comprise administering or continuing to administer the therapeutic agent that treats or prevents decreased bone mineral density in an amount that is the same as or less than a standard dosage amount to a subject that is homozygous for the KREMEN1 variant nucleic acid molecule. The presence of a genotype having the KREMEN1 variant nucleic acid molecule encoding the KREMEN1 predicted loss-of-function polypeptide indicates the subject has a decreased risk of developing decreased bone mineral density. In some embodiments, the subject is KREMEN1 reference. In some embodiments, the subject is heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide.

For subjects that are genotyped or determined to be either KREMEN1 reference or heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide, such subjects can be treated with a KREMEN1 inhibitor, as described herein.

Detecting the presence or absence of a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide in a biological sample from a subject and/or determining whether a subject has a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide can be carried out by any of the methods described herein. In some embodiments, these methods can be carried out *in vitro*. In

some embodiments, these methods can be carried out *in situ*. In some embodiments, these methods can be carried out *in vivo*. In any of these embodiments, the nucleic acid molecule can be present within a cell obtained from the subject.

In some embodiments, when the subject is KREMEN1 reference, the subject is administered a therapeutic agent that treats or prevents decreased bone mineral density in a standard dosage amount. In some embodiments, when the subject is heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide, the subject is administered a therapeutic agent that treats or prevents decreased bone mineral density in a dosage amount that is the same as or less than a standard dosage amount.

In some embodiments, the treatment methods further comprise detecting the presence or absence of a KREMEN1 predicted loss-of-function polypeptide in a biological sample from the subject. In some embodiments, when the subject does not have a KREMEN1 predicted loss-of-function polypeptide, the subject is administered a therapeutic agent that treats or prevents decreased bone mineral density in a standard dosage amount. In some embodiments, when the subject has a KREMEN1 predicted loss-of-function polypeptide, the subject is administered a therapeutic agent that treats or prevents decreased bone mineral density in a dosage amount that is the same as or less than a standard dosage amount.

The present disclosure also provides methods of treating a subject with a therapeutic agent that treats or prevents decreased bone mineral density, wherein the subject has decreased bone mineral density or is at risk of developing decreased bone mineral density. In some embodiments, the subject has decreased bone mineral density. In some embodiments, the subject is at risk of developing decreased bone mineral density. In some embodiments, the method comprises determining whether the subject has a KREMEN1 predicted loss-of-function polypeptide by obtaining or having obtained a biological sample from the subject, and performing or having performed an assay on the biological sample to determine if the subject has a KREMEN1 predicted loss-of-function polypeptide. When the subject does not have a KREMEN1 predicted loss-of-function polypeptide, the therapeutic agent that treats or prevents decreased bone mineral density is administered or continued to be administered to the subject in a standard dosage amount, and/or a KREMEN1 inhibitor is administered to the subject. When the subject has a KREMEN1 predicted loss-of-function polypeptide, the therapeutic agent that treats or prevents decreased bone mineral density is administered or continued to be

administered to the subject in an amount that is the same as or less than a standard dosage amount, and/or a KREMEN1 inhibitor is administered to the subject. The presence of a KREMEN1 predicted loss-of-function polypeptide indicates the subject has a decreased risk of developing decreased bone mineral density. In some embodiments, the subject has a KREMEN1 predicted loss-of-function polypeptide. In some embodiments, the subject does not have a KREMEN1 predicted loss-of-function polypeptide.

Detecting the presence or absence of a KREMEN1 predicted loss-of-function polypeptide in a biological sample from a subject and/or determining whether a subject has a KREMEN1 predicted loss-of-function polypeptide can be carried out by any of the methods described herein. In some embodiments, these methods can be carried out *in vitro*. In some embodiments, these methods can be carried out *in situ*. In some embodiments, these methods can be carried out *in vivo*. In any of these embodiments, the polypeptide can be present within a cell obtained from the subject.

Examples of therapeutic agents that treat or prevent decreased bone mineral density include, but are not limited to: calcium and vitamin D supplementation (vitamin D2, vitamin D3, and cholecalciferol), bisphosphonate medications, such as FOSAMAX®, (alendronate), BONIVA® (ibandronate), RECLAST® (zoledronate), ACTONEL® (risedronate), MIACALCIN®, FORTICAL®, and CALCIMAR® (calcitonin), FORTEO® (teriparatide), PROLIA® (denosumab), hormone replacement therapy with estrogen and progesterone as well as EVISTA® (raloxifene). In some embodiments, the therapeutic agent that treats or prevents decreased bone mineral density is vitamin D2, vitamin D3, cholecalciferol, alendronate, ibandronate, zoledronate, risedronate, calcitonin, teriparatide, denosumab, EVENITY® (romosozumab), or raloxifene. In some embodiments, the therapeutic agent that treats or prevents decreased bone mineral density is vitamin D2. In some embodiments, the therapeutic agent that treats or prevents decreased bone mineral density is vitamin D3. In some embodiments, the therapeutic agent that treats or prevents decreased bone mineral density is cholecalciferol. In some embodiments, the therapeutic agent that treats or prevents decreased bone mineral density is alendronate. In some embodiments, the therapeutic agent that treats or prevents decreased bone mineral density is ibandronate. In some embodiments, the therapeutic agent that treats or prevents decreased bone mineral density is zoledronate. In some embodiments, the therapeutic agent that treats or prevents decreased bone mineral density is risedronate. In some embodiments, the therapeutic agent that treats or prevents decreased bone mineral density is calcitonin. In some embodiments, the

therapeutic agent that treats or prevents decreased bone mineral density is teriparatide. In some embodiments, the therapeutic agent that treats or prevents decreased bone mineral density is denosumab. In some embodiments, the therapeutic agent that treats or prevents decreased bone mineral density is raloxifene.

In some embodiments, the dose of the therapeutic agents that treat or prevent decreased bone mineral density can be decreased by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, by about 60%, by about 70%, by about 80%, or by about 90% for subjects that are heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide (i.e., a less than the standard dosage amount) compared to subjects that are KREMEN1 reference (who may receive a standard dosage amount). In some embodiments, the dose of the therapeutic agents that treat or prevent decreased bone mineral density can be decreased by about 10%, by about 20%, by about 30%, by about 40%, or by about 50%. In addition, the subjects that are heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide can be administered less frequently compared to subjects that are KREMEN1 reference.

In some embodiments, the dose of the therapeutic agents that treat or prevent decreased bone mineral density can be decreased by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, for subjects that are homozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide compared to subjects that are heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide. In some embodiments, the dose of the therapeutic agents that treat or prevent decreased bone mineral density can be decreased by about 10%, by about 20%, by about 30%, by about 40%, or by about 50%. In addition, the dose of therapeutic agents that treat or prevent decreased bone mineral density in subjects that are homozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide can be administered less frequently compared to subjects that are heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide.

Administration of the therapeutic agents that treat or prevent decreased bone mineral density and/or KREMEN1 inhibitors can be repeated, for example, after one day, two days, three days, five days, one week, two weeks, three weeks, one month, five weeks, six weeks, seven weeks, eight weeks, two months, or three months. The repeated administration can be

at the same dose or at a different dose. The administration can be repeated once, twice, three times, four times, five times, six times, seven times, eight times, nine times, ten times, or more. For example, according to certain dosage regimens a subject can receive therapy for a prolonged period of time such as, for example, 6 months, 1 year, or more.

Administration of the therapeutic agents that treat or prevent decreased bone mineral density and/or KREMEN1 inhibitors can occur by any suitable route including, but not limited to, parenteral, intravenous, oral, subcutaneous, intra-arterial, intracranial, intrathecal, intraperitoneal, topical, intranasal, or intramuscular. Pharmaceutical compositions for administration are desirably sterile and substantially isotonic and manufactured under GMP conditions. Pharmaceutical compositions can be provided in unit dosage form (i.e., the dosage for a single administration). Pharmaceutical compositions can be formulated using one or more physiologically and pharmaceutically acceptable carriers, diluents, excipients or auxiliaries. The formulation depends on the route of administration chosen. The term “pharmaceutically acceptable” means that the carrier, diluent, excipient, or auxiliary is compatible with the other ingredients of the formulation and not substantially deleterious to the recipient thereof.

The terms “treat”, “treating”, and “treatment” and “prevent”, “preventing”, and “prevention” as used herein, refer to eliciting the desired biological response, such as a therapeutic and prophylactic effect, respectively. In some embodiments, a therapeutic effect comprises one or more of a decrease/reduction in decreased bone mineral density, a decrease/reduction in the severity of decreased bone mineral density (such as, for example, a reduction or inhibition of development of decreased bone mineral density), a decrease/reduction in symptoms and decreased bone mineral density-related effects, delaying the onset of symptoms and decreased bone mineral density-related effects, reducing the severity of symptoms of decreased bone mineral density-related effects, reducing the number of symptoms and decreased bone mineral density-related effects, reducing the latency of symptoms and decreased bone mineral density-related effects, an amelioration of symptoms and decreased bone mineral density-related effects, reducing secondary symptoms, reducing secondary infections, preventing relapse to decreased bone mineral density, decreasing the number or frequency of relapse episodes, increasing latency between symptomatic episodes, increasing time to sustained progression, speeding recovery, or increasing efficacy of or decreasing resistance to alternative therapeutics, and/or an increased survival time of the affected host animal, following administration of the agent or composition comprising the

agent. A prophylactic effect may comprise a complete or partial avoidance/inhibition or a delay of decreased bone mineral density development/progression (such as, for example, a complete or partial avoidance/inhibition or a delay), and an increased survival time of the affected host animal, following administration of a therapeutic protocol. Treatment of decreased bone mineral density encompasses the treatment of a subject already diagnosed as having any form of decreased bone mineral density at any clinical stage or manifestation, the delay of the onset or evolution or aggravation or deterioration of the symptoms or signs of decreased bone mineral density, and/or preventing and/or reducing the severity of decreased bone mineral density.

The present disclosure also provides methods of identifying a subject having an increased risk of developing decreased bone mineral density. In some embodiments, the method comprises determining or having determined in a biological sample obtained from the subject the presence or absence of a KREMEN1 variant nucleic acid molecule (such as a genomic nucleic acid molecule, mRNA molecule, and/or cDNA molecule) encoding a KREMEN1 predicted loss-of-function polypeptide encoding a KREMEN1 polypeptide. When the subject lacks a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide (i.e., the subject is genotypically categorized as a KREMEN1 reference), then the subject has an increased risk of developing decreased bone mineral density. When the subject has a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide (i.e., the subject is heterozygous or homozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide), then the subject has a decreased risk of developing decreased bone mineral density.

Having a single copy of a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide is more protective of a subject from developing decreased bone mineral density than having no copies of a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide. Without intending to be limited to any particular theory or mechanism of action, it is believed that a single copy of a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide (i.e., heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide) is protective of a subject from developing decreased bone mineral density, and it is also believed that having two copies of a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide (i.e.,

homozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide) may be more protective of a subject from developing decreased bone mineral density, relative to a subject with a single copy. Thus, in some embodiments, a single copy of a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide may not be completely protective, but instead, may be partially or incompletely protective of a subject from developing decreased bone mineral density. While not desiring to be bound by any particular theory, there may be additional factors or molecules involved in the development of decreased bone mineral density that are still present in a subject having a single copy of a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide, thus resulting in less than complete protection from the development of decreased bone mineral density.

Determining whether a subject has a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide in a biological sample from a subject and/or determining whether a subject has a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide can be carried out by any of the methods described herein. In some embodiments, these methods can be carried out *in vitro*. In some embodiments, these methods can be carried out *in situ*. In some embodiments, these methods can be carried out *in vivo*. In any of these embodiments, the nucleic acid molecule can be present within a cell obtained from the subject.

In some embodiments, when a subject is identified as having an increased risk of developing decreased bone mineral density, the subject is treated with a therapeutic agent that treats or prevents decreased bone mineral density, and/or a KREMEN1 inhibitor, as described herein. For example, when the subject is KREMEN1 reference, and therefore has an increased risk of developing decreased bone mineral density, the subject is administered a KREMEN1 inhibitor. In some embodiments, such a subject is also administered a therapeutic agent that treats or prevents decreased bone mineral density. In some embodiments, when the subject is heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide, the subject is administered the therapeutic agent that treats or prevents decreased bone mineral density in a dosage amount that is the same as or less than a standard dosage amount, and is also administered a KREMEN1 inhibitor. In some embodiments, such a subject is also administered a therapeutic agent that treats or prevents decreased bone mineral density. In some embodiments, when the subject is homozygous for a KREMEN1

variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide, the subject is administered the therapeutic agent that treats or prevents decreased bone mineral density in a dosage amount that is the same as or less than a standard dosage amount. In some embodiments, the subject is KREMEN1 reference. In some embodiments, the subject is heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide. In some embodiments, the subject is homozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide.

In some embodiments, any of the methods described herein can further comprise determining the subject's aggregate burden of having a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide, and/or a KREMEN1 predicted loss-of-function variant polypeptide associated with a decreased risk of developing decreased bone mineral density. The gene burden is the aggregate of all variants in the KREMEN1 gene, which can be carried out in an association analysis with bone mineral density. In some embodiments, the subject is homozygous for one or more KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide associated with a decreased risk of developing decreased bone mineral density. In some embodiments, the subject is heterozygous for one or more KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide associated with a decreased risk of developing decreased bone mineral density. The result of the association analysis suggests that KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide are associated with decreased risk of developing decreased bone mineral density. When the subject has a lower aggregate burden, the subject is at a higher risk of developing decreased bone mineral density and the subject is administered or continued to be administered the therapeutic agent that treats or prevents decreased bone mineral density in a standard dosage amount. When the subject has a greater aggregate burden, the subject is at a lower risk of developing decreased bone mineral density and the subject is administered or continued to be administered the therapeutic agent that treats or prevents decreased bone mineral density in an amount that is the same as or less than the standard dosage amount. The greater the aggregate burden, the lower the risk of developing decreased bone mineral density.

The KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide, and/or a KREMEN1 predicted loss-of-function variant polypeptide used

for determining the subject's aggregate burden include, but are not limited to the variants listed in Figure 3, Table 2, or otherwise listed herein.

In some embodiments, the subject's aggregate burden of having any one or more KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide represents a weighted sum of a plurality of any of the KREMEN1 variant nucleic acid molecules encoding a KREMEN1 predicted loss-of-function polypeptide. In some embodiments, the aggregate burden is calculated using at least about 2, at least about 3, at least about 4, at least about 5, at least about 10, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, at least about 70, at least about 80, at least about 100, at least about 120, at least about 150, at least about 200, at least about 250, at least about 300, at least about 400, at least about 500, at least about 1,000, at least about 10,000, at least about 100,000, or at least about or more than 1,000,000 genetic variants present in or around (up to 10 Mb) the KREMEN1 gene where the genetic burden is the number of alleles multiplied by the association estimate with decreased bone mineral density or related outcome for each allele (e.g., a weighted polygenic burden score). This can include any genetic variants, regardless of their genomic annotation, in proximity to the KREMEN1 gene (up to 10 Mb around the gene) that show a non-zero association with bone mineral density-related traits in a genetic association analysis. In some embodiments, when the subject has an aggregate burden above a desired threshold score, the subject has a decreased risk of developing decreased bone mineral density. In some embodiments, when the subject has an aggregate burden below a desired threshold score, the subject has an increased risk of developing decreased bone mineral density.

In some embodiments, the aggregate burden may be divided into quintiles, e.g., top quintile, intermediate quintile, and bottom quintile, wherein the top quintile of aggregate burden corresponds to the lowest risk group and the bottom quintile of aggregate burden corresponds to the highest risk group. In some embodiments, a subject having a greater aggregate burden comprises the highest weighted aggregate burdens, including, but not limited to the top 10%, top 20%, top 30%, top 40%, or top 50% of aggregate burdens from a subject population. In some embodiments, the genetic variants comprise the genetic variants having association with decreased bone mineral density in the top 10%, top 20%, top 30%, top 40%, or top 50% of p-value range for the association. In some embodiments, each of the identified genetic variants comprise the genetic variants having association with decreased bone mineral

density with p-value of no more than about 10^{-2}, about 10^{-3}, about 10^{-4}, about 10^{-5}, about 10^{-6}, about 10^{-7}, about 10^{-8}, about 10^{-9}, about 10^{-10}, about 10^{-11}, about 10^{-12}, about 10^{-13}, about 10^{-14}, about or 10^{-15}. In some embodiments, the identified genetic variants comprise the genetic variants having association with decreased bone mineral density with p-value of less than 5 x 10^{-8}. In some embodiments, the identified genetic variants comprise genetic variants having association with decreased bone mineral density in high-risk subjects as compared to the rest of the reference population with odds ratio (OR) about 1.5 or greater, about 1.75 or greater, about 2.0 or greater, or about 2.25 or greater for the top 20% of the distribution; or about 1.5 or greater, about 1.75 or greater, about 2.0 or greater, about 2.25 or greater, about 2.5 or greater, or about 2.75 or greater. In some embodiments, the odds ratio (OR) may range from about 1.0 to about 1.5, from about 1.5 to about 2.0, from about 2.0 to about 2.5, from about 2.5 to about 3.0, from about 3.0 to about 3.5, from about 3.5 to about 4.0, from about 4.0 to about 4.5, from about 4.5 to about 5.0, from about 5.0 to about 5.5, from about 5.5 to about 6.0, from about 6.0 to about 6.5, from about 6.5 to about 7.0, or greater than 7.0. In some embodiments, high-risk subjects comprise subjects having aggregate burdens in the bottom decile, quintile, or tertile in a reference population. The threshold of the aggregate burden is determined on the basis of the nature of the intended practical application and the risk difference that would be considered meaningful for that practical application.

In some embodiments, when a subject is identified as having an increased risk of developing decreased bone mineral density, the subject is treated with a therapeutic agent that treats or prevents decreased bone mineral density, and/or a KREMEN1 inhibitor, as described herein. For example, when the subject is KREMEN1 reference, and therefore has an increased risk of developing decreased bone mineral density, the subject is administered a KREMEN1 inhibitor. In some embodiments, such a subject is administered a therapeutic agent that treats or prevents decreased bone mineral density. In some embodiments, when the subject is heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide, the subject is administered the therapeutic agent that treats or prevents decreased bone mineral density in a dosage amount that is the same as or less than a standard dosage amount, and is also administered a KREMEN1 inhibitor. In some embodiments, the subject is KREMEN1 reference. In some embodiments, the subject is heterozygous for a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide. Furthermore, when the subject has a lower aggregate burden for having a

KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide, and therefore has an increased risk of developing decreased bone mineral density, the subject is administered a therapeutic agent that treats or prevents decreased bone mineral density. In some embodiments, when the subject has a lower aggregate burden for having a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide, the subject is administered the therapeutic agent that treats or prevents decreased bone mineral density in a dosage amount that is the same as or greater than the standard dosage amount administered to a subject who has a greater aggregate burden for having a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide.

The present disclosure also provides methods of detecting the presence or absence of a KREMEN1 variant nucleic acid molecule (i.e., a genomic nucleic acid molecule, an mRNA molecule, or a cDNA molecule produced from an mRNA molecule) encoding a KREMEN1 predicted loss-of-function polypeptide in a biological sample from a subject. It is understood that gene sequences within a population and mRNA molecules encoded by such genes can vary due to polymorphisms such as single-nucleotide polymorphisms. The sequences provided herein for the KREMEN1 variant genomic nucleic acid molecule, KREMEN1 variant mRNA molecule, and KREMEN1 variant cDNA molecule are only exemplary sequences. Other sequences for the KREMEN1 variant genomic nucleic acid molecule, variant mRNA molecule, and variant cDNA molecule are also possible.

The biological sample can be derived from any cell, tissue, or biological fluid from the subject. The biological sample may comprise any clinically relevant tissue, such as a bone marrow sample, a tumor biopsy, a fine needle aspirate, or a sample of bodily fluid, such as blood, gingival crevicular fluid, plasma, serum, lymph, ascitic fluid, cystic fluid, or urine. In some cases, the sample comprises a buccal swab. The biological sample used in the methods disclosed herein can vary based on the assay format, nature of the detection method, and the tissues, cells, or extracts that are used as the sample. A biological sample can be processed differently depending on the assay being employed. For example, when detecting any KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide, preliminary processing designed to isolate or enrich the biological sample for the genomic DNA can be employed. A variety of techniques may be used for this purpose. When detecting the level of any KREMEN1 variant mRNA molecule, different techniques can be used

enrich the biological sample with mRNA molecules. Various methods to detect the presence or level of an mRNA molecule or the presence of a particular variant genomic DNA locus can be used.

In some embodiments, detecting a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide in a subject comprises performing a sequence analysis on a biological sample obtained from the subject to determine whether a KREMEN1 genomic nucleic acid molecule in the biological sample, and/or a KREMEN1 mRNA molecule in the biological sample, and/or a KREMEN1 cDNA molecule produced from an mRNA molecule in the biological sample, comprises one or more variations that cause a loss-of-function (partial or complete) or are predicted to cause a loss-of-function (partial or complete).

In some embodiments, the methods of detecting the presence or absence of a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide (such as, for example, a genomic nucleic acid molecule, an mRNA molecule, and/or a cDNA molecule produced from an mRNA molecule) in a subject, comprise performing an assay on a biological sample obtained from the subject. The assay determines whether a nucleic acid molecule in the biological sample comprises a particular nucleotide sequence.

In some embodiments, the biological sample comprises a cell or cell lysate. Such methods can further comprise, for example, obtaining a biological sample from the subject comprising a KREMEN1 genomic nucleic acid molecule or mRNA molecule, and if mRNA, optionally reverse transcribing the mRNA into cDNA. Such assays can comprise, for example determining the identity of these positions of the particular KREMEN1 nucleic acid molecule. In some embodiments, the method is an *in vitro* method.

In some embodiments, the determining step, detecting step, or sequence analysis comprises sequencing at least a portion of the nucleotide sequence of the KREMEN1 genomic nucleic acid molecule, the KREMEN1 mRNA molecule, or the KREMEN1 cDNA molecule in the biological sample, wherein the sequenced portion comprises one or more variations that cause a loss-of-function (partial or complete) or are predicted to cause a loss-of-function (partial or complete).

In some embodiments, the assay comprises sequencing the entire nucleic acid molecule. In some embodiments, only a KREMEN1 genomic nucleic acid molecule is analyzed. In some embodiments, only a KREMEN1 mRNA is analyzed. In some embodiments, only a KREMEN1 cDNA obtained from KREMEN1 mRNA is analyzed.

Alteration-specific polymerase chain reaction techniques can be used to detect mutations such as SNPs in a nucleic acid sequence. Alteration-specific primers can be used because the DNA polymerase will not extend when a mismatch with the template is present.

In some embodiments, the nucleic acid molecule in the sample is mRNA and the mRNA is reverse-transcribed into a cDNA prior to the amplifying step. In some embodiments, the nucleic acid molecule is present within a cell obtained from the subject.

In some embodiments, the assay comprises contacting the biological sample with a primer or probe, such as an alteration-specific primer or alteration-specific probe, that specifically hybridizes to a KREMEN1 variant genomic sequence, variant mRNA sequence, or variant cDNA sequence and not the corresponding KREMEN1 reference sequence under stringent conditions, and determining whether hybridization has occurred.

In some embodiments, the determining step, detecting step, or sequence analysis comprises: a) amplifying at least a portion of the nucleic acid molecule that encodes the KREMEN1 polypeptide; b) labeling the amplified nucleic acid molecule with a detectable label; c) contacting the labeled nucleic acid molecule with a support comprising an alteration-specific probe; and d) detecting the detectable label.

In some embodiments, the assay comprises RNA sequencing (RNA-Seq). In some embodiments, the assays also comprise reverse transcribing mRNA into cDNA, such as by the reverse transcriptase polymerase chain reaction (RT-PCR).

In some embodiments, the methods utilize probes and primers of sufficient nucleotide length to bind to the target nucleotide sequence and specifically detect and/or identify a polynucleotide comprising a KREMEN1 variant genomic nucleic acid molecule, variant mRNA molecule, or variant cDNA molecule. The hybridization conditions or reaction conditions can be determined by the operator to achieve this result. The nucleotide length may be any length that is sufficient for use in a detection method of choice, including any assay described or exemplified herein. Such probes and primers can hybridize specifically to a target nucleotide sequence under high stringency hybridization conditions. Probes and primers may have complete nucleotide sequence identity of contiguous nucleotides within the target nucleotide sequence, although probes differing from the target nucleotide sequence and that retain the ability to specifically detect and/or identify a target nucleotide sequence may be designed by conventional methods. Probes and primers can have about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about

99%, or 100% sequence identity or complementarity with the nucleotide sequence of the target nucleic acid molecule.

Illustrative examples of nucleic acid sequencing techniques include, but are not limited to, chain terminator (Sanger) sequencing and dye terminator sequencing. Other methods involve nucleic acid hybridization methods other than sequencing, including using labeled primers or probes directed against purified DNA, amplified DNA, and fixed cell preparations (fluorescence in situ hybridization (FISH)). In some methods, a target nucleic acid molecule may be amplified prior to or simultaneous with detection. Illustrative examples of nucleic acid amplification techniques include, but are not limited to, polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA), and nucleic acid sequence based amplification (NASBA). Other methods include, but are not limited to, ligase chain reaction, strand displacement amplification, and thermophilic SDA (tSDA).

In hybridization techniques, stringent conditions can be employed such that a probe or primer will specifically hybridize to its target. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target sequence to a detectably greater degree than to other non-target sequences, such as, at least 2-fold, at least 3-fold, at least 4-fold, or more over background, including over 10-fold over background. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target nucleotide sequence to a detectably greater degree than to other nucleotide sequences by at least 2-fold. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target nucleotide sequence to a detectably greater degree than to other nucleotide sequences by at least 3-fold. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target nucleotide sequence to a detectably greater degree than to other nucleotide sequences by at least 4-fold. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target nucleotide sequence to a detectably greater degree than to other nucleotide sequences by over 10-fold over background. Stringent conditions are sequence-dependent and will be different in different circumstances.

Appropriate stringency conditions which promote DNA hybridization, for example, 6X sodium chloride/sodium citrate (SSC) at about 45°C., followed by a wash of 2X SSC at 50°C, are known or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Typically, stringent conditions for hybridization and detection will be those

in which the salt concentration is less than about 1.5 M Na^+ ion, typically about 0.01 to 1.0 M Na^+ ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (such as, for example, 10 to 50 nucleotides) and at least about 60°C for longer probes (such as, for example, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Optionally, wash buffers may comprise about 0.1% to about 1% SDS. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours. The duration of the wash time will be at least a length of time sufficient to reach equilibrium.

In some embodiments, such isolated nucleic acid molecules comprise or consist of at least about 5, at least about 8, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 55, at least about 60, at least about 65, at least about 70, at least about 75, at least about 80, at least about 85, at least about 90, at least about 95, at least about 100, at least about 200, at least about 300, at least about 400, at least about 500, at least about 600, at least about 700, at least about 800, at least about 900, at least about 1000, at least about 2000, at least about 3000, at least about 4000, or at least about 5000 nucleotides. In some embodiments, such isolated nucleic acid molecules comprise or consist of at least about 5, at least about 8, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, or at least about 25 nucleotides. In some embodiments, the isolated nucleic acid molecules comprise or consist of at least about 18 nucleotides. In some embodiments, the isolated nucleic acid molecules comprise or consists of at least about 15 nucleotides. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 10 to about 35, from about 10 to about 30, from about 10 to about 25, from about 12 to about 30, from about 12 to about 28, from about 12 to about 24, from about 15 to about 30, from about 15 to about 25, from about 18 to about 30, from about 18 to about 25, from about 18 to about 24, or from about 18 to about 22 nucleotides. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 18 to about 30 nucleotides. In some

embodiments, the isolated nucleic acid molecules comprise or consist of at least about 15 nucleotides to at least about 35 nucleotides.

In some embodiments, such isolated nucleic acid molecules hybridize to KREMEN1 variant nucleic acid molecules (such as genomic nucleic acid molecules, mRNA molecules, and/or cDNA molecules) under stringent conditions. Such nucleic acid molecules can be used, for example, as probes, primers, alteration-specific probes, or alteration-specific primers as described or exemplified herein, and include, without limitation primers, probes, antisense RNAs, shRNAs, and siRNAs, each of which is described in more detail elsewhere herein, and can be used in any of the methods described herein.

In some embodiments, the isolated nucleic acid molecules hybridize to at least about 15 contiguous nucleotides of a nucleic acid molecule that is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to KREMEN1 variant genomic nucleic acid molecules, KREMEN1 variant mRNA molecules, and/or KREMEN1 variant cDNA molecules. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 15 to about 100 nucleotides, or from about 15 to about 35 nucleotides. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 15 to about 100 nucleotides. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 15 to about 35 nucleotides.

In some embodiments, the alteration-specific probes and alteration-specific primers comprise DNA. In some embodiments, the alteration-specific probes and alteration-specific primers comprise RNA.

In some embodiments, the probes and primers described herein (including alteration-specific probes and alteration-specific primers) have a nucleotide sequence that specifically hybridizes to any of the nucleic acid molecules disclosed herein, or the complement thereof. In some embodiments, the probes and primers specifically hybridize to any of the nucleic acid molecules disclosed herein under stringent conditions.

In some embodiments, the primers, including alteration-specific primers, can be used in second generation sequencing or high throughput sequencing. In some instances, the primers, including alteration-specific primers, can be modified. In particular, the primers can comprise various modifications that are used at different steps of, for example, Massive Parallel Signature Sequencing (MPSS), Polony sequencing, and 454 Pyrosequencing. Modified primers

can be used at several steps of the process, including biotinylated primers in the cloning step and fluorescently labeled primers used at the bead loading step and detection step. Polony sequencing is generally performed using a paired-end tags library wherein each molecule of DNA template is about 135 bp in length. Biotinylated primers are used at the bead loading step and emulsion PCR. Fluorescently labeled degenerate nonamer oligonucleotides are used at the detection step. An adaptor can contain a 5'-biotin tag for immobilization of the DNA library onto streptavidin-coated beads.

The probes and primers described herein can be used to detect a nucleotide variation within any of the KREMEN1 variant genomic nucleic acid molecules, KREMEN1 variant mRNA molecules, and/or KREMEN1 variant cDNA molecules disclosed herein. The primers described herein can be used to amplify KREMEN1 variant genomic nucleic acid molecules, KREMEN1 variant mRNA molecules, or KREMEN1 variant cDNA molecules, or a fragment thereof.

In the context of the disclosure “specifically hybridizes” means that the probe or primer (such as, for example, the alteration-specific probe or alteration-specific primer) does not hybridize to a nucleic acid sequence encoding a KREMEN1 reference genomic nucleic acid molecule, a KREMEN1 reference mRNA molecule, and/or a KREMEN1 reference cDNA molecule.

In some embodiments, the probes (such as, for example, an alteration-specific probe) comprise a label. In some embodiments, the label is a fluorescent label, a radiolabel, or biotin.

The present disclosure also provides supports comprising a substrate to which any one or more of the probes disclosed herein is attached. Solid supports are solid-state substrates or supports with which molecules, such as any of the probes disclosed herein, can be associated. A form of solid support is an array. Another form of solid support is an array detector. An array detector is a solid support to which multiple different probes have been coupled in an array, grid, or other organized pattern. A form for a solid-state substrate is a microtiter dish, such as a standard 96-well type. In some embodiments, a multiwell glass slide can be employed that normally contains one array per well.

The nucleotide sequence of a KREMEN1 reference genomic nucleic acid molecule is set forth in SEQ ID NO:1 (ENSG00000183762.13; ENST00000327813.9; chr22:29073118-29168333 in the GRCh38/hg38 human genome assembly; alternately, chr22:29073035-29168333 or chr22:29073077-29168333).

The nucleotide sequence of a KREMEN1 reference mRNA molecule is set forth in SEQ ID NO:2. The nucleotide sequence of another KREMEN1 reference mRNA molecule is set forth

in SEQ ID NO:3. The nucleotide sequence of another KREMEN1 reference mRNA molecule is set forth in SEQ ID NO:4. The nucleotide sequence of another KREMEN1 reference mRNA molecule is set forth in SEQ ID NO:5. The nucleotide sequence of another KREMEN1 reference mRNA molecule is set forth in SEQ ID NO:6. The nucleotide sequence of another KREMEN1 reference mRNA molecule is set forth in SEQ ID NO:7.

The nucleotide sequence of a KREMEN1 reference cDNA molecule is set forth in SEQ ID NO:8. The nucleotide sequence of another KREMEN1 reference cDNA molecule is set forth in SEQ ID NO:9. The nucleotide sequence of another KREMEN1 reference cDNA molecule is set forth in SEQ ID NO:10. The nucleotide sequence of another KREMEN1 reference cDNA molecule is set forth in SEQ ID NO:11. The nucleotide sequence of another KREMEN1 reference cDNA molecule is set forth in SEQ ID NO:12. The nucleotide sequence of another KREMEN1 reference cDNA molecule is set forth in SEQ ID NO:13.

The amino acid sequence of a KREMEN1 reference polypeptide is set forth in SEQ ID NO:14, and is 492 amino acids in length. The nucleotide sequence of another KREMEN1 reference polypeptide is set forth in SEQ ID NO:15, and is 458 amino acids in length. The nucleotide sequence of another KREMEN1 reference polypeptide is set forth in SEQ ID NO:16, and is 473 amino acids in length.

The genomic nucleic acid molecules, mRNA molecules, and cDNA molecules can be from any organism. For example, the genomic nucleic acid molecules, mRNA molecules, and cDNA molecules can be human or an ortholog from another organism, such as a non-human mammal, a rodent, a mouse, or a rat. It is understood that gene sequences within a population can vary due to polymorphisms such as single-nucleotide polymorphisms. The examples provided herein are only exemplary sequences. Other sequences are also possible.

Also provided herein are functional polynucleotides that can interact with the disclosed nucleic acid molecules. Examples of functional polynucleotides include, but are not limited to, antisense molecules, aptamers, ribozymes, triplex forming molecules, and external guide sequences. The functional polynucleotides can act as effectors, inhibitors, modulators, and stimulators of a specific activity possessed by a target molecule, or the functional polynucleotides can possess a *de novo* activity independent of any other molecules.

The isolated nucleic acid molecules disclosed herein can comprise RNA, DNA, or both RNA and DNA. The isolated nucleic acid molecules can also be linked or fused to a heterologous nucleic acid sequence, such as in a vector, or a heterologous label. For example, the isolated

nucleic acid molecules disclosed herein can be within a vector or as an exogenous donor sequence comprising the isolated nucleic acid molecule and a heterologous nucleic acid sequence. The isolated nucleic acid molecules can also be linked or fused to a heterologous label. The label can be directly detectable (such as, for example, fluorophore) or indirectly detectable (such as, for example, hapten, enzyme, or fluorophore quencher). Such labels can be detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. Such labels include, for example, radiolabels, pigments, dyes, chromogens, spin labels, and fluorescent labels. The label can also be, for example, a chemiluminescent substance; a metal-containing substance; or an enzyme, where there occurs an enzyme-dependent secondary generation of signal. The term “label” can also refer to a “tag” or hapten that can bind selectively to a conjugated molecule such that the conjugated molecule, when added subsequently along with a substrate, is used to generate a detectable signal. For example, biotin can be used as a tag along with an avidin or streptavidin conjugate of horseradish peroxidate (HRP) to bind to the tag, and examined using a calorimetric substrate (such as, for example, tetramethylbenzidine (TMB)) or a fluorogenic substrate to detect the presence of HRP. Exemplary labels that can be used as tags to facilitate purification include, but are not limited to, myc, HA, FLAG or 3XFLAG, 6XHis or polyhistidine, glutathione-S-transferase (GST), maltose binding protein, an epitope tag, or the Fc portion of immunoglobulin. Numerous labels include, for example, particles, fluorophores, haptens, enzymes and their calorimetric, fluorogenic and chemiluminescent substrates and other labels.

The isolated nucleic acid molecules, or the complement thereof, can also be present within a host cell. In some embodiments, the host cell can comprise the vector that comprises any of the nucleic acid molecules described herein, or the complement thereof. In some embodiments, the nucleic acid molecule is operably linked to a promoter active in the host cell. In some embodiments, the promoter is an exogenous promoter. In some embodiments, the promoter is an inducible promoter. In some embodiments, the host cell is a bacterial cell, a yeast cell, an insect cell, or a mammalian cell. In some embodiments, the host cell is a bacterial cell. In some embodiments, the host cell is a yeast cell. In some embodiments, the host cell is an insect cell. In some embodiments, the host cell is a mammalian cell.

The disclosed nucleic acid molecules can comprise, for example, nucleotides or non-natural or modified nucleotides, such as nucleotide analogs or nucleotide substitutes. Such nucleotides include a nucleotide that contains a modified base, sugar, or phosphate group, or

that incorporates a non-natural moiety in its structure. Examples of non-natural nucleotides include, but are not limited to, dideoxynucleotides, biotinylated, aminated, deaminated, alkylated, benzylated, and fluorophor-labeled nucleotides.

The nucleic acid molecules disclosed herein can also comprise one or more nucleotide analogs or substitutions. A nucleotide analog is a nucleotide which contains a modification to either the base, sugar, or phosphate moieties. Modifications to the base moiety include, but are not limited to, natural and synthetic modifications of A, C, G, and T/U, as well as different purine or pyrimidine bases such as, for example, pseudouridine, uracil-5-yl, hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl. Modified bases include, but are not limited to, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo (such as, for example, 5-bromo), 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine, 7-methyladenine, 8-azaguanine, 8-azaadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, and 3-deazaadenine.

Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety include, but are not limited to, natural modifications of the ribose and deoxy ribose as well as synthetic modifications. Sugar modifications include, but are not limited to, the following modifications at the 2’ position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl, and alkynyl may be substituted or unsubstituted $C_{1\text{-}10}$alkyl or $C_{2\text{-}10}$alkenyl, and $C_{2\text{-}10}$alkynyl. Exemplary 2’ sugar modifications also include, but are not limited to, $-O[(CH_2)_nO]_mCH_3$, $-O(CH_2)_nOCH_3$, $-O(CH_2)_nNH_2$, $-O(CH_2)_nCH_3$, $-O(CH_2)_n$-ONH_2, and $-O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m, independently, are from 1 to about 10. Other modifications at the 2’ position include, but are not limited to, $C_{1\text{-}10}$alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar modifications may also be made at other

positions on the sugar, particularly the 3’ position of the sugar on the 3’ terminal nucleotide or in 2’-5’ linked oligonucleotides and the 5’ position of 5’ terminal nucleotide. Modified sugars can also include those that contain modifications at the bridging ring oxygen, such as CH_2 and S. Nucleotide sugar analogs can also have sugar mimetics, such as cyclobutyl moieties in place of the pentofuranosyl sugar.

Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include, but are not limited to, those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3’-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3’-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. These phosphate or modified phosphate linkage between two nucleotides can be through a 3’-5’ linkage or a 2’-5’ linkage, and the linkage can contain inverted polarity such as 3’-5’ to 5’-3’ or 2’-5’ to 5’-2’. Various salts, mixed salts, and free acid forms are also included. Nucleotide substitutes also include peptide nucleic acids (PNAs).

The present disclosure also provides vectors comprising any one or more of the nucleic acid molecules disclosed herein. In some embodiments, the vectors comprise any one or more of the nucleic acid molecules disclosed herein and a heterologous nucleic acid. The vectors can be viral or nonviral vectors capable of transporting a nucleic acid molecule. In some embodiments, the vector is a plasmid or cosmid (such as, for example, a circular double-stranded DNA into which additional DNA segments can be ligated). In some embodiments, the vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Expression vectors include, but are not limited to, plasmids, cosmids, retroviruses, adenoviruses, adeno-associated viruses (AAV), plant viruses such as cauliflower mosaic virus and tobacco mosaic virus, yeast artificial chromosomes (YACs), Epstein-Barr (EBV)-derived episomes, and other expression vectors known in the art.

Desired regulatory sequences for mammalian host cell expression can include, for example, viral elements that direct high levels of polypeptide expression in mammalian cells, such as promoters and/or enhancers derived from retroviral LTRs, cytomegalovirus (CMV) (such as, for example, CMV promoter/enhancer), Simian Virus 40 (SV40) (such as, for example, SV40 promoter/enhancer), adenovirus, (such as, for example, the adenovirus major late promoter

(AdMLP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. Methods of expressing polypeptides in bacterial cells or fungal cells (such as, for example, yeast cells) are also well known. A promoter can be, for example, a constitutively active promoter, a conditional promoter, an inducible promoter, a temporally restricted promoter (such as, for example, a developmentally regulated promoter), or a spatially restricted promoter (such as, for example, a cell-specific or tissue-specific promoter).

Percent identity (or percent complementarity) between particular stretches of nucleotide sequences within nucleic acid molecules or amino acid sequences within polypeptides can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs (Altschul *et al.*, J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656) or by using the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489). Herein, if reference is made to percent sequence identity, the higher percentages of sequence identity are preferred over the lower ones.

As used herein, the phrase “corresponding to” or grammatical variations thereof when used in the context of the numbering of a particular nucleotide or nucleotide sequence or position refers to the numbering of a specified reference sequence when the particular nucleotide or nucleotide sequence is compared to a reference sequence (such as, for example, SEQ ID NO:1). In other words, the residue (such as, for example, nucleotide or amino acid) number or residue (such as, for example, nucleotide or amino acid) position of a particular polymer is designated with respect to the reference sequence rather than by the actual numerical position of the residue within the particular nucleotide or nucleotide sequence. For example, a particular nucleotide sequence can be aligned to a reference sequence by introducing gaps to optimize residue matches between the two sequences. In these cases, although the gaps are present, the numbering of the residue in the particular nucleotide or nucleotide sequence is made with respect to the reference sequence to which it has been aligned.

The nucleotide and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three-letter code for amino acids. The nucleotide sequences follow the standard convention of beginning at the 5’ end of the sequence and proceeding forward (i.e., from left to right in each line) to the 3’ end.

Only one strand of each nucleotide sequence is shown, but the complementary strand is understood to be included by any reference to the displayed strand. The amino acid sequence follows the standard convention of beginning at the amino terminus of the sequence and proceeding forward (i.e., from left to right in each line) to the carboxy terminus.

The present disclosure also provides therapeutic agents that treat or prevent decreased bone mineral density for use in the treatment or prevention of decreased bone mineral density in a subject having: a KREMEN1 variant genomic nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide; a KREMEN1 variant mRNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; or a KREMEN1 variant cDNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide. Any of the therapeutic agents that treat or prevent decreased bone mineral density described herein can be used in these methods. The subject can have or have a risk of developing decreased bone mineral density, osteopenia, Type I osteoporosis, Type II osteoporosis, or secondary osteoporosis.

The present disclosure also provides uses of therapeutic agents that treat or prevent decreased bone mineral density for use in the preparation of a medicament for treating or preventing decreased bone mineral density in a subject having: a KREMEN1 variant genomic nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide; a KREMEN1 variant mRNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; or a KREMEN1 variant cDNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide. Any of the therapeutic agents that treat or prevent decreased bone mineral density described herein can be used in these methods. The subject can have or have a risk of developing decreased bone mineral density, osteopenia, Type I osteoporosis, Type II osteoporosis, or secondary osteoporosis.

The present disclosure also provides KREMEN1 inhibitors for use in the treatment or prevention of decreased bone mineral density in a subject that: a) is reference for a KREMEN1 genomic nucleic acid molecule, a KREMEN1 mRNA molecule, or a KREMEN1 cDNA molecule; or b) is heterozygous for: i) a KREMEN1 variant genomic nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide; ii) a KREMEN1 variant mRNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; or iii) a KREMEN1 variant cDNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide. Any of the KREMEN1 inhibitors described herein can be used in these methods. The subject can have or have a risk of

developing decreased bone mineral density, osteopenia, Type I osteoporosis, Type II osteoporosis, or secondary osteoporosis.

The present disclosure also provides uses of KREMEN1 inhibitors in the preparation of a medicament for treating or preventing decreased bone mineral density in a subject that: a) is reference for a KREMEN1 genomic nucleic acid molecule, a KREMEN1 mRNA molecule, or a KREMEN1 cDNA molecule; or b) is heterozygous for: i) a KREMEN1 variant genomic nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide; ii) a KREMEN1 variant mRNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; or iii) a KREMEN1 variant cDNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide. Any of the KREMEN1 inhibitors described herein can be used in these methods. The subject can have or have a risk of developing decreased bone mineral density, osteopenia, Type I osteoporosis, Type II osteoporosis, or secondary osteoporosis.

All patent documents, websites, other publications, accession numbers and the like cited above or below are incorporated by reference in their entirety for all purposes to the same extent as if each individual item were specifically and individually indicated to be so incorporated by reference. If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing date of a priority application referring to the accession number if applicable. Likewise, if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect of the present disclosure can be used in combination with any other feature, step, element, embodiment, or aspect unless specifically indicated otherwise. Although the present disclosure has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

The following examples are provided to describe the embodiments in greater detail. They are intended to illustrate, not to limit, the claimed embodiments. The following examples provide those of ordinary skill in the art with a disclosure and description of how the compounds, compositions, articles, devices and/or methods described herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the scope of

any claims. Efforts have been made to ensure accuracy with respect to numbers (such as, for example, amounts, temperature, etc.), but some errors and deviations may be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

Examples

Example 1: General Methodology

Cohort description

The United Kingdom (UK) Biobank (UKB) is a population-based cohort of individuals aged between 40 to 69 years at baseline and recruited via 22 testing centers in the UK between 2006-2010 (Bycroft et al., Nature, 2018, 562, 203-209). Genetic and phenotypic data from close to 420,000 European-ancestry participants in UKB was used. This study was approved by relevant ethics committees and participants provided informed consent for participation in UKB.

Phenotype definition

Data pertaining to quantitative ultrasound of the heel were extracted from UKB. eBMD trait values (in g/cm^2) were derived using a combination of speed of sound (SOS) and bone ultrasound attenuation (BUA; eBMD = 0.002592 × (BUA + SOS) − 3.687). Sex-specific quality control measures were implemented for SOS (subjects were excluded if SOS ≤1,450 or ≥1,700 m/s for men, ≤1,455 or ≥1,700 m/s for women), BUA (exclude if BUA ≤27 or ≥138 dB/MHz for men, ≤22 or ≥138 dB/MHz for women), and eBMD (exclude if ≤0.18 or ≥1.06 g/cm^2 for men, ≤0.12 or ≥1.025 g/cm^2 for women). Phenotypic values for eBMD were first transformed using rank-based inverse normal transformation, applied within each ancestry group and separately in men and women, and adjusted for fine-mapped common (MAF >= 0.01) genetic variants associated with eBMD.

Genotype data

High coverage whole exome sequencing was performed as previously described (Dewey et al., Science, 2016, 354, 6319:aaf6814; and Van Hout et al., Nature, 2020, 586, 749-756) and as summarized below. A modified version of the xGen design available from Integrated DNA Technologies (IDT) was used for target sequence capture of the exome. A unique 10 bp barcode (IDT) was added to each DNA fragment during library preparation to facilitate multiplexed exome capture and sequencing. Equal amounts of sample were pooled

prior to exome capture. Sequencing was performed using 75 bp paired-end reads on Illumina NovaSeq instruments. Sequencing had a coverage depth (i.e., number of sequence-reads covering each nucleotide in the target areas of the genome) sufficient to provide greater than 20x coverage over 90% of targeted bases in 99% of IDT samples. Data processing steps included sample de-multiplexing using Illumina software, alignment to the GRCh38 Human Genome reference sequence including generation of binary alignment and mapping files (BAM), processing of BAM files (e.g., marking of duplicate reads and other read mapping evaluations). Variant calling was performed using the GLNexus system (Lin et al., bioRxiv, 2018, 343970). Variant mapping and annotation were based on the GRCh38 Human Genome reference sequence and Ensembl v85 gene definitions using the snpEff software. The snpEff predictions that involve protein-coding transcripts with an annotated start and stop were then combined into a single functional impact prediction by selecting the most deleterious functional effect class for each gene. The hierarchy (from most to least deleterious) for these annotations was frameshift, stop-gain, stop-loss, splice acceptor, splice donor, stop-lost, in-frame indel, missense, other annotations. Predicted LoF genetic variants included: a) insertions or deletions resulting in a frameshift, b) insertions, deletions or single nucleotide variants resulting in the introduction of a premature stop codon or in the loss of the transcription start site or stop site, and c) variants in donor or acceptor splice sites. Missense variants were classified for likely functional impact according to the number of *in silico* prediction algorithms that predicted deleteriousness using SIFT (Vaser et al., Nature Protocols, 2016, 11, 1-9), Polyphen2_HDIV and Polyphen2_HVAR (Adzhubei et al., Nat. Methods, 2010, 7, 248-249), LRT (Chun et al., Genome Res., 2009, 19, 1553-1561) and MutationTaster (Schwarz et al., Nat. Methods, 2010, 7, 575-576). For each gene, the alternative allele frequency (AAF) and functional annotation of each variant determined inclusion into 7 gene burden exposures: 1) pLoF variants with AAF < 1%; 2) pLoF or missense variants predicted deleterious by 5/5 algorithms with AAF < 1%; 3) pLoF or missense variants predicted deleterious by 5/5 algorithms with AAF < 0.1%; 4) pLoF or missense variants predicted deleterious by at least 1/5 algorithms with AAF < 1%; 5) pLoF or missense variants predicted deleterious by at least 1/5 algorithms with AAF < 0.1%; 6) pLoF or any missense with AAF < 1%; 7) pLoF or any missense variants with AAF < 0.1%. The results described elsewhere in this document as pertaining to “pLoF or predicted deleterious missense variants” refer to analysis performed using the aggregate burden of pLoF variants or missense variants predicted to by deleterious by 5/5 algorithms.

Association analysis of gene burden of rare pLoF and missense variation in KREMEN1

Association between the burden of rare pLoF or missense variants in a given gene and eBMD was examined by fitting a linear regression model adjusted for a polygenic score that approximates a genomic kinship matrix, using REGENIE v1.0 (Mbatchou et al., Nature Genetics, 2021). Analyses were adjusted for age, age^2, sex, age-by-sex and age^2-by-sex interaction terms, experimental batch-related covariates, ten common variant-derived principal components, and twenty rare variant-derived principal components. Association analyses were performed using single variants, and using gene burden tests. In gene burden tests, all individuals are labelled as heterozygotes if they carry one or more qualifying rare variant (as described above based on frequency and functional annotation) and as homozygotes if they carry any qualifying variant in the homozygous state. This "composite genotype" is then used to test for association.

Example 2: Loss-of-Function of *KREMEN1* is Associated with Higher Estimated Bone Mineral Density

Whole exome sequencing of 419,737 European-ancestry individuals in the UK Biobank (UKB) was performed to identify protein-coding variants in each gene in the genome. The association of each sequenced gene and genetic variant with estimated bone mineral density (eBMD, measured using ultrasound of the heel) was examined. eBMD is a commonly used biomarker of bone density and strength, and is highly correlated with bone mineral density as measured using dual-energy X-ray absorptiometry (DXA) technology. Lower levels of bone density are strongly associated with a higher risk of osteoporotic fractures.

The exome-wide analysis in UKB found that the burden of rare (alternative allele frequency [AAF] < 1%) predicted loss-of-function (pLoF) or predicted deleterious missense variants (with predicted deleteriousness for missense variants based on agreement between five *in silico* prediction algorithms) in the *KREMEN1* gene was associated with 0.13 standard deviation units (or 0.015 g/cm^2 units) higher eBMD (P-value=2.1x10^{-7}, meeting a Bonferroni-corrected, exome-wide statistical significance threshold of P<3.6×10^{-7} (correction for 20,000 genes and seven variant aggregation models at an alpha of 0.05)) (see, Figure 1).

A nominally significant association was observed between the aggregate burden of *KREMEN1* pLoF variants only (excluding missense variants) and higher eBMD (see, Figure 2). The effect estimate for the burden of pLoF variants (0.18 SD or 0.022 g/cm^2 higher eBMD per *KREMEN1* allele copy, as shown in Figure 2) was similar to the effect of the burden of pLoF or

predicted deleterious missense variants (0.13 SD or 0.015 g/cm^2 higher eBMD per *KREMEN1* allele copy, as shown in Figure 1). This suggests that most of the missense variants included in the analysis result in *KREMEN1* loss-of-function, and that the association with higher eBMD can be attributed to *KREMEN1* loss-of-function.

Figure 3 shows all pLoF and predicted deleterious missense variants included in the *KREMEN1* gene burden analyses of eBMD.

Example 3: Converging Evidence from Exome Sequencing and Common Variants Implicates *KREMEN1* for Osteoporosis

UKB cohort

From within the UKB, a total of 291,932 participants (278,807 of European ancestry and 13,125 of African, East-Asian, or South Asian ancestry) with available whole-exome sequencing and eBMD data were included in the analyses.

Whole exome sequencing in UKB

Sample preparation and sequencing of the UKB samples were performed and briefly summarized below. A modified version of the xGen exome design available from Integrated DNA Technologies was used for target DNA capture. Sequencing was performed using 75 bp paired-end reads on Illumina NovaSeq instruments. Sequencing had a coverage depth sufficient to provide greater than 20x coverage over 90% of targeted bases in 99% of samples. Variant calling and annotation were based on the GRCh38 Human Genome reference sequence and Ensembl v85 gene definitions using the snpEff software. Variants were annotated according to the most deleterious functional effect in this order (of descending deleteriousness): frameshift, stop-gain, stop-loss, splice acceptor, splice donor, in-frame indel, missense, other annotations. Predicted LOF variants included: a) insertions or deletions resulting in a frameshift, b) insertions, deletions or single nucleotide variants resulting in the introduction of a premature stop codon or in the loss of the transcription start site or stop site, and c) variants in donor or acceptor splice sites. Missense variants were classified for predicted functional impact using a number of *in silico* prediction algorithms that predicted deleteriousness (SIFT, PolyPhen2 (HDIV), PolyPhen2 (HVAR), LRT, and MutationTaster). For each gene, the alternative allele frequency (AAF) and functional annotation of each variant determined inclusion into seven gene burden exposures as previously described (Akbari et al., 2021, Science 373, eabf8683): 1) pLOF variants with AAF < 1%; 2) pLOF or missense variants predicted deleterious by 5/5

algorithms with AAF < 1%; 3) pLOF or missense variants predicted deleterious by 5/5 algorithms with AAF < 0.1%; 4) pLOF or missense variants predicted deleterious by at least 1/5 algorithms with AAF < 1%; 5) pLOF or missense variants predicted deleterious by at least 1/5 algorithms with AAF < 0.1%; 6) pLOF or any missense with AAF < 1%; 7) pLOF or any missense variants with AAF < 0.1%. SNP array genotyping and imputation was performed in the UKB as previously described.

Phenotype definition in UKB

eBMD of the heel was derived from quantitative ultrasound SOS and broadband ultrasound attenuation using a previously described model (Morris et al., Nat. Genet., 2018, 51, 258-66). An in-depth data curation pipeline yielded high quality eBMD data while maximizing the number of participants compared to using direct bone-densitometry of the heel reported in UKB as reported in a previous study. eBMD is used as a surrogate of bone mineral density (BMD) because of eBMD's high correlation with dual-energy X-ray absorptiometry (DXA)-derived BMD (Pearson's correlation r=0.69) and eBMD's strong association with risk of osteoporotic fracture. Before analysis, rank-inverse normal transformation of the eBMD phenotype, by sex and within each ancestry, was performed.

Exome-wide association analyses in UKB

The association of genetic variants or their gene burden with eBMD by fitting mixed-effects regression models using REGENIE v1.0.6.8 was estimated. REGENIE accounts for relatedness, polygenicity, and population structure by approximating the genomic kinship matrix using predictions of individual trait values that are based on genotypes from across the genome. Then, the association of genetic variants or their burden is estimated conditional upon that polygenic predictor along with other covariates. Covariates in association models included age, age^2, sex, age-by-sex interaction term, age^2-by-sex interaction term, experimental batch-related covariates, ten common-variant derived principal components, and twenty rare-variant derived principal components. To ensure that rare coding variant or gene-burden associations were statistically independent of eBMD-associated common genetic variants, exome association analyses for sentinel common variants (MAF≥1%) identified by fine-mapping genome-wide associations of common alleles with eBMD were further adjusted as previously described (Akbari et al., 2021, Science 373, eabf8683). Meta-analysis between subgroup results were performed using fixed-effect inverse-variance weighted models. The exome-wide level of statistical significance for the gene burden analysis was defined as $p<3.6x10^{-7}$, a Bonferroni

correction at the type I error rate of 0.05 which assumes 20,000 genes and accounts for the seven variant selection models used per gene (Akbari et al., 2021, Science 373, eabf8683). In a secondary analysis, the association with eBMD of individual nonsynonymous and/or pLOF variants (minor allele frequency <1% and minor allele count ≥ 25) identified by exome sequencing was estimated. The threshold of $p<5x10^{-8}$, which is a Bonferroni correction based on one million effective number of independent tests at the type I error rate of 0.05, was used to identify exome-wide significant single variants as described (Akbari et al., 2021, Science 373, eabf8683).

For all secondary analyses involving false discovery rate (FDR)-corrected results, FDR-adjusted p-values were obtained by first preselecting for each gene and each gene-burden exposures with the strongest associations (lowest p value) and then correcting for multiple testing using the Benjamini-Hochberg approach across all genes in this subset. Hence, the reported FDR threshold of 1% (corresponding to an unadjusted p-value threshold of $1.49x10^{-5}$) is applied to 18,866 genes, after selection of the best gene-burden exposure per gene. This translates to an FDR threshold of 2.05%, if the FDR correction had been applied to the overall analysis, and not a preselected subset.

Fine-mapping of GWAS common variants

eBMD-associated common variants were identified by performing a genome-wide association study based on imputed genetic variants. Imputation was based on the HRC reference panel supplemented with UK10K. Genome-wide association analyses were performed in the UKB by fitting mixed-effects linear regression models using REGENIE v1.0.6.8. Within each ancestry, fine-mapping was performed using the FINEMAP software at genomic regions harboring genetic variants associated with eBMD at the genome-wide significance threshold of $p < 5\times10^{-8}$. Linkage disequilibrium was estimated using genetic data from the exact set of individuals included in each ancestry-specific genome-wide association analyses.

Test of association with fracture and osteoporosis

The association with fracture and osteoporosis in UK Biobank was tested for genes that met the exome-wide level of statistical significance in the gene burden analysis of eBMD. Fracture cases were defined as individuals with a history of electronic health record-coded or self-reported fracture (not including, where possible, fractures of the skull, facial bones, hands, or toes), and individuals with a history of any type of fracture were excluded from the control group. Osteoporosis cases were defined as individuals with a history of electronic health

record-coded or self-reported osteoporosis. Individuals with a self-reported history of osteopaenia were further excluded from the control group.

Test of Enrichment for Positive Control Genes for Osteoporosis

To evaluate the ability of WES to detect effector genes for osteoporosis, a set of positive control genes for this disease was identified. Fifty-six protein coding genes which are either known drug targets for osteoporosis or whose perturbation causes a Mendelian form of osteoporosis or bone mass disease, resulting in changes to bone density, bone mineralization or bone mass, were included as positive control genes (Morris et al., Nat. Genet., 2018, 51, 258-66). A Fisher's test was used to estimate the enrichment for positive control genes among the exome-wide significant genes in the gene burden analysis.

Effector Index for eBMD Effector Genes

The development of Effector index (Ei) was recently described (Forgetta et al., Hum. Genet., 2022, (world wide web "doi.org/10.1007/s00439-022-02434-z"). A goal of the Ei is to generate a probability of causality for each protein coding gene at a genome-wide association study (GWAS) locus, assigning a score from zero to one. GWAS loci were defined by 500kb around the lead GWAS SNP following linkage disequilibrium (LD) clumping (Forgetta et al., Hum. Genet., 2022, world wide web at "doi.org/10.1007/s00439-022-02434-z"). Protein coding genes with at least 50% of their gene body located in a GWAS locus were included, and overlapping GWAS loci were merged. In short, to generate Ei scores for eBMD, positive control genes for 12 diseases and traits (type 2 diabetes, low-density lipoprotein cholesterol level, adult height, calcium level, hypothyroidism, triglyceride level, eBMD, glucose level, red blood cell count systolic blood pressure, diastolic blood pressure, and direct bilirubin level) were selected. GWAS followed by fine-mapping was performed for each disease, and genomic annotations at GWAS loci were used as features to predict positive control genes. This was achieved by first training a gradient boosted trees algorithm (XGBoost) to generate the probability of causality for genes in GWAS loci for 11 diseases and traits (excluding eBMD), and then applying this trained algorithm to derive Ei scores from eBMD GWAS data. Generalized linear models implemented in R were used to assess the association of the Ei score with the odds of being an exome-wide significant gene. A further, complementary gene prioritization method called Polygenic Priority Score (PoPS) was used to identify effector genes for eBMD from GWAS data (Weeks et al., medRxiv,2020, world wide web at "doi:10.1101/2020.09.08.20190561."

Test of Enrichment for Ei prioritized genes within loci identified using exome-wide gene-burden results for Osteoporosis

2x2 contingency tables were generated comparing genes prioritized by Ei to genes identified from the exome-wide analyses per locus. The data were then aggregated across these loci and tested for enrichment using a stratified Fisher's exact test approach. Estimation of the odds ratio and its confidence interval were then based on the conditional Maximum Likelihood Estimate and estimation of the exact confidence bounds using the tail approach for discrete distributions, respectively.

Two-sample Mendelian randomization

Two-sample Mendelian randomization (MR) analyses were performed to identify circulating proteins that influence eBMD. Two-sample MR uses genetic variants strongly and specifically associated with circulating protein levels (pQTLs) as instrumental variables to estimate the causal relationship between a given protein and an outcome (in this case eBMD). This approach is less affected by confounding and reverse causality than observational epidemiology biomarker studies. The MR framework is based on three main assumptions: First, the SNPs are robustly associated with the exposure. Second, the SNPs are not associated with factors that confound the relationship between the exposure and the outcome. Third, the SNPs have no effect on the outcome that is independent of the exposure (i.e. a lack of horizontal pleiotropy). Of these, the most challenging to assess is the third assumption since the biological mechanistic effect of SNPs on outcomes like eBMD is most often not known. However, in the case of circulating proteins, SNPs that are associated with the protein level and close to the gene that encodes the protein are more likely to have an effect via the protein level by influencing the transcription or translation of the gene into the protein. Such SNPs are called cis-SNPs and may help to reduce potential bias from horizontal pleiotropy.

To select genetic instruments for circulating proteins, summary-level data were used from two proteomic GWAS studies that both measured serum protein levels on the SOMAlogic platform. For the primary analysis, the INTERVAL study was used as a source of pQTL data, which included the measurement of 1,478 serum proteins in 3,301 individuals. In a replication analysis, the AGES study was used, which included measurement of 4,137 serum proteins in 3,200 individuals. Proteins were selected for inclusion in the analysis if the proteins had *cis*-acting associated SNPs ("*cis*-SNPs"), because such instruments may be less likely to be affected by horizontal pleiotropy (Swerdlow et al., Int. J. Epidemiol. 2016, 45, 1600-16). The *cis*-SNPs

from INTERVAL were independent, genome-wide significant SNPs ($P < 1.5 \times 10^{-11}$, the multiple-testing corrected genome-wide significance threshold previously adopted in INTERVAL) within 1 Mb of the transcription start site (TSS) of the gene encoding the protein. To select these *cis*-SNPs, PLINK and the 1000 Genomes Project European population reference panel (1KG EUR) were used to clump and select independent SNPs ($R^2<0.001$, distance 1000 kb) for each protein. The *cis*-SNPs from AGES were the sentinel *cis*-SNPs (genome-wide significant SNPs of $P < 5 \times 10^{-8}$ and with the lowest P value for each protein) within 300 kb of the corresponding protein-coding gene (Milsson et al., Science, 2018, 1327, 1-12). The association of each cis-SNP with eBMD (i.e., the outcome in the MR analysis) was taken from a recent eBMD GWAS, including 426,824 white British individuals (Surakka et al., Nat. Commun., 2020, 11, 4093). Palindromic *cis*-SNPs with minor allele frequency (MAF) > 0.42 (as recommended by the TwoSampleMR R package) were removed prior to MR to prevent allele-mismatches. For *cis*-SNPs that were not present in the eBMD GWAS, SNPs with LD $R^2>0.8$ and with MAF < 0.42 were selected as proxies. For the alignment of SNP proxies, MAF > 0.3 was used as a threshold for removal of palindromic SNPs.

After matching of the *cis*-SNPs of proteins with eBMD GWAS and the removal of palindromic SNPs, 550 SOMAmer reagents (517 proteins) from INTERVAL (including 515 matching *cis*-SNPs and 59 LD-proxy *cis*-SNPs) and 749 circulating proteins from AGES (including 706 unique matching *cis*-SNPs, 41 LD-proxy *cis*-SNPs, and 2 *cis*-SNPs each for two proteins) were included in the MR analyses. Independent cis-pQTL data were selected from INTERVAL data ($p<1.5 \times 10^{-11}$).

MR analyses were performed using the TwoSampleMR package in R, using the Wald ratio ($\beta_{eBMD}/\beta_{protein}$) to estimate the effect of each circulating protein on eBMD. For any proteins with multiple independent *cis*-SNPs, the inverse variance weighted (IVW) method was used to meta-analyze their combined effects[64]. A Bonferroni correction was used to control for the number proteins tested in INTERVAL and AGES independently.

Results

Whole-exome sequencing was performed in nearly 300,000 people from the UK Biobank cohort (UKB and, for each gene in the genome, estimated associations with eBMD for the burden of rare nonsynonymous and/or pLOF variants. In the larger European ancestry subset of UKB (N=278,807), *KREMEN1* was identified ($p<3.6 \times 10^{-7}$). This association did not arise from common genetic variants since these WES analyses were designed to be independent of

eBMD-associated fine-mapped common alleles. An exome-wide multi-ancestry meta-analysis identified two additional genes (*WNT5B* and *KREMEN1*) at exome-wide significance (Figures 4 and 5), providing an exome-wide significant gene. Table 2 shows all variants in the *KREMEN1* gene burden test that were observed in only one ancestry.

Table 2

Ancestry	Genetic exposure, variant type; allele frequency cut-off in %	CPRA	RSID	AAF, fraction of 1	Beta (95% CI) per allele in SD units of eBMD	Beta (95% CI) per allele in g/cm2 units of eBMD	p	Protein effect
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121465:G:A	rs200023748	0.0002026	0.25 (0.081, 0.41)	0.03 (0.0099, 0.05)	3.39E-03	p.Arg154Gln:p.Arg152Gln:p.Arg154Gln
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125344:G:A	rs745457999	0.0001184	0.11 (-0.11, 0.32)	0.013 (-0.013, 0.039)	3.22E-01	p.Glu187Lys:p.Glu185Lys:p.Glu187Lys
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125417:G:T	rs369977022	9.15E-05	0.23 (-0.016, 0.47)	0.028 (-0.0019, 0.057)	6.64E-02	
EUR	pLOF plus deleterious missense	22:29138713:G:A	rs201316832	8.61E-05	-0.05 (-0.3, 0.2)	-0.0061 (-0.037, 0.025)	6.97E-01	p.Ala352Thr:p.Ala350Th

	(5/5); AAF < 0.1%							r:p.Ala352Thr
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125368:G:T	rs778844831	7.89E-05	0.059 (-0.2, 0.32)	0.0071 (-0.025, 0.039)	6.63E-01	p.Asp195Tyr:p.Asp193Tyr:p.Asp195Tyr
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125365:G:A	rs774154713	6.81E-05	0.38 (0.1, 0.67)	0.047 (0.012, 0.081)	8.00E-03	p.Gly194Arg:p.Gly192Arg:p.Gly194Arg
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138812:GC:G	rs780654165	6.10E-05	0.11 (-0.19, 0.41)	0.014 (-0.023, 0.05)	4.59E-01	p.Ala383fs:p.Ala385fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138753:GC:G	rs751192135	5.92E-05	0.14 (-0.17, 0.44)	0.017 (-0.02, 0.054)	3.72E-01	p.Ser367fs:p.Ser365fs:p.Ser367fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121464:C:T	rs377056181	4.66E-05	0.052 (-0.29, 0.39)	0.0063 (-0.035, 0.048)	7.66E-01	p.Arg154Trp:p.Arg152Trp:p.Arg154Trp
EUR	pLOF plus deleterious missense	22:29137540:C:T	rs971202099	2.51E-05	0.27 (-0.2, 0.74)	0.033 (-0.024, 0.089)	2.57E-01	p.Thr277Ile:p.Thr275Ile

	(5/5); AAF < 0.1%							:p.Thr277Ile
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121372:G:T	rs768929560	2.51E-05	0.096 (-0.37, 0.56)	0.012 (-0.045, 0.068)	6.88E-01	p.Gly123Val:p.Gly121Val:p.Gly123Val
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125372:A:T	rs372602179	2.51E-05	-0.058 (-0.52, 0.41)	-0.007 (-0.064, 0.05)	8.08E-01	p.His196Leu:p.His194Leu:p.His196Leu
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125270:G:A		2.15E-05	0.05 (-0.45, 0.55)	0.0061 (-0.055, 0.067)	8.46E-01	p.Gly162Glu:p.Gly160Glu:p.Gly162Glu
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121359:C:A	rs1316057401	1.97E-05	-0.31 (-0.83, 0.22)	-0.037 (-0.1, 0.027)	2.53E-01	p.Pro119Thr:p.Pro117Thr:p.Pro119Thr
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098884:C:T		1.97E-05	-0.094 (-0.62, 0.43)	-0.011 (-0.075, 0.052)	7.27E-01	p.Pro95Ser:p.Pro93Ser:p.Pro95Ser
EUR	pLOF plus deleterious missense	22:29137657:T:C	rs752867471	1.79E-05	0.89 (0.33, 1.4)	0.11 (0.04, 0.17)	1.67E-03	p.Phe316Ser:p.Phe314Se

	(5/5); AAF < 0.1%							r:p.Phe316Ser
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138740:G:C	rs1374143255	1.79E-05	0.0068 (-0.55, 0.56)	0.00083 (-0.066, 0.068)	9.81E-01	p.Val361Leu:p.Val359Leu:p.Val361Leu
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125297:G:T	rs766694351	1.61E-05	-0.16 (-0.74, 0.43)	-0.019 (-0.09, 0.052)	5.97E-01	p.Cys171Phe:p.Cys169Phe:p.Cys171Phe
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125392:G:A	rs775655154	1.61E-05	-0.032 (-0.61, 0.55)	-0.0039 (-0.074, 0.067)	9.15E-01	p.Asp203Asn:p.Asp201Asn:p.Asp203Asn
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29140311:C:T		1.43E-05	-0.35 (-0.97, 0.26)	-0.043 (-0.12, 0.032)	2.60E-01	p.Leu385Phe:p.Leu400Phe:p.Leu402Phe
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29140294:A:G	rs765361896	1.43E-05	0.17 (-0.45, 0.79)	0.021 (-0.054, 0.096)	5.90E-01	p.Tyr379Cys:p.Tyr394Cys:p.Tyr396Cys

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29140308:C:G	rs763307050	1.26E-05	0.61 (-0.049, 1.3)	0.074 (-0.0059, 0.15)	6.96E-02	p.Leu384Val:p.Leu399Val:p.Leu401Val
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29167043:G:A	rs1350020628	1.26E-05	0.28 (-0.38, 0.94)	0.034 (-0.046, 0.11)	4.07E-01	
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29142018:T:G	rs1196930418	1.26E-05	0.12 (-0.54, 0.78)	0.014 (-0.066, 0.094)	7.28E-01	p.Ile428Ser:p.Ile443Ser:p.Ile445Ser
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098926:A:G		1.08E-05	-0.18 (-0.9, 0.53)	-0.022 (-0.11, 0.064)	6.11E-01	p.Lys109Glu:p.Lys107Glu:p.Lys109Glu
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098885:C:T		8.97E-06	-0.41 (-1.2 ,0.37)	-0.049 (-0.14, 0.045)	3.07E-01	p.Pro95Leu:p.Pro93Leu:p.Pro95Leu
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098940:A:G	rs746388017	8.97E-06	-0.28 (-1.1 ,0.5)	-0.034 (-0.13, 0.061)	4.85E-01	p.Ile113Met:p.Ile111Met:p.Ile113Met

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137539:ACC:A		7.17E-06	0.97 (0.098, 1.8)	0.12 (0.012, 0.22)	2.92E-02	p.His278fs:p.His276fs:p.His278fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098878:G:A	rs1237728918	7.17E-06	0.71 (-0.17, 1.6)	0.086 (-0.02, 0.19)	1.12E-01	p.Val93Met:p.Val91Met:p.Val93Met
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137404:T:C	rs754510885	7.17E-06	0.54 (-0.33, 1.4)	0.066 (-0.04, 0.17)	2.25E-01	p.Phe232Leu:p.Phe230Leu:p.Phe232Leu
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094276:G:A	rs776500404	7.17E-06	-0.25 (-1.1, 0.62)	-0.03 (-0.14, 0.076)	5.74E-01	p.Gly39Asp:p.Gly37Asp:p.Gly39Asp
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121416:A:G	rs377416427	5.38E-06	0.73 (-0.28, 1.7)	0.088 (-0.034, 0.21)	1.58E-01	p.Ser138Gly:p.Ser136Gly:p.Ser138Gly
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137671:C:T		5.38E-06	0.59 (-0.42, 1.6)	0.072 (-0.051, 0.19)	2.51E-01	p.Gln321*:p.Gln319*:p.Gln321*

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094342:A:G		5.38E-06	-0.57 (-1.6, 0.44)	-0.069 (-0.19, 0.053)	2.69E-01	p.Asn61Ser:p.Asn59Ser:p.Asn61Ser
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094396:G:T	rs777119022	5.38E-06	0.5 (-0.51, 1.5)	0.061 (-0.062, 0.18)	3.32E-01	p.Gly79Val:p.Gly77Val:p.Gly79Val
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094282:A:G	rs774706235	5.38E-06	-0.47 (-1.5, 0.54)	-0.057 (-0.18, 0.066)	3.64E-01	p.Asp41Gly:p.Asp39Gly:p.Asp41Gly
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138723:C:T		5.38E-06	0.37 (-0.64, 1.4)	0.045 (-0.078, 0.17)	4.75E-01	p.Ser355Phe:p.Ser353Phe:p.Ser355Phe
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137557:C:T	rs764867793	3.59E-06	1.2 (-0.032, 2.4)	0.15 (-0.0038, 0.3)	5.62E-02	p.Arg283Cys:p.Arg281Cys:p.Arg283Cys
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138716:G:T	rs769585716	3.59E-06	0.87 (-0.37, 2.1)	0.11 (-0.045, 0.25)	1.69E-01	p.Ala353Ser:p.Ala351Ser:p.Ala353Ser

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137654:G:A		3.59E-06	0.77 (-0.47, 2)	0.093 (-0.056, 0.24)	2.22E-01	p.Gly315Glu:p.Gly313Glu:p.Gly315Glu
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094270:C:A		3.59E-06	-0.69 (-1.9, 0.55)	-0.083 (-0.23, 0.067)	2.76E-01	p.Ala37Asp:p.Ala35Asp:p.Ala37Asp
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094267:C:T	rs1399637574	3.59E-06	0.65 (-0.58, 1.9)	0.079 (-0.071, 0.23)	3.00E-01	p.Thr36Ile:p.Thr34Ile:p.Thr36Ile
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138806:ATGGGGGCT:A	rs1211200038	3.59E-06	-0.65 (-1.9, 0.59)	-0.078 (-0.23, 0.071)	3.05E-01	p.Met381fs:p.Met383fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138818:A:AT		3.59E-06	-0.65 (-1.9, 0.59)	-0.078 (-0.23, 0.071)	3.05E-01	p.Ser385fs:p.Ser387fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138623:G:A	rs750903841	3.59E-06	-0.46 (-1.7, 0.78)	-0.056 (-0.21, 0.094)	4.68E-01	

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137559:CT:C		3.59E-06	-0.45 (-1.7, 0.79)	-0.054 (-0.2, 0.096)	4.79E-01	p.Phe284fs:p.Phe282fs:p.Phe284fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121450:G:A	rs1250219980	3.59E-06	0.39 (-0.85, 1.6)	0.047 (-0.1, 0.2)	5.37E-01	p.Cys149Tyr:p.Cys147Tyr:p.Cys149Tyr
AFR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137398:C:T	rs1484654267	0.0001957	-0.31 (-1.6, 1)	-0.037 (-0.2, 0.12)	6.49E-01	p.Pro230Ser:p.Pro228Ser:p.Pro230Ser
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137600:C:T		3.59E-06	-0.26 (-1.5, 0.97)	-0.032 (-0.18, 0.12)	6.78E-01	p.Ser297Phe:p.Ser295Phe:p.Ser297Phe
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121444:A:T		3.59E-06	-0.24 (-1.5, 1)	-0.029 (-0.18, 0.12)	7.05E-01	p.Gln147Leu:p.Gln145Leu:p.Gln147Leu
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098869:G:C	rs1326783697	3.59E-06	0.23 (-1, 1.5)	0.028 (-0.12, 0.18)	7.11E-01	p.Asp90His:p.Asp88His:p.Asp90His

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29141987:C:T		3.59E-06	0.21 (-1, 1.4)	0.025 (-0.12, 0.17)	7.44E-01	p.Gln418*:p.Gln433*:p.Gln435*
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29073134:G:GCGCCGCC		3.59E-06	-0.081 (- 1.3, 1.2)	-0.0098 (-0.16, 0.14)	8.98E-01	p.Arg7fs:p.Arg7fs:p.Arg7fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138687:C:T	rs558712379	3.59E-06	-0.062 (-1.3, 1.2)	-0.0075 (-0.16, 0.14)	9.22E-01	p.Thr343Met:p.Thr341Met:p.Thr343Met
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29142019:T:G		3.59E-06	-0.046 (-1.3, 1.2)	-0.0056 (-0.16, 0.14)	9.42E-01	p.Ile428Met:p.Ile443Met:p.Ile445Met
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29167083:CA:C		3.59E-06	0.021 (-1.2, 1.3)	0.0025 (-0.15, 0.15)	9.74E-01	p.Gln486fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125296:T:C		3.59E-06	-0.014 (-1.2, 1.2)	-0.0018 (-0.15, 0.15)	9.82E-01	p.Cys171Arg:p.Cys169Arg:p.Cys171Arg

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138738:A:G	rs766331382	1.79E-06	2.4 (0.62, 4.1)	0.29 (0.075, 0.5)	7.99E-03	p.Tyr360Cys:p.Tyr358Cys:p.Tyr360Cys
AFR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138764:C:G		9.79E-05	2.2 (0.37, 4.1)	0.27 (0.045, 0.5)	1.91E-02	p.Pro369Ala:p.Pro367Ala:p.Pro369Ala
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29140318:TCA:T		1.79E-06	2 (0.21, 3.7)	0.24 (0.025, 0.45)	2.83E-02	p.Thr388fs:p.Thr403fs:p.Thr405fs
AFR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094420:G:A		9.79E-05	1.9 (0.015, 3.8)	0.23 (0.0019, 0.46)	4.81E-02	p.Arg87Lys:p.Arg85Lys:p.Arg87Lys
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121428:A:C	rs370491326	1.79E-06	-1.6 (-3.4, 0.098)	-0.2 (-0.41, 0.012)	6.43E-02	p.Asn142His:p.Asn140His:p.Asn142His
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121443:C:T		1.79E-06	1.6 (-0.19, 3.3)	0.19 (-0.023, 0.4)	8.03E-02	p.Gln147*:p.Gln145*:p.Gln147*

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29073228:G:T		1.79E-06	1.6 (-0.2, 3.3)	0.19 (-0.024, 0.4)	8.18E-02	
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137521:G:A	rs1282517348	1.79E-06	-1.5 (-3.2, 0.26)	-0.18 (-0.39, 0.031)	9.48E-02	p.Glu271Lys:p.Glu269Lys:p.Glu271Lys
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094391:G:C		1.79E-06	1.4 (-0.3, 3.2)	0.18 (-0.036, 0.39)	1.04E-01	p.Glu77Asp:p.Glu75Asp:p.Glu77Asp
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125383:T:C		1.79E-06	1.4 (-0.3, 3.2)	0.18 (-0.036, 0.39)	1.04E-01	p.Cys200Arg:p.Cys198Arg:p.Cys200Arg
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29140285:G:A		1.79E-06	1.2 (-0.54, 3)	0.15 (-0.065, 0.36)	1.75E-01	p.Trp376*:p.Trp391*:p.Trp393*
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121372:G:A		1.79E-06	1.2 (-0.54, 3)	0.15 (-0.066, 0.36)	1.76E-01	p.Gly123Asp:p.Gly121Asp:p.Gly123Asp

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125288:C:T	rs568747143	1.79E-06	1.2 (-0.56, 2.9)	0.14 (-0.067, 0.36)	1.82E-01	p.Ala168Val:p.Ala166Val:p.Ala168Val
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121371:G:C		1.79E-06	1.2 (-0.56, 2.9)	0.14 (-0.068, 0.36)	1.84E-01	p.Gly123Arg:p.Gly121Arg:p.Gly123Arg
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137351:G:A		1.79E-06	1.1 (-0.61, 2.9)	0.14 (-0.073, 0.35)	2.00E-01	p.Gly214Asp:p.Gly212Asp:p.Gly214Asp
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138770:C:T		1.79E-06	1.1 (-0.62, 2.9)	0.14 (-0.075, 0.35)	2.07E-01	p.Gln371*:p.Gln369*:p.Gln371*
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29142029:C:G	rs140385700	1.79E-06	-1.1 (-2.8, 0.69)	-0.13 (-0.34, 0.083)	2.34E-01	p.Pro432Ala:p.Pro447Ala:p.Pro449Ala
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094354:A:G	rs1329701509	1.79E-06	-1.1 (-2.8, 0.69)	-0.13 (-0.34, 0.084)	2.36E-01	p.Gln65Arg:p.Gln63Arg:p.Gln65Arg

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121477:TC:T		1.79E-06	1.1 (-0.69, 2.8)	0.13 (-0.084, 0.34)	2.37E-01	p.Phe158fs:p.Phe156fs:p.Phe158fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29073133:G:T		1.79E-06	0.99 (-0.76, 2.7)	0.12 (-0.092, 0.33)	2.68E-01	p.Met1?:p.Met1?:p.Met1?
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098866:C:A	rs763361689	1.79E-06	0.88 (-0.86, 2.6)	0.11 (-0.1, 0.32)	3.21E-01	p.Pro89Thr:p.Pro87Thr:p.Pro89Thr
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137492:T:G	rs759044199	1.79E-06	0.88 (-0.86, 2.6)	0.11 (-0.1, 0.32)	3.21E-01	p.Phe261Cys:p.Phe259Cys:p.Phe261Cys
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098925:G:A		1.79E-06	0.87 (-0.88, 2.6)	0.11 (-0.11, 0.32)	3.28E-01	p.Trp108*:p.Trp106*:p.Trp108*
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121405:T:A		1.79E-06	0.85 (-0.9, 2.6)	0.1 (-0.11, 0.32)	3.40E-01	p.Leu134Gln:p.Leu132Gln:p.Leu134Gln

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094257:G:A		1.79E-06	-0.82 (-2.6, 0.93)	-0.099 (-0.31, 0.11)	3.58E-01	
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29140320:A:C		1.79E-06	-0.8 (-2.5, 0.95)	-0.096 (-0.31, 0.12)	3.72E-01	p.Thr388Pro:p.Thr403Pro:p.Thr405Pro
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137340:A:G		1.79E-06	0.79 (-0.96, 2.5)	0.096 (-0.12, 0.31)	3.76E-01	
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094363:A:G		1.79E-06	-0.77 (-2.5, 0.98)	-0.093 (-0.31, 0.12)	3.89E-01	p.Tyr68Cys:p.Tyr66Cys:p.Tyr68Cys
AFR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137483:T:TC		9.79E-05	-0.81 (-2.7, 1.1)	-0.098 (-0.33, 0.13)	3.98E-01	p.Leu260fs:p.Leu258fs:p.Leu260fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138834:G:A		1.79E-06	-0.74 (-2.5, 1)	-0.09 (-0.3, 0.12)	4.05E-01	

EAS	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098903:A:G		0.0003592	0.69 (-1.1, 2.5)	0.084 (-0.13, 0.3)	4.46E-01	p.Glu101Gly:p.Glu99Gly:p.Glu101Gly
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125389:G:A		1.79E-06	0.59 (-1.2, 2.3)	0.071 (-0.14, 0.28)	5.10E-01	p.Gly202Ser:p.Gly200Ser:p.Gly202Ser
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137447:T:TCC	rs752591633	1.79E-06	-0.58 (-2.3, 1.2)	-0.07 (-0.28, 0.14)	5.17E-01	p.Arg247fs:p.Arg245fs:p.Arg247fs
EAS	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138691:GCAGGCCAACCT:G	rs777458364	0.0003592	0.59 (-1.2, 2.4)	0.071 (-0.15, 0.29)	5.20E-01	p.Ala346fs:p.Ala344fs:p.Ala346fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29167104:T:A		1.79E-06	0.53 (-1.2, 2.3)	0.065 (-0.15, 0.28)	5.49E-01	p.Ter493Argext*?
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121359:C:T		1.79E-06	-0.53 (-2.3, 1.2)	-0.064 (-0.28, 0.15)	5.54E-01	p.Pro119Ser:p.Pro117Ser:p.Pro119Ser

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138753:G:T		1.79E-06	-0.5 (-2.2, 1.3)	-0.06 (-0.27, 0.15)	5.78E-01	p.Ser365Ile:p.Ser363Ile:p.Ser365Ile
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094348:CTT:C		1.79E-06	-0.49 (-2.2, 1.3)	-0.059 (-0.27, 0.15)	5.83E-01	p.Phe64fs:p.Phe62fs:p.Phe64fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137417:A:G	rs777755270	1.79E-06	-0.48 (-2.2, 1.3)	-0.058 (-0.27, 0.15)	5.93E-01	p.Tyr236Cys:p.Tyr234Cys:p.Tyr236Cys
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125325:C:G		1.79E-06	0.46 (-1.3, 2.2)	0.056 (-0.16, 0.27)	6.03E-01	p.Tyr180*:p.Tyr178*:p.Tyr180*
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137495:A:C		1.79E-06	0.42 (-1.3, 2.2)	0.051 (-0.16, 0.26)	6.38E-01	p.Asp262Ala:p.Asp260Ala:p.Asp262Ala
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29142029:C:A	rs140385700	1.79E-06	-0.42 (-2.2, 1.3)	-0.051 (-0.26, 0.16)	6.40E-01	p.Pro432Thr:p.Pro447Thr:p.Pro449Thr

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094328:AT:A		1.79E-06	0.39 (-1.4, 2.1)	0.047 (-0.17, 0.26)	6.65E-01	p.Cys57fs:p.Cys55fs:p.Cys57fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098922:C:A		1.79E-06	-0.36 (-2.1, 1.4)	-0.044 (-0.26, 0.17)	6.82E-01	p.Tyr107*:p.Tyr105*:p.Tyr107*
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29140284:T:C		1.79E-06	0.36 (-1.4, 2.1)	0.044 (-0.17, 0.26)	6.84E-01	p.Trp376Arg:p.Trp391Arg:p.Trp393Arg
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098893:T:C		1.79E-06	0.36 (-1.4, 2.1)	0.043 (-0.17, 0.26)	6.89E-01	p.Tyr98His:p.Tyr96His:p.Tyr98His
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121482:G:C		1.79E-06	-0.36 (-2.1, 1.4)	-0.043 (-0.25, 0.17)	6.90E-01	
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098911:G:C		1.79E-06	0.34 (-1.4, 2.1)	0.041 (-0.17, 0.25)	7.07E-01	p.Asp104His:p.Asp102His:p.Asp104His

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094324:A:T		1.79E-06	-0.33 (-2.1, 1.4)	-0.039 (-0.25, 0.17)	7.15E-01	p.Lys55Met:p.Lys53Met:p.Lys55Met
EAS	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29121358:G:T	rs7804 69996	0.0003592	0.32 (-1.5, 2.1)	0.039 (-0.18, 0.26)	7.24E-01	p.Met118Ile:p.Met116Ile:p.Met118Ile
SAS	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098861:G:T		7.55E-05	-0.32 (-2.2, 1.5)	-0.038 (-0.26, 0.19)	7.38E-01	
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098866:C:G		1.79E-06	-0.28 (-2, 1.5)	-0.034 (-0.25, 0.18)	7.55E-01	p.Pro89Ala:p.Pro87Ala:p.Pro89Ala
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094282:A:T		1.79E-06	0.26 (-1.5, 2)	0.032 (-0.18, 0.24)	7.70E-01	p.Asp41Val:p.Asp39Val:p.Asp41Val
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094296:CAG:C		1.79E-06	-0.25 (-2, 1.5)	-0.031 (-0.24, 0.18)	7.76E-01	p.Asn47fs:p.Asn45fs:p.Asn47fs

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29142017:A:AT		1.79E-06	0.24 (-1.5, 2)	0.03 (-0.18, 0.24)	7.84E-01	p.Tyr430fs:p.Tyr445fs:p.Tyr447fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138755:C:G	rs758662506	1.79E-06	-0.24 (-2, 1.5)	-0.029 (-0.24, 0.18)	7.90E-01	p.Pro366Ala:p.Pro364Ala:p.Pro366Ala
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125269:G:A		1.79E-06	0.23 (-1.5, 2)	0.028 (-0.18, 0.24)	7.96E-01	p.Gly162Arg:p.Gly160Arg:p.Gly162Arg
AFR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098935:G:GA		9.79E-05	0.22 (-1.7, 2.1)	0.027 (-0.2, 0.25)	8.15E-01	p.Ile113fs:p.Ile111fs:p.Ile113fs
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29142057:A:T		1.79E-06	0.19 (-1.6, 1.9)	0.023 (-0.19, 0.24)	8.28E-01	p.Lys441Met:p.Lys456Met:p.Lys458Met
EAS	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138796:G:A	rs1270896219	0.0003592	0.2 (-1.6, 2)	0.024 (-0.19, 0.24)	8.29E-01	p.Trp377*:p.Trp379*

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137674:G:C		1.79E-06	-0.19 (-1.9, 1.6)	-0.022 (-0.23, 0.19)	8.36E-01	p.Ala322Pro:p.Ala320Pro:p.Ala322Pro
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094256:A:G		1.79E-06	-0.17 (-1.9, 1.6)	-0.021 (-0.23, 0.19)	8.49E-01	
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137353:G:C		1.79E-06	0.15 (-1.6, 1.9)	0.018 (-0.19, 0.23)	8.70E-01	p.Ala215Pro:p.Ala213Pro:p.Ala215Pro
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29140318:T:A		1.79E-06	0.13 (-1.6, 1.9)	0.015 (-0.2, 0.23)	8.87E-01	p.Leu387His:p.Leu402His:p.Leu404His
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138765:C:T		1.79E-06	0.12 (-1.6, 1.9)	0.015 (-0.2, 0.23)	8.93E-01	p.Pro369Leu:p.Pro367Leu:p.Pro369Leu
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29098939:T:C	rs1348515584	1.79E-06	0.1 (-1.6, 1.8)	0.012 (-0.2, 0.22)	9.09E-01	p.Ile113Thr:p.Ile111Thr:p.Ile113Thr

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137575:C:T	rs757200121	1.79E-06	0.083 (-1.7, 1.8)	0.01 (-0.2, 0.22)	9.25E-01	p.Arg289Cys:p.Arg287Cys:p.Arg289Cys
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29142101:G:A		1.79E-06	0.081 (-1.7, 1.8)	0.0098 (-0.2, 0.22)	9.28E-01	
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094281:G:C	rs1255710108	1.79E-06	0.075 (-1.7, 1.8)	0.0091 (-0.2, 0.22)	9.33E-01	p.Asp41His:p.Asp39His:p.Asp41His
SAS	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137518:G:A	rs1208119541	7.55E-05	-0.063 (-1.9, 1.8)	-0.0076 (-0.23, 0.22)	9.47E-01	p.Val270Met:p.Val268Met:p.Val270Met
EAS	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29138740:G:A		0.0003592	-0.055 (-1.8, 1.7)	-0.0067 (-0.22, 0.21)	9.52E-01	p.Val361Ile:p.Val359Ile:p.Val361Ile
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29125319:G:A	rs1395949514	1.79E-06	0.044 (-1.7, 1.8)	0.0054 (-0.21, 0.22)	9.60E-01	p.Trp178*:p.Trp176*:p.Trp178*

EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29137644:C:A		1.79E-06	-0.03 (-1.8, 1.7)	-0.0037 (-0.22, 0.21)	9.73E-01	p.Gln312Lys:p.Gln310Lys:p.Gln312Lys
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094257:G:T	rs1171737622	1.79E-06	-0.023 (-1.8, 1.7)	-0.0028 (-0.21, 0.21)	9.79E-01	
EUR	pLOF plus deleterious missense (5/5); AAF < 0.1%	22:29094333:T:G		1.79E-06	-0.015 (-1.8, 1.7)	-0.0019 (-0.21, 0.21)	9.86E-01	p.Leu58Arg:p.Leu56Arg:p.Leu58Arg

Abbreviations: pLOF, predicted loss of function; CPRA, chromosome position reference alternative; RR, reference homozygote genotype; RA, reference-alternative genotype; AA, alternative homozygote genotype; SD, standard deviation; CI, confidence interval; p, P-value; AAF alternative allele frequency; AAC, alternate allele count.

A distinct GWAS effector gene prioritization method, the gene-level Polygenic Priority Score (PoPS), yielded similar results to the Ei.

KREMEN1 as a gene associated with eBMD at exome-wide significance and their evidence from common variant GWAS, predicted by PoPS. “Positive control” indicates whether a gene is among a subset of 56 expert-curated genes implicated in bone mineral density by Mendelian genetics or pharmacological validation. eBMD PoP scores were calculated for all genes in the genome, whereas the PoPS rank was only derived for genes in GWAS loci (a total of 857 eBMD GWAS loci are included).

Table 3 shows that KREMEN1 was discovered exclusively in multi-ancestry meta-analysis of eBMD (Genetic exposure, variant type; frequency cutoff in % = pLOF plus deleterious missense (5/5); AAF < 1%). Abbreviations: European, EUR; African, AFR; South asians, SAS; East asians, EAS; predicted loss of function, pLOF; alternative allele frequency, AAF; confidence

interval, CI; standard deviation, SD; estimated bone mineral density, eBMD; P-value, p; reference-reference genotype, RR; reference-alternative genotype, RA; alternative-alternative genotype, AA; grams per square centimeter, g/cm^2; ratio of true heterogeneity to total observed variation, I2.

Table 3

Ancestry	AAF, fraction of 1	Beta (95% CI) per allele in SD units of eBMD	Beta (95% CI) per allele in g/cm^2 units of eBMD	p
EUR	0.0016	0.1382(0.0802,0.1961)	0.0168(0.0097,0.0238)	2.96E-06
AFR	0.0019	0.5045(0.0727,0.9363)	0.0612(0.0088,0.1136)	2.20E-02
EAS	0.0029	0.2963(-0.3345,0.9271)	0.0359(-0.0406,0.1125)	3.57E-01
SAS	0.0020	0.316(-0.0411,0.6731)	0.0383(-0.005,0.0816)	8.29E-02

Table 3 (cont.)

Ancestry	Genotype counts, RR|RA|AA genotypes	p-value for heterogeneity in effect estimates between ancestries	Multi-ancestry beta (95% CI) per allele in SD units of eBMD	Multi-ancestry p	I^2
EUR	277,895|912|0	2.86E-01	0.1502(0.0937,0.2066)	1.87485E-07	20.76%
AFR	5,090|19|0				
EAS	1,384|8|0				
SAS	6,597|27|0				

Mendelian randomization of circulating protein abundances with eBMD

Large-scale proteomics data were leveraged to provide further evidence implicating *KREMEN1* in bone mineral density. Two-sample Mendelian randomization (MR; Davey Smith et

al., Int. J. Epidemiol., 2003, 32, 1-22) to identify circulating proteins genetically-associated with eBMD. First, *cis*-SNPs associated with 863 circulating protein levels from two proteomic GWAS were identified, the INTERVAL study and the AGES study. Both studies measured circulating proteins using the SomaScan platform, and included 3,301 and 3,200 European ancestry individuals, respectively. MR analyses revealed that genetically-predicted concentrations of KREMEN1 from INTERVAL ($P<9.2x10^{-5}$, corresponding to a Bonferroni correction for KREMEN1 tested in INTERVAL) and KREMEN1 from AGES ($P<6.5x10^{-5}$, corresponding to a Bonferroni correction for KREMEN1 tested in AGES) were associated with eBMD. In addition, KREMEN1 as a protein was significantly associated with eBMD from INTERVAL (MR pval$<9.2 \times 10^{-5}$) and AGES (MR pval$<6.45 \times 10^{-5}$). Beta: effect estimate for eBMD in SD units, per SD unit increase in protein level.

Various modifications of the described subject matter, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. Each reference (including, but not limited to, journal articles, U.S. and non-U.S. patents, patent application publications, international patent application publications, gene bank accession numbers, and the like) cited in the present application is incorporated herein by reference in its entirety and for all purposes.

What is Claimed is:

1. Use of a Kringle Containing Transmembrane Protein 1 (KREMEN1) inhibitor in the preparation of a medicament for treating decreased bone mineral density or risk of development of decreased bone mineral density in a subject, wherein the KREMEN1 inhibitor comprises an inhibitory nucleic acid molecule that hybridizes to a KREMEN1 nucleic acid molecule, and wherein the inhibitory nucleic acid molecule comprises an antisense nucleic acid molecule, a small interfering RNA (siRNA), or a short hairpin RNA (shRNA).

2. The use according to claim 1, wherein the decreased bone mineral density comprises osteopenia, Type I osteoporosis, Type II osteoporosis, or secondary osteoporosis.

3. The use according to claim 1 or 2, further comprising detecting the presence or absence of a KREMEN1 variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide in a biological sample from the subject.

4. The use according to claim 3, wherein a therapeutic agent that treats decreased bone mineral density is to be further administered in a standard dosage amount to a subject wherein the KREMEN1 variant nucleic acid molecule is absent from the biological sample, wherein the therapeutic agent is chosen from alendronate, ibandronate, zoledronate, risedronate, calcitonin, teriparatide, denosumab, estrogen and progesterone, raloxifene, or any combination thereof.

5. The use according to claim 3, wherein a therapeutic agent that treats decreased bone mineral density is to be administered in a dosage amount that is the same as or less than a standard dosage amount to a subject that is heterozygous for the KREMEN1 variant nucleic acid molecule.

6. The use according to any one of claims 3 to 5, wherein the KREMEN1 predicted variant nucleic acid molecule is a splice-site variant, a stop-gain variant, a start-loss variant, a stop-loss variant, a frameshift variant, or an in-frame indel variant, or a variant that encodes a truncated KREMEN1 predicted loss-of-function polypeptide.

7. The use according to any one of claims 3 to 6, wherein the KREMEN1 predicted loss-of-function variant nucleic acid molecule is listed in Figure 3 or Table 2, or an mRNA molecule produced therefrom, or a cDNA molecule produced from the mRNA molecule.

8. The use according to claim 6, wherein the KREMEN1 variant nucleic acid molecule encodes a truncated KREMEN1 predicted loss-of-function polypeptide.

9. Use of a therapeutic agent that treats decreased bone mineral density in the preparation of a medicament for treating a subject that has decreased bone mineral density or is at risk of developing decreased bone mineral density, wherein the subject has been determined to have a Kringle Containing Transmembrane Protein 1 (KREMEN1) variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide by:

performing or having performed a sequence analysis on a biological sample obtained or having been obtained from the subject to determine if the subject has a genotype comprising the KREMEN1 variant nucleic acid molecule; and

wherein the therapeutic agent that treats decreased bone mineral density is to be administered or continued to be administered in a standard dosage amount to a subject that is KREMEN1 reference, and/or a KREMEN1 inhibitor is to be administered to the subject;

wherein the therapeutic agent that treats decreased bone mineral density is to be administered or continued to be administered in an amount that is the same as or less than a standard dosage amount to a subject that is heterozygous for the KREMEN1 variant nucleic acid molecule, and/or a KREMEN1 inhibitor is to be administered to the subject; or

wherein the therapeutic agent that treats decreased bone mineral density is to be administered or continued to be administered in an amount that is the same as or less than a standard dosage amount to a subject that is homozygous for the KREMEN1 variant nucleic acid molecule;

wherein the presence of a genotype having the KREMEN1 variant nucleic acid molecule encoding the KREMEN1 predicted loss-of-function polypeptide indicates the subject has a decreased risk of developing decreased bone mineral density;

wherein the therapeutic agent is chosen from alendronate, ibandronate, zoledronate, risedronate, calcitonin, teriparatide, denosumab, estrogen and progesterone, raloxifene, or any combination thereof; and

wherein the KREMEN1 inhibitor comprises an inhibitory nucleic acid molecule that hybridizes to a KREMEN1 nucleic acid molecule, and wherein the inhibitory nucleic acid molecule comprises an antisense nucleic acid molecule, a small interfering RNA (siRNA), or a short hairpin RNA (shRNA).

10. The use according to claim 9, wherein the subject is KREMEN1 reference, and the subject is to be administered or continued to be administered the therapeutic agent that treats

decreased bone mineral density in a standard dosage amount, and is to be administered a KREMEN1 inhibitor.

11. The use according to claim 9, wherein the subject is heterozygous for a KREMEN1 variant nucleic acid molecule, and the subject is to be administered or continued to be administered the therapeutic agent that treats decreased bone mineral density in an amount that is the same as or less than a standard dosage amount, and is to be administered a KREMEN1 inhibitor.

12. The use according to any one of claims 9 to 11, wherein the KREMEN1 variant nucleic acid molecule is a splice-site variant, a stop-gain variant, a start-loss variant, a stop-loss variant, a frameshift variant, or an in-frame indel variant, or a variant that encodes a truncated KREMEN1 predicted loss-of-function polypeptide.

13. The use according to any one of claims 9 to 12, wherein the KREMEN1 predicted loss-of-function variant nucleic acid molecule is listed in Figure 3 or Table 2, or an mRNA molecule produced therefrom, or a cDNA molecule produced from the mRNA molecule.

14. The use according to any one of claims 9 to 12, wherein the KREMEN1 variant nucleic acid molecule encodes a truncated KREMEN1 predicted loss-of-function polypeptide.

15. The use according to any one of claims 9 to 14, wherein the decreased bone mineral density is an osteopenia, Type I osteoporosis, Type II osteoporosis, or secondary osteoporosis.

16. A method of identifying a subject having an increased risk of developing decreased bone mineral density, the method comprising:

determining or having determined the presence or absence of a Kringle Containing Transmembrane Protein 1 (KREMEN1) variant nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide in a biological sample obtained from the subject;

wherein:

when the subject is KREMEN1 reference, then the subject has an increased risk of developing decreased bone mineral density; and

when the subject is heterozygous or homozygous for the KREMEN1 variant nucleic acid molecule encoding the KREMEN1 predicted loss-of-function polypeptide, then the subject has a decreased risk of developing decreased bone mineral density.

17. The method according to claim 16, wherein the KREMEN1 variant nucleic acid molecule is a splice-site variant, a stop-gain variant, a start-loss variant, a stop-loss variant, a

frameshift variant, or an in-frame indel variant, or a variant that encodes a truncated KREMEN1 predicted loss-of-function polypeptide.

18. The method according to claim 16 or claim 17, wherein the KREMEN1 predicted loss-of-function variant nucleic acid molecule is listed in Figure 3 or Table 2, or an mRNA molecule produced therefrom, or a cDNA molecule produced from the mRNA molecule.

19. The method according to claim 16 or claim 17, wherein the KREMEN1 variant nucleic acid molecule encodes a truncated KREMEN1 predicted loss-of-function polypeptide.

20. The use according to any one of claims 16 to 19, wherein the decreased bone mineral density is an osteopenia, Type I osteoporosis, Type II osteoporosis, or secondary osteoporosis.

21. Use of a therapeutic agent that treats decreased bone mineral density in the preparation of a medicament for use in treating decreased bone mineral density in a subject having:

a Kringle Containing Transmembrane Protein 1 (KREMEN1) variant genomic nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide;

a KREMEN1 variant mRNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; or

a KREMEN1 variant cDNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; and

wherein the therapeutic agent is chosen from alendronate, ibandronate, zoledronate, risedronate, calcitonin, teriparatide, denosumab, estrogen and progesterone, raloxifene, or any combination thereof.

22. The use according to claim 21, wherein the decreased bone mineral density is an osteopenia, Type I osteoporosis, Type II osteoporosis, or secondary osteoporosis.

23. Use of a Kringle Containing Transmembrane Protein 1 (KREMEN1) inhibitor in the preparation of a medicament for treating decreased bone mineral density in a subject that:

a) is reference for a KREMEN1 genomic nucleic acid molecule, a KREMEN1 mRNA molecule, or a KREMEN1 cDNA molecule; or

b) is heterozygous for:

i) a KREMEN1 variant genomic nucleic acid molecule encoding a KREMEN1 predicted loss-of-function polypeptide;

ii) a KREMEN1 variant mRNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; or

iii) a KREMEN1 variant cDNA molecule encoding a KREMEN1 predicted loss-of-function polypeptide; and

wherein the KREMEN1 inhibitor comprises an inhibitory nucleic acid molecule that hybridizes to a KREMEN1 nucleic acid molecule, and wherein the inhibitory nucleic acid molecule comprises an antisense nucleic acid molecule, a small interfering RNA (siRNA), or a short hairpin RNA (shRNA).

24. The use according to claim 23, wherein the decreased bone mineral density is an osteopenia, Type I osteoporosis, Type II osteoporosis, or secondary osteoporosis.

25. The use according to any one of claims 21 to 24, wherein the KREMEN1 predicted loss-of-function variant nucleic acid molecule is listed in Figure 3 or Table 2, or an mRNA molecule produced therefrom, or a cDNA molecule produced from the mRNA molecule.