

The New England Journal of Medicine

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VOLUME 346

MAY 16, 2002

NUMBER 20



HIGH BONE DENSITY DUE TO A MUTATION IN LDL-RECEPTOR-RELATED PROTEIN 5

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ABSTRACT

Background Osteoporosis is a major public health problem of largely unknown cause. Loss-of-function mutations in the gene for low-density lipoprotein receptor-related protein 5 (*LRP5*), which acts in the Wnt signaling pathway, have been shown to cause osteoporosis-pseudoglioma.

Methods We performed genetic and biochemical analyses of a kindred with an autosomal dominant syndrome characterized by high bone density, a wide and deep mandible, and torus palatinus.

Results Genetic analysis revealed linkage of the syndrome to chromosome 11q12-13 (odds of linkage, >1 million to 1), an interval that contains *LRP5*. Affected members of the kindred had a mutation in this gene, with valine substituted for glycine at codon 171 (*LRP5*_{V171}). This mutation segregated with the trait in the family and was absent in control subjects. The normal glycine lies in a so-called propeller motif that is highly conserved from fruit flies to humans. Markers of bone resorption were normal in the affected subjects, whereas markers of bone formation such as osteocalcin were markedly elevated. Levels of fibronectin, a known target of signaling by Wnt, a developmental protein, were also elevated. In vitro studies showed that the normal inhibition of Wnt signaling by another protein, Dickkopf-1 (*Dkk-1*), was defective in the presence of *LRP5*_{V171} and that this resulted in increased signaling due to unopposed Wnt activity.

Conclusions The *LRP5*_{V171} mutation causes high bone density, with a thickened mandible and torus palatinus, by impairing the action of a normal antagonist of the Wnt pathway and thus increasing Wnt signaling. These findings demonstrate the role of altered *LRP5* function in high bone mass and point to *Dkk* as a potential target for the prevention or treatment of osteoporosis. (N Engl J Med 2002;346:1513-21.)

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OSTEOPOROSIS is a major public health problem, and its prevalence is increasing.¹⁻³ In the United States, nearly 1 million fractures occur annually in people over the age of 65 years, the majority of which are due to osteoporosis.^{1,4} Osteoporotic fractures are associated with substantial morbidity, and the estimated rate of death in the first year after a hip fracture is 25 to 30 percent.^{5,6}

Bone mass, a major determinant of the risk of osteoporotic fracture, increases during childhood and adolescence, reaching a peak at about the age of 20 years.⁷ Twin and family studies indicate that genetic factors account for approximately 75 percent of the variation in peak bone mass,⁷ although the genes that contribute to this variation are largely unknown.

In this setting, the investigation of rare mendelian disorders may identify pathways that affect the trait. One disorder characterized by increased bone density with largely normal bone is an autosomal dominant disease with variable clinical features, including entrapment neuropathies, increased levels of alkaline phosphatase, a square jaw, and torus palatinus.⁸⁻¹⁵ In 1997, Johnson et al. reported the linkage of a gene causing nonsyndromic high bone mass in a single family to a 30-cM region of 11q12-13 with a lod score of 5.74.¹⁶ Because few kindreds have been studied, the question of whether these varied clinical manifestations arise from mutations in the same gene or in different genes has not been answered.

Osteoporosis-pseudoglioma, an autosomal recessive disease characterized by low bone mass, with

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childhood fractures and abnormal eye development, has also been mapped to 11q12–13.¹⁷ The disorder has recently been shown to be due to an inherited loss of function of the gene for low-density lipoprotein receptor–related protein 5 (*LRP5*).¹⁸ This protein is involved in the Wnt signaling pathway, acting as a coreceptor for Wnt, a developmental protein, and as a target for the inhibitory effects of Dickkopf (Dkk), another developmental protein, on Wnt signaling.^{19–21} These findings suggest a link between Wnt signaling through *LRP5* and bone density, and they raise the question of whether gain-of-function mutations in the *LRP5* gene cause high bone mass. We performed genetic and biochemical analyses in a kindred with an autosomal dominant syndrome characterized by high bone density, a wide and deep mandible, and torus palatinus.

METHODS

Subjects

Twenty members of a kindred of white ancestry from Connecticut participated in the study. The protocol was approved by the Human Investigation Committee at Yale University School of Medicine. All the subjects (or their guardians, in the case of minors) provided written informed consent. Assent was also obtained from minors. The subjects provided a medical history and a blood sample for DNA preparation. Bone density was measured in 16 of the 20 kindred members. The four members in whom bone density was not measured included two children (10 and 13 years old), one person who died before the study, and one person who declined to participate. We performed detailed serum and urinary biochemical measurements in four kindred members with very high bone density; these values were compared with the values in nine healthy control subjects.

Bone-Density Measurements

We measured bone mineral density in the lumbar spine, femoral neck, and total body, using dual-energy x-ray absorptiometry with a densitometer (QDR 4500W, Hologic, or DPXL, Lunar). The results are expressed as z scores (the number of standard deviations from the mean value for persons in the general population matched for age, sex, and race).

Biochemical Evaluation

Serum levels of parathyroid hormone and tartrate-resistant acid phosphatase and plasma levels of vitamin D metabolites were measured as previously reported.^{22,23} Serum calcium was measured with the use of flame atomic absorptiometry. Serum creatinine and phosphate levels and levels of the brain isoform of creatine kinase were measured with the use of an AutoAnalyzer (model 747-200, Hitachi) in the clinical-chemistry laboratory of Yale–New Haven Hospital in New Haven, Connecticut. Serum osteocalcin, bone-specific alkaline phosphatase, urinary N-telopeptide of type I collagen, the receptor for activation of nuclear factor- κ B ligand, osteoprotegerin, transforming growth factor β 1 (TGF- β 1), and fibronectin were measured with the use of commercial kits. Mean (\pm SD) values in affected subjects and controls were compared with the use of unpaired two-tailed t-tests.

Genetic Studies

Polymorphic markers on 11q were genotyped with the use of a polymerase-chain-reaction (PCR) assay and specific fluorescent

primers and genomic DNA from members of the kindred as the template. The products were fractionated by electrophoresis on an ABI 3700 DNA analyzer (Applied Biosystems), and genotypes were determined with the use of Genotyper software. We analyzed linkage with the use of the Linkage program, specifying high bone density as an autosomal dominant trait with a disease-allele frequency of 0.00001, complete penetrance, and a phenocopy prevalence of 0.00001. Unstudied obligate carriers were classified as “phenotype unknown.” Altering the specified model had minor effects on the lod score.

LRP5 mutations in members of the kindred were sought by PCR amplification of short segments spanning the coding region and intron–exon boundaries of *LRP5*; the products were analyzed by electrophoresis under nondenaturing conditions. Identified variants were subjected to DNA sequencing. The controls were 210 unrelated white persons not known to have abnormalities in bone density.

In Vitro Biochemical Studies

We used a well-characterized signaling system in the mouse fibroblast NIH3T3 cell line to examine the effect of mutant *LRP5* on Wnt signaling.¹⁹ In each experiment, plasmids encoding LEF-1 (a transcription factor activated by Wnt signaling) constitutively expressed from a cytomegalovirus promoter, luciferase under control of an LEF-1–responsive promoter, and green fluorescent protein were introduced by transfection into cells seeded in 24-well plates. In addition, plasmids encoding wild-type (normal) *LRP5* or *LRP5*_{V171}, Wnt-1, and Dkk type 1 (Dkk-1) were transfected in indicated combinations. The total amount of DNA transfected in each experiment was kept constant (0.5 μ g per well) by the addition of varying amounts of a plasmid encoding β -galactosidase (LacZ). One day after transfection, the cells were lysed, and the levels of luciferase activity and green fluorescent protein were measured; luciferase activity was normalized according to the green fluorescent protein level in order to account for variation in the efficiency of transfection. Each experiment was conducted in triplicate. Values are reported as means \pm SD.

RESULTS

Identification of the Kindred

The kindred was identified when two persons found on clinical screening to have extremely high bone density (Subjects 3 and 6) were incidentally noted to be related to one another. In addition to high bone density, both had a strikingly wide and deep mandible (Fig. 1A and 1B) and torus palatinus (Fig. 1C). Radiographic studies showed normal skeletal morphology except for dramatically thickened mandibular ramii (Fig. 1D), marked cortical thickening in long bones (Fig. 1E), and dense vertebrae (Fig. 1F). There was no radiographic evidence of osteopetrosis, and the shape of the vertebral bodies was normal. There were no cranial-nerve palsies. Both persons were asymptomatic but noted difficulty staying afloat while swimming. They reported that other family members had the same facial features, prompting an investigation of the kindred.

Bone Density

A total of seven members of the kindred had strikingly elevated age- and sex-adjusted bone mineral density in the lumbar spine, femoral neck, and total body, whereas nine had normal bone density at all

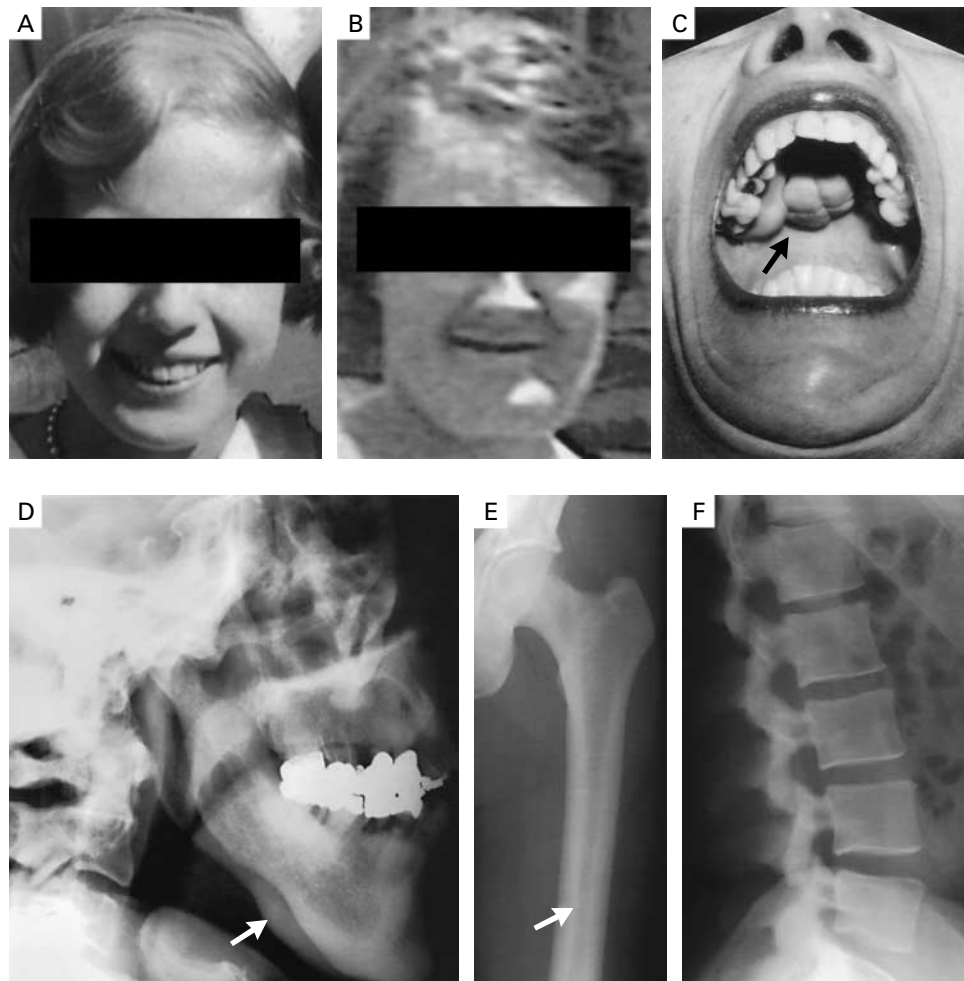


Figure 1. Clinical and Radiographic Features of Affected Members of the Kindred.

Photographs of an affected member at the ages of 12 years (Panel A) and 45 years (Panel B) show the development of the wide, deep mandible that was characteristic of all affected members of the kindred. A large, lobulated torus palatinus in an affected member (Panel C, arrow) was also characteristic of all affected kindred members. Characteristic radiographic findings included an abnormally thick mandibular ramus (arrow, Panel D); a markedly thickened cortex and narrowed medullary cavity (arrow) in the femur (Panel E), which was otherwise normal; and dense but otherwise normal-appearing vertebrae (Panel F).

sites (Table 1). Phenotypic classification of bone density as either very high (indicating affected subjects) or normal (indicating unaffected subjects) was unambiguous. In affected subjects, age- and sex-adjusted bone density of the lumbar spine was at least 5 SD above the population mean, and the mean z scores for bone density in the lumbar spine, femoral neck, and total body were 6.83, 4.42, and 4.78, respectively. In contrast, none of the unaffected subjects had a z score at any site that was greater than 2.71, and the mean z scores for bone density in the lumbar spine, femoral neck, and total body were 0.40, -0.61, and

-0.08, respectively. All affected subjects had torus palatinus and the striking square jaw; none of the unaffected subjects had either of these features. None of the affected subjects had a history of bone fracture.

The pattern of elevated bone density with torus palatinus and a square jaw was characteristic of autosomal dominant transmission with high penetrance (Fig. 2). The trait was present in successive generations, affected or obligate-carrier parents had approximately equal numbers of affected and unaffected offspring overall, and there was male-to-male transmission. There were four deceased obligate gene car-

TABLE 1. CLINICAL PHENOTYPES IN THE KINDRED.

SUBJECT No.	AGE	SEX	BONE DENSITY			TORUS PALATINUS
			L1-L4	FEMORAL NECK	TOTAL BODY	
	yr		z score			
Affected						
1	77	F	6.83	2.39	4.53	Yes
3	53	F	6.94	3.83	5.11	Yes
5	49	M	8.08	6.52	—	Yes
6	69	M	5.83	3.16	4.60	Yes
7	41	M	5.04	7.10	4.45	Yes
10	71	M	7.73	3.76	5.20	Yes
16*	77	M	—	—	—	Yes
20	50	M	7.37	4.19	—	Yes
Mean±SD			6.83±1.07	4.42±1.74	4.78±0.35	
Unaffected						
2	52	M	-0.71	0.16	-0.93	No
4	56	M	-0.20	-0.38	0.07	No
8	38	F	-1.33	-1.47	-0.95	No
9	73	F	1.48	-0.63	-0.74	No
11	23	M	1.14	1.15	0.56	No
12	49	F	0.39	-1.97	-0.23	No
13	65	M	0.15	-1.30	-0.59	No
14	38	F	-0.03	-0.11	0.64	No
15	45	F	2.71	-0.97	1.43	No
17	47	M	—	—	—	No
18	13	M	—	—	—	No
19	10	F	—	—	—	No
Mean±SD			0.40±1.22	-0.61±0.95	-0.08±0.82	

*The subject died before the study was performed.

riers, one of whom (Subject 16) was examined before death and found to have torus palatinus and the characteristic mandible. Three living family members (Subjects 17, 18, and 19) did not undergo bone-density measurements, but they did not have torus palatinus or the characteristic facies.

Biochemical Findings

The mean serum calcium and phosphate levels were normal in the affected subjects; the urinary calcium level was at the high end of the normal range (Table 2). Serum levels of parathyroid hormone and vitamin D metabolites were also normal. In addition, the mean serum level of tartrate-resistant acid phosphatase was normal (1.2 ± 2 U per liter), and serum levels of the brain isoform of creatine kinase were undetectable; these two biochemical markers may be elevated in patients with osteopetrosis.^{24,25}

The mean level of urinary N-telopeptide of type I collagen, a marker of bone resorption, was normal in the affected subjects (Table 2). Circulating levels of the receptor for activation of nuclear factor- κ B ligand and osteoprotegerin, cytokines that regulate rates of bone resorption,²⁶ were also normal. As noted above, the level of tartrate-resistant acid phosphatase, a marker

of osteoclast activity, was normal. In contrast, the mean level of serum osteocalcin, a marker of bone formation, was markedly elevated — more than three times the value in the controls. The values for bone-specific alkaline phosphatase were not elevated in the affected subjects (mean value, 25 ± 6 U per liter; normal range, 15 to 41). The levels of TGF- β 1 were markedly elevated. These findings suggested that increased bone formation with unaltered bone resorption was the mechanism of high bone density in affected members of this family.

Point Mutation in *LRP5*

Because of prior reports of linkage of both high-bone-density and low-bone-density phenotypes to 11q12-13,^{16,17,27,28} we performed genotyping for 37 polymorphic genetic markers across a 40-cM segment of this interval (Fig. 2). Analysis of the relation between inheritance of this chromosome segment and high bone density provided strong evidence of linkage, with a multipoint lod score of 5.30. When the three subjects in whom bone density was not measured but who did not have torus palatinus or a square jaw were included in the analysis as unaffected subjects, the lod score was 6.21 (odds of linkage

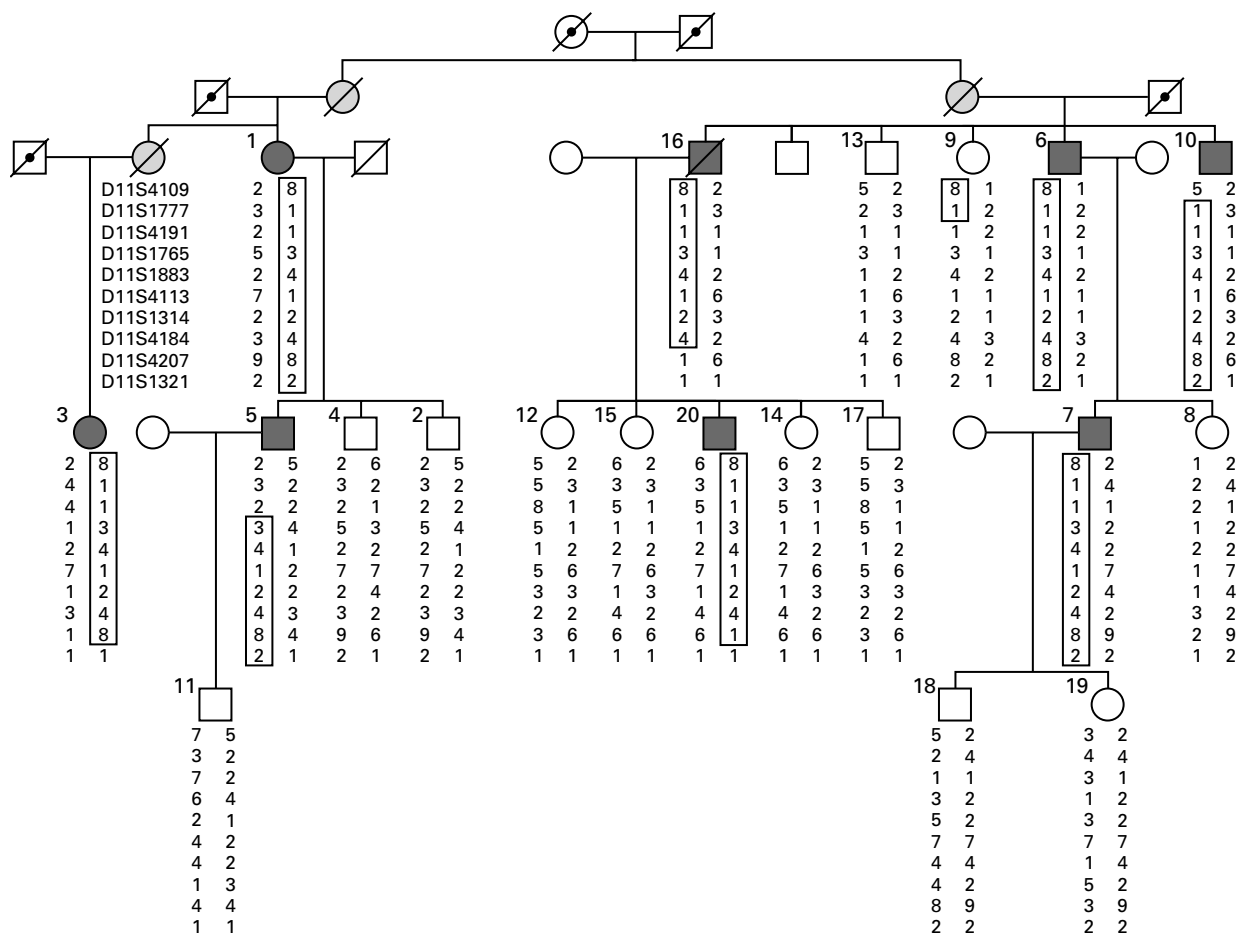


Figure 2. Pedigree of the Kindred Showing the Linkage of Bone Density to 11q12–13. Solid symbols indicate affected members of the kindred, open symbols unaffected members, shaded symbols obligate carriers, symbols with dots members who were not evaluated, squares male members, circles female members, and slashes deceased members. The members are numbered in the order in which blood samples were received at the laboratory. Below each symbol, the genotypes for a subgroup of genetic markers at 11q12–13 are shown in their chromosomal order. Chromosome segments that cosegregate with the affected status are enclosed by boxes. All affected members shared a chromosome segment extending from locus *D11S1765* to *D11S4184*.

to 11q12–13, >1 million to 1). Meiotic recombination events in affected subjects localized the disease gene to the 16-cM interval flanked by loci *D11S4191* and *D11S4207*. This linked interval contains *LRP5*, the gene for osteoporosis–pseudoglioma.¹⁸

The protein encoded by *LRP5* spans the plasma membrane once and contains a large extracellular segment with four domains that are each predicted to form a structure resembling a propeller with six blades (Fig. 3A). The amino acid sequence YWTD or a variant of this sequence is found as a conserved element (a YWTD repeat) in each propeller blade.^{29,30}

We examined the coding sequence of *LRP5* for DNA-sequence variants and identified one variant in all the affected subjects (Fig. 3B). This variant intro-

duced a single base substitution that resulted in a missense mutation, with valine substituted for glycine at residue 171 (*LRP5*_{V171}) (Fig. 3C). The residue lies in the fourth blade of the first propeller, two amino acids beyond the aspartate residue of the YWTD sequence (Fig. 3A and 3D).

The heterozygous *LRP5*_{V171} mutation precisely cosegregated with high bone density in the kindred (Fig. 3B); *LRP5*_{V171} is not a simple polymorphism, since it was absent in 420 control chromosomes from unrelated, unaffected subjects. The normal glycine residue shows extraordinary evolutionary conservation (Fig. 3D). Glycine is found at the same position in the fourth blade of the first three propellers of *LRP5* and *LRP6* in humans and mice, as well as in the sin-

TABLE 2. INDEXES OF MINERAL METABOLISM AND MARKERS OF BONE TURNOVER IN FOUR AFFECTED SUBJECTS AND NINE CONTROLS.*

INDEX OR MARKER	AFFECTED SUBJECTS	NORMAL CONTROLS†	NORMAL RANGE
Serum calcium (mg/dl)	9.7±0.6	9.5±0.2	8.8–10.2
Serum phosphate (mg/dl)	3.8±0.6	3.3±0.3	3.1–4.5
Parathyroid hormone (pg/ml)	36±8	31±6	10–65
25-Hydroxyvitamin D (ng/ml)	22±2	27±4	>15
1,25-Dihydroxyvitamin D (pg/ml)	49±10	47±6	20–65
Urinary calcium (mg/24 hr)	277±90	147±30	<300
Osteocalcin (ng/ml)	32.3±7.4‡	9.8±1.8	5.1–12.4
Urinary NTX (nM bone collagen equivalent/mM creatine)	25±6	52±21	18–50
RANKL (pmol/liter)	1.1±0.6	1.5±0.6	NA
Osteoprotegerin (pmol/liter)	3.1±1.6	4.7±1.2	NA
TGF-β1 (ng/ml)	13.0±7.6‡	2.3±0.6	1.6–3.2
Fibronectin (μg/ml)	66±10‡	33±9	NA

*Plus-minus values are means ±SD. NTX denotes N-telopeptide of type 1 collagen, RANKL receptor for activation of nuclear factor-κB ligand, TGF-β1 transforming growth factor β1, and NA not available. To convert the values for serum calcium to millimoles per liter, multiply by 0.250. To convert the values for phosphate to millimoles per liter, multiply by 0.3229. To convert the values for urinary calcium to millimoles per 24 hours, multiply by 0.02495.

†The controls included four men (mean age, 54 years) and five women (mean age, 57 years).

‡P<0.001 for the comparison with the controls.

gle propeller of the low-density lipoprotein (LDL) receptor in humans, mice, rats, pigs, hamsters, and rabbits. Moreover, glycine is also found at this position in the first propeller of the *Drosophila melanogaster* LDL-receptor-related protein homologue, *arrow*. In addition, glycine is present at this position in a wide range of other YWTD propellers, including those in other LDL-receptor-related proteins, as well as those in the epidermal growth factor precursor, the very-low-density lipoprotein receptor, and the vitellogenin receptor in fruit flies and mosquitos (protein sequences are available at <http://www.ncbi.nlm.nih.gov/entrez>). The evolutionary conservation of this glycine residue is strong evidence of the functional importance of its mutation in our kindred.

Molecular Studies

If this mutation indeed causes gain of LRP5 function and increased Wnt signaling, downstream target genes in the Wnt signaling pathway should show increased expression in vivo. A direct transcriptional target of Wnt signaling is the extracellular matrix protein fibronectin.³¹ Fibronectin levels were markedly elevated in the affected members of our kindred, with

a mean level that was more than 3 SD above the mean level in controls (Table 2).

Possible mechanisms of increased Wnt signaling include constitutive signaling in the absence of a ligand, increased signaling in response to a ligand, or loss of action of a normal inhibitor of signaling. To identify the mechanism, we performed in vitro studies using the mouse fibroblast NIH3T3 cell line, in which the expression of normal LRP5 potentiates Wnt signaling.¹⁹ We found that the expression of LRP5_{V171} did not activate signaling in the absence of Wnt-1, a finding that ruled out constitutive signaling as the mechanism (Fig. 4A). Activation of the signaling pathway in response to Wnt-1 was the same with normal and mutant LRP5; this finding ruled out increased activation by a ligand as the mechanism (Fig. 4A). Finally, we tested the action of the endogenous antagonist of Wnt signaling, Dkk-1. Although Dkk-1 inhibited Wnt signaling in conjunction with wild-type LRP5, Dkk-1 inhibition of Wnt signaling was virtually abolished in cells expressing LRP5_{V171} (Fig. 4B). These findings indicated that LRP5_{V171} results in increased Wnt signaling because of loss of Dkk antagonism.

DISCUSSION

Recent work has established that loss of function of *LRP5* leads to reduced bone mass.¹⁸ Our study shows that a gain-of-function mutation in *LRP5* causes an autosomal dominant disorder characterized by high bone density, torus palatinus, and a wide, deep mandible.

Our in vitro and in vivo studies show that the *LRP5*_{V171} mutation increases Wnt signaling. The mutation impairs antagonism of Wnt signaling by Dkk-1 in vitro, and the levels of fibronectin, a downstream target of Wnt signaling, are increased in vivo in patients with this mutation. These findings indicate that unopposed Wnt signaling due to loss of action of a normal antagonist is the molecular mechanism that accounts for the effect of the mutation.

Nonsyndromic high bone mass has been linked to 11q12–13 in another kindred.¹⁶ While we were preparing this report, Little et al. identified an *LRP5* mutation in the other kindred.³² Remarkably, this mutation is identical to the *LRP5*_{V171} mutation in our kindred. To our knowledge, these families do not share a common ancestor, suggesting that the mutations have arisen independently; however, the possibility of a very remote common ancestor cannot be excluded. It is striking that the same mutation is associated with nonsyndromic high bone mass in one family and syndromic high bone mass in the other. These findings suggest that alleles of other genes or environmental factors influence phenotypic manifestations of the mutation and that other phenotypes in kindreds with autosomal dominant high bone mass may also arise from

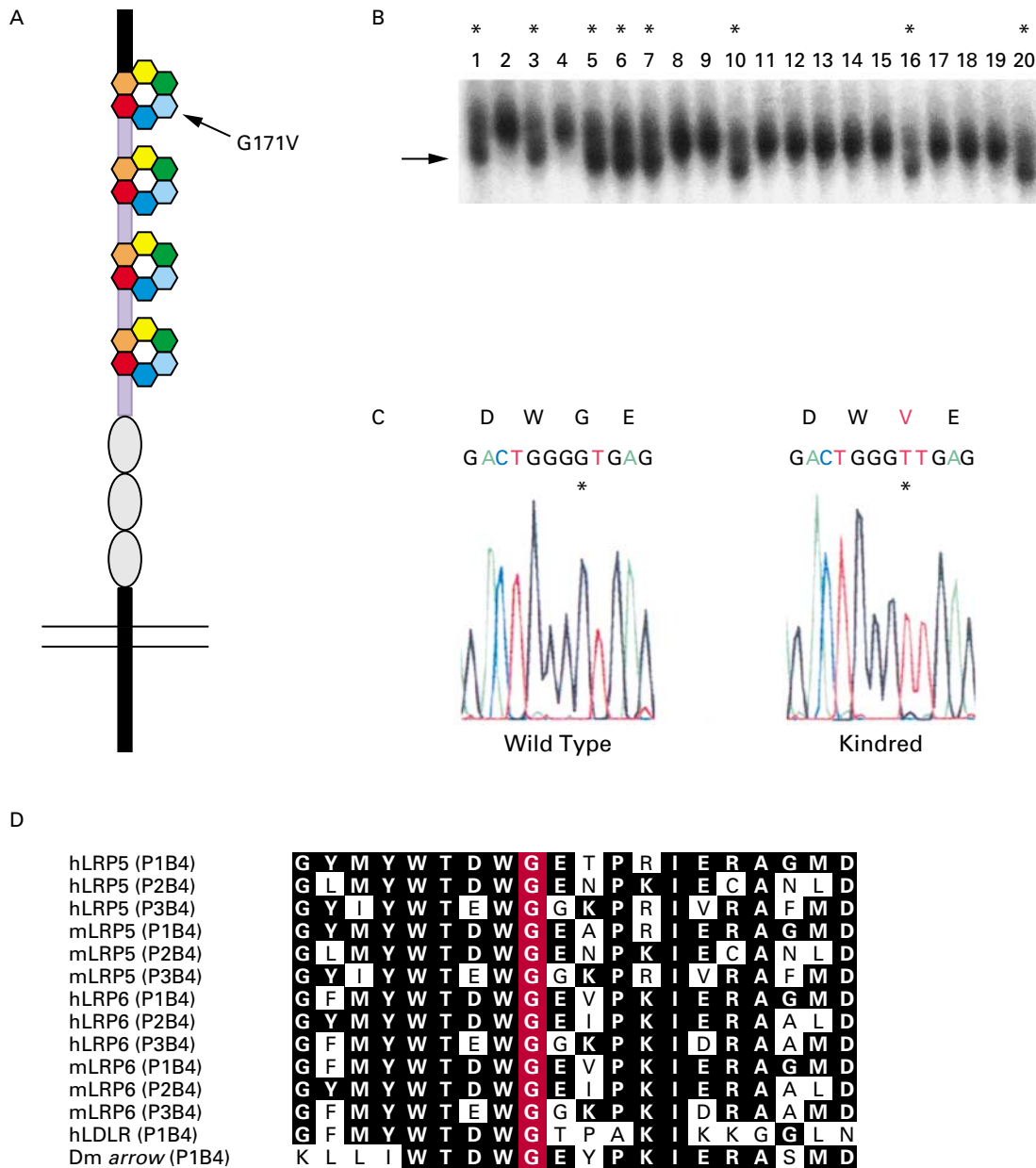


Figure 3. Mutation in *LRP5*.

Panel A is a schematic of the structure of *LRP5*. A number of motifs are identified by their homology to other proteins, although the precise function of each is uncertain. There is an amino-terminal signal sequence for targeting the protein to the membrane (black), with four propeller structures (shown as hexagons), each followed by an epidermal-growth-factor-like repeat (purple); three low-density lipoprotein (LDL) receptor-like ligand binding domains (gray); a single transmembrane domain (black); and a C-terminal cytoplasmic tail (black). The location of the G171V mutation identified in the kindred is shown. Panel B shows the novel *LRP5* variant in the kindred. A segment of exon 3 was amplified from kindred members by polymerase-chain-reaction assay, fractionated by electrophoresis under nondenaturing conditions, and exposed to x-ray film. Kindred members are numbered as in Figure 2, and affected members are indicated by asterisks. Affected members had a novel fragment not found in unaffected members, located below the common fragment (arrow). Panel C shows the sequence of the variant in the kindred. The normal DNA sequence of a segment of *LRP5* spanning codons 169 through 172 is shown at the left; the encoded amino acids are indicated by the single letters above. The sequence of the same segment from the novel variant in affected members of the kindred is shown at the right. There is a single base substitution (G to T) (asterisks), resulting in the substitution of valine for glycine at codon 171. Panel D shows the conservation of glycine in propeller structures. A partial amino acid sequence of the fourth blade of a number of propellers of the YWTD family is shown. Glycine is highly conserved at this position in the first three propellers of *LRP5* and *LRP6* in humans (h) and mice (m), as well as in the only YWTD propeller of the LDL receptor (*LDLR*) and the first propeller of the *Drosophila melanogaster* (*Dm*) homologue, *arrow* (red).

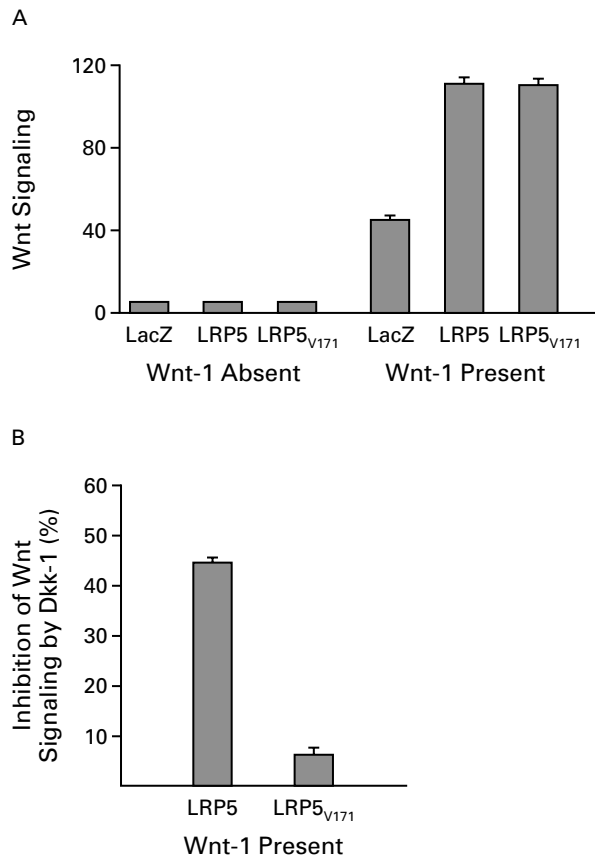


Figure 4. Level of Wnt Signaling with Normal and Mutant LRP5. DNA encoding the indicated proteins was introduced (transfected) into the mouse fibroblast NIH3T3 cell line. The level of Wnt signaling was determined by measuring the activity of the luciferase enzyme expressed under control of a promoter specifically activated by Wnt signaling. Panel A shows the potentiation of Wnt signaling by normal and mutant LRP5. In the absence of Wnt-1, neither normal LRP5 nor LRP5_{V171} activated Wnt signaling above the level of that with a control protein, β -galactosidase (LacZ), demonstrating that LRP5_{V171} does not result in constitutive Wnt signaling. In the presence of Wnt-1, signaling was potentiated to an equal degree with normal LRP5 and LRP5_{V171}, indicating that LRP5_{V171} does not simply increase signaling in response to Wnt-1. Wnt signaling in each experiment is represented as the relative level compared with the expression of the β -galactosidase control in the absence of LRP5 and Wnt-1. The control value was arbitrarily defined as 1. Panel B shows the level of Dkk-1 inhibition of Wnt signaling. The inhibition of Wnt signaling was expressed as the percent reduction in signaling in the presence of Dkk-1 as compared with signaling in its absence. Whereas Dkk-1 inhibited Wnt signaling in the presence of normal LRP5, this inhibition was almost lost in the presence of LRP5_{V171}. The T bars indicate standard deviations.

the same mutation. If this is correct, all these disorders could be diagnosed with the use of a simple genetic test. The findings also suggest that the target for gain-of-function mutations in LRP5 is very small, possibly indicating a site critical for Dkk binding or action.

Our findings provide evidence that the Wnt signaling pathway alters bone mass through a primary effect on bone formation. Biochemical markers of bone resorption were unaltered in affected members of our kindred, whereas levels of specific markers of osteoblast activity were strikingly elevated. These findings indicate an uncoupling of bone turnover in favor of increased bone formation. The observation that LRP5 is expressed at high levels in osteoblasts is consistent with its having a role in this axis.³³ The elevated fibronectin levels are also consistent with an effect on bone formation, since fibronectin is an early scaffolding protein in osteoid formation and enhances the survival and differentiation of osteoblasts.³⁴⁻³⁷ Finally, the observed elevation in TGF- β 1 levels is noteworthy. TGF- β 1 has stimulatory effects on osteoblasts, and targeted disruption in mice results in reduced bone mass.³⁸⁻⁴⁰

Given the established role of rare LRP5 mutations in abnormal bone density, it is of particular interest that variation in bone density in the general population may be linked to the chromosome segment containing LRP5.²⁸ This finding raises the possibility that common variants that alter the expression or function of LRP5 will be found to have a role in the risk of osteoporosis in the general population. Further studies will be required to investigate this possibility. Finally, the observation that the impaired action of Dkk at LRP5 increases bone density suggests that pharmacologic antagonism of Dkk action, either at the pre-receptor level or through the inhibition of binding or action at LRP5, may have a role in the prevention or treatment of osteoporosis.

Supported by grants from the National Institutes of Health (AG15345, to Dr. Insogna, and CA85420, to Dr. Wu), the Danbury Hospital Medical Education Center, the Yale Core Center for Musculoskeletal Disorders (AR46032, to Dr. Insogna), the Yale Bone Center, and the Yale General Clinical Research Center (National Center for Research Resources grant RR00125). Dr. Lifton is an Investigator of the Howard Hughes Medical Institute.

We are indebted to the members of the kindred who participated in the study for making this work possible, and to Carol Nelson-Williams, Ana Quinones, Diane Wall, and the staff of the Yale General Clinical Research Center, for technical assistance and helpful discussions.

REFERENCES

1. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. *JAMA* 2001;285:785-95.
2. Lips P. Epidemiology and predictors of fractures associated with osteoporosis. *Am J Med* 1997;103:Suppl:3S-8S.

3. Kannus P, Palvanen M, Niemi S, Parkkari J, Jarvinen M. Epidemiology of osteoporotic pelvic fractures in elderly people in Finland: sharp increase in 1970-1997 and alarming projections for the new millennium. *Osteoporos Int* 2000;11:443-8.
4. Incidence and costs to Medicare of fractures among Medicare beneficiaries aged ≥ 65 years — United States, July 1991–June 1992. *MMWR Morb Mortal Wkly Rep* 1996;45:877-83.
5. Zuckerman JD. Hip fracture. *N Engl J Med* 1996;334:1519-25.
6. Poor G, Atkinson EJ, O'Fallon WM, Melton LJ. Determinants of reduced survival following hip fractures in men. *Clin Orthop* 1995;319:260-5.
7. Heaney RP, Abrams S, Dawson-Hughes B, et al. Peak bone mass. *Osteoporos Int* 2000;11:985-1009.
8. Worth HM, Wollin DG. Hyperostosis cotalialis generalisata congenita. *J Can Assoc Radiol* 1966;17:67-74.
9. Russell WJ, Bizzozero OJ Jr, Omori Y. Idiopathic osteosclerosis: a report of 6 related cases. *Radiology* 1968;90:70-6.
10. Maroteaux P, Fontaine G, Scharfman W, Farriaux JP. L'hyperostose corticale generalisee a transmission dominante (type Worth). *Arch Fr Pediatr* 1971;28:685-98.
11. Owen RH. Van Buchem's disease (Hyperostosis corticalis generalisata). *Br J Radiol* 1976;49:126-32.
12. Gelman MI. Autosomal dominant osteosclerosis. *Radiology* 1977;125:289-96.
13. Gorlin RJ, Glass L. Autosomal dominant osteosclerosis. *Radiology* 1977;125:547-8.
14. Perez-Vicente JA, Rodriguez de Castro E, Lafuente J, Mateo MMD, Gimenez-Roldan S. Autosomal dominant endosteal hyperostosis: report of a Spanish family with neurological involvement. *Clin Genet* 1987;31:161-9.
15. Ades LC, Morris LL, Burns R, Haan EA. Neurological involvement in Worth type endosteal hyperostosis: report of a family. *Am J Med Genet* 1994;51:46-50.
16. Johnson ML, Gong G, Kimberling W, Recker SM, Kimmel DB, Recker RB. Linkage of a gene causing high bone mass to human chromosome 11 (11q12-13). *Am J Hum Genet* 1997;60:1326-32.
17. Gong Y, Vikkula M, Boon L, et al. Osteoporosis-pseudoglioma syndrome, a disorder affecting skeletal strength and vision, is assigned to chromosome region 11q12-13. *Am J Hum Genet* 1996;59:146-51.
18. Gong Y, Slee RB, Fukai N, et al. LDL receptor-related protein 5 (*LRP5*) affects bone accrual and eye development. *Cell* 2001;107:513-23.
19. Mao J, Wang J, Liu B, et al. Low-density lipoprotein receptor-related protein-5 binds to Axin and regulates the canonical Wnt signaling pathway. *Mol Cell* 2001;7:801-9.
20. Semenov MV, Tamai K, Brott BK, Kuhl M, Sokol S, He X. Head inducer Dickkopf-1 is a ligand for Wnt coreceptor LRP6. *Curr Biol* 2001;11:951-61.
21. Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA. Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. *Nat Cell Biol* 2001;3:683-6.
22. Kerstetter JE, Caseria DM, Mitnick ME, et al. Increased circulating concentrations of parathyroid hormone in healthy, young women consuming a protein-restricted diet. *Am J Clin Nutr* 1997;66:1188-96.
23. Meric F, Yap P, Bia MJ. Etiology of hypercalcemia in hemodialysis patients on calcium carbonate therapy. *Am J Kidney Dis* 1990;16:459-64.
24. Whyte MP, Chines A, Silva DP Jr, Landt Y, Ladenson JH. Creatine kinase brain isoenzyme (BB-CK) presence in serum distinguishes osteopetroses among the sclerosing bone disorders. *J Bone Miner Res* 1996;11:1438-43.
25. Takacs I, Cooper H, Weaver DD, Econs MJ. Bone mineral density and laboratory evaluation of a type II autosomal dominant osteopetrosis carrier. *Am J Med Genet* 1999;85:9-12.
26. Teitelbaum SL. Bone resorption by osteoclasts. *Science* 2000;289:1504-8.
27. Heaney C, Shalev H, Elbedour K, et al. Human autosomal recessive osteopetrosis maps to 11q13, a position predicted by comparative mapping of the murine osteosclerosis (*oc*) mutation. *Hum Mol Genet* 1998;7:1407-10.
28. Koller DL, Rodriguez LA, Christian JC, et al. Linkage of a QTL contributing to normal variation in bone mineral density to chromosome 11q12-13. *J Bone Miner Res* 1998;13:1903-8.
29. Springer TA. An extracellular β -propeller module predicted in lipoprotein and scavenger receptors, tyrosine kinases, epidermal growth factor precursor, and extracellular matrix components. *J Mol Biol* 1998;283:837-62.
30. Jeon H, Meng W, Takagi J, Eck MJ, Springer TA, Blacklow SC. Implications for familial hypercholesterolemia from the structure of the LDL receptor YWTD-EGF domain pair. *Nat Struct Biol* 2001;8:499-504.
31. Graddl D, Kuhl M, Wedlich D. The Wnt/Wg signal transducer β -catenin controls fibronectin expression. *Mol Cell Biol* 1999;19:5576-87.
32. Little RD, Carulli JP, Del Mastro RG, et al. A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. *Am J Hum Genet* 2002;70:11-9.
33. Dong Y, Lathrop W, Weaver D, et al. Molecular cloning and characterization of LR3, a novel LDL receptor family protein with mitogenic activity. *Biochem Biophys Res Commun* 1998;251:784-90.
34. Eielson C, Kaplan D, Mitnick MA, Paliwal I, Insogna K. Estrogen modulates parathyroid hormone-induced fibronectin production in human and rat osteoblast-like cells. *Endocrinology* 1994;135:1639-44.
35. Sun BH, Mitnick M, Eielson C, Yao GQ, Paliwal I, Insogna K. Parathyroid hormone increases circulating levels of fibronectin *in vivo*: modulating effect of ovariectomy. *Endocrinology* 1997;138:3918-24.
36. Globus RK, Doty SB, Lull JC, Holmuhamedov E, Humphries MJ, Damsky CH. Fibronectin is a survival factor for differentiated osteoblasts. *J Cell Sci* 1998;111:1385-93.
37. Moursi AM, Damsky CH, Lull J, et al. Fibronectin regulates calvarial osteoblast differentiation. *J Cell Sci* 1996;109:1369-80.
38. Verhaar HJ, Damen CA, Duursma SA, Scheven BA. Comparison of the action of 17 beta-estradiol and progestins with insulin-like growth factors-I/-II and transforming growth factor-beta 1 on the growth of normal adult human bone-forming cells. *Maturitas* 1995;21:237-43.
39. Centrella M, McCarthy TL, Canalis E. Glucocorticoid regulation of transforming growth factor beta 1 activity and binding in osteoblast-enriched cultures from fetal rat bone. *Mol Cell Biol* 1991;11:4490-6.
40. Geiser AG, Zeng QQ, Sato M, Helvering LM, Hirano T, Turner CH. Decreased bone mass and bone elasticity in mice lacking the transforming growth factor-beta1 gene. *Bone* 1998;23:87-93.

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