ORIGINAL ARTICLE

JBMR

Patients With Sclerosteosis and Disease Carriers: Human Models of the Effect of Sclerostin on Bone Turnover

Antoon H van Lierop,[1] Neveen AT Hamdy,[1] Herman Hamersma,[2] Rutger L van Bezooijen,[1] Jon Power,[3] Nigel Loveridge,[3] and Socrates E Papapoulos[1]

[1]Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, The Netherlands
[2]Flora Clinic, Roodenpoort, South Africa
[3]Departments of Medicine and Orthopedic Research Unit, University of Cambridge, Cambridge, United Kingdom

ABSTRACT

Sclerosteosis is a rare bone sclerosing dysplasia, caused by loss-of-function mutations in the *SOST* gene, encoding sclerostin, a negative regulator of bone formation. The purpose of this study was to determine how the lack of sclerostin affects bone turnover in patients with sclerosteosis and to assess whether sclerostin synthesis is decreased in carriers of the *SOST* mutation and, if so, to what extent this would affect their phenotype and bone formation. We measured sclerostin, procollagen type 1 amino-terminal propeptide (P1NP), and cross-linked C-telopeptide (CTX) in serum of 19 patients with sclerosteosis, 26 heterozygous carriers of the C69T *SOST* mutation, and 77 healthy controls. Chips of compact bone discarded during routine surgery were also examined from 6 patients and 4 controls. Sclerostin was undetectable in serum of patients but was measurable in all carriers (mean 15.5 pg/mL; 95% confidence interval [CI] 13.7 to 17.2 pg/mL), in whom it was significantly lower than in healthy controls (mean 40.0 pg/mL; 95% CI 36.9 to 42.7 pg/mL; $p < 0.001$). P1NP levels were highest in patients (mean 153.7 ng/mL; 95% CI 100.5 to 206.9 ng/mL; $p = 0.01$ versus carriers, $p = 0.002$ versus controls), but carriers also had significantly higher P1NP levels (mean 58.3 ng/mL; 95% CI 47.0 to 69.6 ng/mL) than controls (mean 37.8 ng/mL; 95% CI 34.9 to 42.0 ng/mL; $p = 0.006$). In patients and carriers, P1NP levels declined with age, reaching a plateau after the age of 20 years. Serum sclerostin and P1NP were negatively correlated in carriers and age- and gender-matched controls ($r = 0.40$, $p = 0.008$). Mean CTX levels were well within the normal range and did not differ between patients and disease carriers after adjusting for age ($p = 0.22$). Our results provide in vivo evidence of increased bone formation caused by the absence or decreased synthesis of sclerostin in humans. They also suggest that inhibition of sclerostin can be titrated because the decreased sclerostin levels in disease carriers did not lead to any of the symptoms or complications of the disease but had a positive effect on bone mass. Further studies are needed to clarify the role of sclerostin on bone resorption. © 2011 American Society for Bone and Mineral Research.

KEY WORDS: BONE FORMATION; BONE RESORPTION; SCLEROSTEOSIS; CARRIERS; SCLEROSTIN

Introduction

Sclerosteosis is a rare autosomal recessive bone sclerosing dysplasia characterized by generalized osteosclerosis.[1] It is caused by loss-of-function mutations in the *SOST* gene encoding for sclerostin,[2–4] a protein produced in bone by osteocytes[5] that decreases bone formation by inhibiting the Wnt signaling pathway in osteoblasts.[6,7] In patients with sclerosteosis, the lack of sclerostin leads to unrestrained bone formation resulting in generalized osteosclerosis.

The clinical manifestations of sclerosteosis have been well described since it was first identified in 1958.[8] They include cranial nerve deficits and increased intracranial pressure as a result of excessive growth of the skull bones.[9,10] Bone mineral density (BMD) is markedly increased at the spine and the hip,[11] but data on the rate of bone turnover are scarce in these patients. Interestingly, heterozygous carriers of the *SOST* mutation have high normal or increased BMD,[11] suggesting that having one affected allele of the *SOST* gene would also have an effect on sclerostin synthesis, albeit milder than was observed in the homozygous state. Whether this may also be associated with clinical manifestations has not been studied systematically.

To address these questions, we conducted a study in a cohort of patients with sclerosteosis and their relatives who were heterozygous carriers of the *SOST* mutation, with the following specific aims: first, to determine how the lack of sclerostin affects parameters of bone turnover in patients with sclerosteosis; second, to assess whether sclerostin synthesis is decreased in

Received in original form June 20, 2011; revised form July 4, 2011; accepted July 7, 2011. Published online July 22, 2011.
Address correspondence to: Socrates E Papapoulos, MD, PhD, Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands. E-mail: m.v.iken@lumc.nl

Journal of Bone and Mineral Research, Vol. 26, No. 12, December 2011, pp 2804–2811
DOI: 10.1002/jbmr.474

carriers of the *SOST* mutation and, if so, to what extent this would affect their phenotype and bone turnover.

Material and Methods

Nineteen South African patients with sclerosteosis and 30 mostly first-degree relatives living within a 250-km radius from Johannesburg were invited to participate in our study. None of the patients or relatives studied used any drugs that could affect bone metabolism. One patient was on long-term treatment for hypothyroidism with thyroid replacement therapy.

Physical examination, focusing on clinical features of sclerosteosis and on neurological deficits, was conducted at the Ear-Nose-and-Throat Department of the Flora Clinic in Johannesburg or at the patients' homes by AvL and HH. Because patients with sclerosteosis are descendants of Dutch settlers and because South African normative data are lacking, height *Z*-scores were calculated relative to Dutch normative data, using Growth Analyzer 3.5 (Dutch Growth Foundation, Rotterdam, The Netherlands). Results of hearing tests were obtained from the patients' hospital records. Nonfasting blood samples were collected when convenient, but samples from patients and their family members were collected at the same time of the day. Full blood samples and separated serum and plasma were frozen and transported to The Netherlands on dry ice for biochemical analysis and DNA extraction.

The study was conducted according to the principles of the Declaration of Helsinki, was approved by the Medical Ethical Committee of the Leiden University Medical Center, and informed consent was obtained from all subjects included in the study.

DNA analysis

DNA was isolated from full blood samples using the Insorb Spin Blood Maxi Kit (Invitek GmbH, Berlin, Germany). The first exon of the *SOST* gene was replicated by PCR, using primers 5′-AAGGAACTTGCCCAAGATGA-3′ and 5′-AAGGCTCGAGCCCAA-GATGA-3′. PCR products were purified with MicroSpin S-400 HR Columns (GE Healthcare, Buckinghamshire, UK) and sent to the Leiden Genome Technology Centre for sequencing. DNA sequences were analyzed with Chromas 2.33 (Technelysium Pty. Ltd., Helensvale, Australia), focusing on the presence of a single cysteine to tyrosine substitution 69 base pairs downstream of the predicted translation initiation site (C69T), previously found to be the underlying mutation in South African patients with sclerosteosis.[3]

Serum biochemistry

All biochemical measurements were performed at the Clinical Chemistry Laboratory of the Leiden University Medical Center (Leiden, The Netherlands). Serum calcium adjusted for albumin, phosphate, and creatinine were measured by semiautomated techniques. Alkaline phosphatase (ALP) was measured using a fully automated P800 modulator system (Roche BV, Woerden, The Netherlands). Procollagen type 1 amino-terminal propeptide (P1NP) and β-cross-linked C-telopeptide (β-CTX) were determined by the E-170 system (Roche BV). 25-hydroxyvitamin D (25-OHD) was measured by the LIAISON 25-OH Vitamin D TOTAL assay (DiaSorin S.A./N.V., Brussels, Belgium).

Sclerostin assay

Sclerostin was measured in serum by an electrochemiluminescense assay (MSD 96-Well MULTI-ARRAY Human Sclerostin Assay, Meso-Scale Discoveries, Gaithersburg, MD, USA) as described previously.[12] The assay is very sensitive (detection limit $\pm$ 1 pg/mL), with a broad detection range (1 to 10,000 pg/mL) and an intra-assay precision of 6% and an inter-assay precision of 10%. The recovery of sclerostin in serum spiked with sclerostin 250, 125, and 65 pg/mL was 82% to 93%.

Sclerostin is a glycoprotein containing 190 residues, which form a three-looplike structure, with a cystine knot at the base and long, highly flexible C- and N-terminal regions.[13] Bioavailability of circulating form(s) of sclerostin is currently unknown, and the specificity of commercial assays for different domains of the molecule has not been reported. We performed, therefore, additional experiments to obtain more insight into the specificity of the MSD assay for sclerostin.

First, to validate the sclerostin standard used in this assay, we compared it with that of recombinant sclerostin prepared by the University of Würzburg, Germany, kindly provided by Prof Thomas Mueller within the TALOS research consortium. This recombinant sclerostin was produced in an *Escherichia coli* strain, and the protein concentration was accurately determined by spectrophotometry. Serial dilutions (10 to 1000 pg/mL) of both these sclerostin preparations were measured. Values of the two peptides were highly concordant at every concentration. The mean ratio of the concentrations was 1.02 (range 0.8 to 1.3). Second, to characterize the specificity of the antibodies used in the assay, we performed epitope mapping of the polyclonal detection and capture antibodies according to Pepscan's Epitope Mapping Technology (kindly performed by Jaap Willem Back and Peter Timmerman of Pepscan Therapeutics [Lelystad, The Netherlands] within the TALOS research consortium). Pepscan's Epitope Mapping Technology uses microarrays of overlapping peptides, covering the complete sequence of a given protein. Antibody binding studies subsequently identify the peptides representing the protein interaction of interest (http://www.pepscantherapeutics.com/technology/epitope-mapping). The epitope mapping revealed two binding sites of the capture antibody for human sclerostin, one on the N terminus and one on the distal end of the C terminus. For the detection antibody, there were three apparent epitopes: at the N terminus, at the third loop, and at the C terminus of the protein. We further validated these results by assessing reactivity of sclerostin fragments of different sizes (provided by Jaap Willem Back of Pepscan Therapeutics). We tested three fragments comprising the three loops without the N terminus and C terminus; the first and third loops without C and N terminus and the second loop alone. All fragments were added at high concentrations (10 ng/mL). As was expected from the epitope mapping, all fragments were undetectable, suggesting that the assay detects the whole sclerostin molecule.

Serum sclerostin and bone turnover marker values were compared with those obtained in a cohort of healthy volunteers, who were used as controls as previously described.[12] This group consisted of 77 healthy volunteers (30 males and 47 females) with a mean age of 50.3 years (range 20 to 77 years) and a mean body mass index (BMI) of 25.2 kg/m^2. All had normal serum calcium and phosphate concentrations, normal renal function, and biochemical markers of bone turnover.

Bone biopsies

Chips of compact bone, obtained from 6 patients with sclerosteosis (4 reported here) on a different occasion during surgical procedures and from 4 control subjects, were examined. The samples were fixed in 10% neutral buffered formalin and were embedded in methyl methacrylate without prior decalcification.[14,15] Ten-micron sections were cut by a Jung Polycut E microtome (Leica, Milton Keynes, UK) and stained using solochrome cyanine R and Goldner's protocols.[15] Images for the quantification of bone remodeling parameters were acquired under bright field illumination at x5 objective magnification using Surveyor software (Objective Imaging, Ltd., Cambridge, UK). The samples were essentially compact bone, which was being remodeled in a manner similar to cortical bone so that assessment of bone formation and resorption was done in the same way as that reported by Bell and colleagues.[15] Canals within the bone were denoted as undergoing formation (presence of an osteoid seam) or resorption (presence of a crenelated surface), and the data were expressed as a proportion of the total number of canals within the specimen.

Statistical analysis

Statistical analysis was performed using the SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). Group differences in levels of sclerostin and other biochemical markers were assessed by ANOVA. Because of inequality in sample sizes, a Games-Howell post hoc test was used. To adjust for effect of age on procollagen type 1 amino-terminal propeptide (P1NP) levels, we also matched carriers to healthy controls according to gender and age (within 5 years). Correlations between sclerostin and bone turnover markers were determined by Pearson's correlation tests. P1NP and sclerostin data were log-transformed because of skewness. A p value < 0.05 was considered significant.

Results

Subjects

The diagnosis of sclerosteosis was confirmed in all 19 patients studied by DNA analysis, on the basis of the demonstration of a C69T substitution in both alleles of the *SOST* gene. Of the 30 relatives, 26 were heterozygous carriers of the C69T mutation and were included in the analysis. Median age of the patients was 23 years (range 9 to 70 years) and of the carriers was 44 years (range 13 to 70 years). Characteristics of patients and their heterozygous relatives are shown in Table 1.

Table 1. Characteristics and Parameters of Calcium Metabolism in Patients With Sclerosteosis and Heterozygous Disease Carriers

	Patients ($n = 19$)	Carriers ($n = 26$)	p Value
Male:female	11:8	7:19	0.16
Age (years)	28.32 ± 15.0	39.9 ± 15.0	0.014
Height (cm)	179.8 ± 12.0	164.0 ± 37.0	0.100
Height Z-score (SD)	$+0.83 \pm 0.73$	-0.48 ± 0.89	<0.001
BMI (kg/m^2)	25.6 ± 5.7	25.7 ± 5.3	0.93
Calcium (mmol/L)	2.33 ± 0.07	2.27 ± 0.11	0.06
Phosphate (mmol/L)	1.37 ± 0.32	1.20 ± 0.32	0.10
25(OH)D (nmol/L)	48.9 ± 13.1	41.6 ± 15.0	0.10
Creatinine (μmol/L)	62.2 ± 20.4	55.2 ± 12.1	0.20

Values given as mean $\pm$ SD.
BMI = body mass index; 25(OH)D = 25-hydroxyvitamin D.

Clinical features

At the time of the study, 3 of the 19 patients had symptoms related to increased intracranial pressure in the form of severe and persistent headaches, which were worse in the morning and associated with dizziness and nausea. Two of these 3 patients had already undergone decompressive surgery to relieve the increased intracranial pressure. Thirteen of the remaining 16 patients had undergone decompressive surgery, 10 after experiencing symptoms of increased intracranial pressure and 3 after a diagnosis of increased intracranial pressure on routine screening. Decompressive surgery consisted of anterior and/or posterior craniotomy, as described previously.[16] The median age at which this procedure was performed was 15 years. Of the 7 patients who had undergone this procedure at a younger age, 4 (57%) had to be reoperated because of recurrence of signs of increased intracranial pressure after 2 to 11 years. Of the 8 patients in whom the operation was performed after the age of 15 years, only 1 (13%) patient had to be reoperated after an interval of 2 years.

The majority of patients (89%) had experienced recurrent episodes of facial palsy, usually occurring before the age of 4 years, although unilateral facial paresis was already present at birth in 1 patient. Surgical decompression of the facial nerve was conducted in all cases, with the unaffected side also decompressed prophylactically. Despite these interventions, facial palsy recurred in 6 of the previously operated cases. Hearing loss was present in all cases, having been recognized in early childhood and progressing into adulthood. Operations to improve hearing, such as widening of the external bony ear canal or freeing of fixed ossicles, had been performed in 13 patients. Eleven patients used hearing aids. Other complaints associated with cranial nerve compression were decreased sensation of the face (trigeminal nerve) in 2 cases and a visual field defect in one eye in 1 patient (optic nerve). None of the patients reported sustaining a bone fracture.

Overview of the medical history of the whole group provided insight into the natural history of the disorder. Disease manifestations first appear during childhood and adolescence and progress through to the third decade of life, appearing to

stabilize thereafter. In the majority of patients, no recurrence or progression of symptoms were observed after the age of 25 years.

On clinical examination, the majority of patients were of tall stature. The average height of adult male patients was 190.6 cm and of adult females 175.7 cm. Mean weight and BMI were, respectively, 104.4 kg and 28.8 kg/m^2 for adult males, and 73.7 kg and 24.7 kg/m^2 for adult females. Mean height *Z*-score of patients was above zero and was significantly higher than that of their relatives ($p < 0.001$) (Table 1). Patients had a sclerosteosis phenotype, as previously described, of variable degree of severity. The facial deformities of bossing of the forehead and enlargement of the mandible were observed in 47% and 68% of subjects, respectively, and had generally developed by the time puberty was reached. Although these features were not present in the 2 youngest patients, both 9 years old, they were already clearly noticeable in 2 young male patients, aged 12 and 13 years. Six patients had undergone corrective surgery of the mandible. One of these patients needed a second corrective surgery 7 years later because of further enlargement of the mandible. Syndactyly of fingers or toes was present in 52% of the cases. All but 1 of the remaining patients did, however, display other digit abnormalities, such as nail dysplasia or radial deviation of the phalanges.

On neurological examination, movement of the facial muscles was impaired in 73% of patients, bilateral in 52% of these cases, and of moderate (House-Brackmann score grade III) to moderately severe (grade IV) degree. Previously performed hearing tests were abnormal in all patients, showing conductive hearing loss of a moderately severe (50 to 70 dB) to severe (70 to 90 dB) grade in all, with an additional sensorineural component in 36% of the cases. In 2 patients, hearing loss was complete. Cognitive function was normal in all patients.

None of the carriers reported having any of the above-mentioned symptoms, and none had abnormal findings on clinical examination. Similar to patients, none of the studied carriers had ever sustained a fracture.

Serum chemistry

There was no difference in serum calcium, phosphate, and 25-hydroxyvitamin D [25(OH)D] concentrations between patients and carriers, and all had normal renal function (Table 1).

Markers of bone turnover

Serum P1NP levels declined with age in both patients and carriers and appeared to reach a plateau after the age of 20 years (Fig. 1). Thirteen of 14 adult patients (18 years or older) and 7 of 22 adult carriers had serum P1NP values $>$ 65 ng/mL. Because of the clear effect of age on serum P1NP levels and the low number of young individuals in the carrier group, we compared serum P1NP between adult patients and carriers. P1NP levels were significantly different between groups (ANOVA $p < 0.001$). Compared with carriers and controls, patients with sclerosteosis had significantly higher serum P1NP values (153.7 ng/mL; 95% confidence interval [CI] 100.5 to 206.9 ng/mL; $p = 0.01$ versus carriers, $p = 0.002$ versus controls), whereas carriers (58.3 ng/mL; 95% CI 47.0 to 69.6 ng/mL) had significantly higher values than controls (37.8 ng/mL; 95% CI 34.5 to 41.0 ng/mL; $p = 0.006$) (see also Fig. 2). These differences remained after adjusting the P1NP values for age.

Similar to serum P1NP, serum CTX values declined with age, reaching a plateau around the age of 20 years (Fig. 1). However, in all but 1 adult patient, values were lower than 600 pg/mL. There was a difference in mean serum CTX between adult patients (213 pg/mL; 95% CI 103 to 323 pg/mL) and carriers (126 pg/mL; 95% CI 84 to 167 pg/mL; $p = 0.02$), but this was no longer significant after adjusting for age ($p = 0.22$). Although absolute values for serum CTX should be interpreted with caution because not all samples were obtained in the fasting state, comparison of serum CTX between groups is valid because samples were obtained from patients and carriers under identical conditions. Serum P1NP and CTX values were significantly

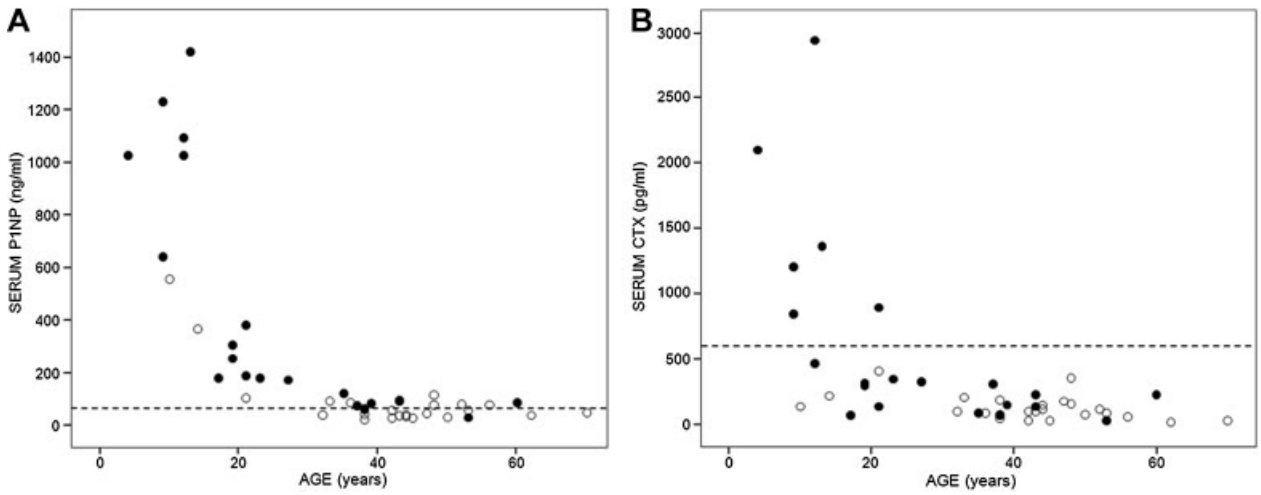

Fig. 1. Relation between serum P1NP levels and age (*A*) and CTX levels and age (*B*) in patients with sclerosteosis and heterozygous disease carriers. Closed circles represent homozygous patients; open circles represent heterozygous carriers. The dotted lines represent the upper limit of the normal adult reference range (65 ng/mL for P1NP; 600 pg/mL for CTX).

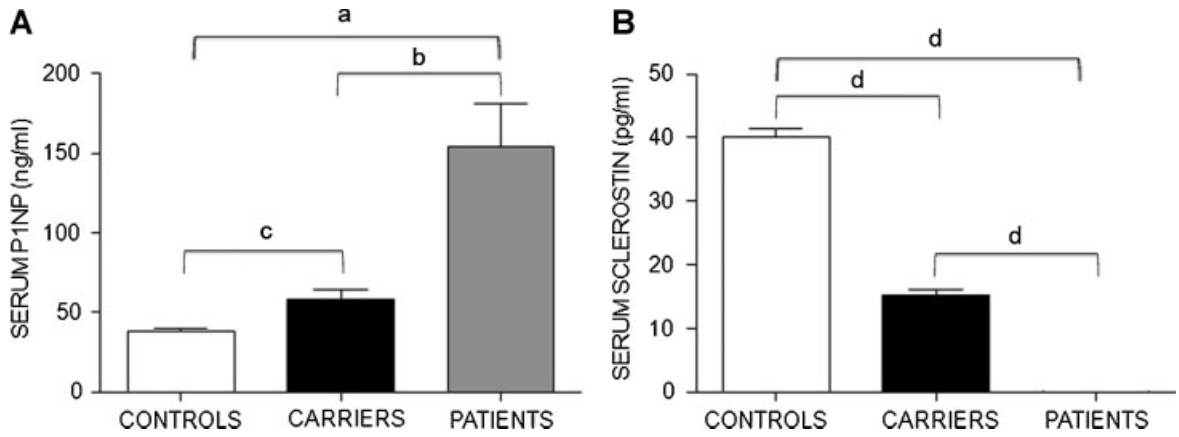

Fig. 2. Serum P1NP (*A*) and sclerostin (*B*) levels in healthy controls and adult patients with sclerosteosis and heterozygous disease carriers. Bars represent SEM. a: $p = 0.002$; b: $p = 0.01$; c: $p = 0.006$; d: $p < 0.001$.

correlated both in patients ($r = 0.86$, $p < 0.001$) and carriers ($r = 0.46$, $p = 0.025$).

Serum sclerostin

Serum sclerostin was undetectable in all 19 patients. In contrast, sclerostin was measurable in the serum of all carriers, although the mean value (15.5 pg/mL; 95% CI 13.4 to 16.9 pg/mL) was significantly lower than that of healthy controls (40.0 pg/mL; 95% CI 37.2 to 42.9 pg/mL; $p < 0.001$) (Fig. 2), and the difference remained significant after adjusting values for age. There was no correlation between serum sclerostin and either P1NP or CTX in carriers. However, when values of carriers and controls were pooled together, there was a significant negative correlation ($r = -0.23$, $p = 0.02$) between sclerostin and P1NP, which improved further ($r = -0.40$, $p = 0.008$) when carriers were analyzed together with age and gender-matched controls (Fig. 3).

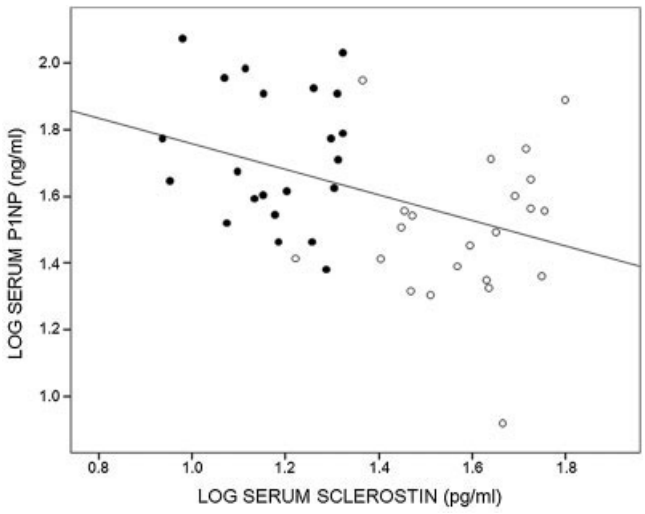

Fig. 3. Relation between serum P1NP and sclerostin levels in age-matched healthy controls (open circles) and heterozygous carriers of sclerosteosis (closed circles). $r = -0.40$, $p = 0.008$.

Histomorphometric analysis of bone remodeling

The results of histomorphometric analysis of bone samples are shown in Table 2. Bone from patients with sclerosteosis showed evidence of increased formation in that there was a higher proportion of canals with an osteoid seam compared with bone from similar sites in control subjects (sclerosteosis 2.6% to 21.1%; controls 1.1% to 3.7%; Table 2). The proportion of canals undergoing resorption was similar in both groups (sclerosteosis 0.6% to 3.0%; controls 0% to 0.9%). In the patients, as with the relation between serum P1NP and age, bone formation activity appeared to reach a peak at puberty. Direct comparison of histomorphometric values is, however, not appropriate because of differences in age between patients and controls.

Discussion

Patients with sclerosteosis and heterozygous disease carriers form a unique model to study the role of sclerostin on bone metabolism in humans. In this study, we show that there is a gene-dose effect of the sclerosteosis mutation on circulating sclerostin, with absent sclerostin in serum of patients and decreased sclerostin levels in disease carriers. These differences in circulating sclerostin were accompanied by different levels of the bone formation marker serum P1NP between patients, carriers, and controls.

The recognition of the role of sclerostin in bone metabolism and the development of commercial assays for measuring it in serum have led to a series of studies that explored the association between serum sclerostin and indices of bone metabolism in healthy individuals and in patients with various bone disorders.[12,17–23] Caution is needed, however, in interpreting the results of these studies and in deriving conclusions about the pathophysiological significance of observed associations. Sclerostin is produced in bone by osteocytes, acts on osteoblasts, and is released in the circulation. At present, neither the bioactivity of circulating sclerostin nor the specificity of the antibodies used in existing assays for the protein are known. Moreover, there are differences in measured values, and

Table 2. Histomorphometry of Patients With Sclerosteosis and Controls

Biopsy	Source	Age (Years)	Sex	Formation (Active/Total)	Resorption (Active/Total)
Controls					
1	Mastoid	37	Female	1/41 (2.4%)	0/54 (0.0%)
2	Mastoid	49	Female	1/37 (2.7%)	0/37 (0.0%)
3	Mastoid	49	Male	1/94 (1.1%)	0/113 (0.0%)
4	Mastoid	64	Male	4/108 (3.7%)	1/117 (0.9%)
Patients					
1	Mastoid	4	Male	8/311 (2.6%)	2/347 (0.6%)
2	Mastoid	8	Female	49/654 (7.5%)	9/686 (1.3%)
3	Mastoid	8	Male	10/66 (15.1%)	1/68 (1.5%)
4	External hearing canal	14	Male	11/52 (21.1%)	2/67 (3.0%)
5	Mastoid	18	Male	7/49 (14.2%)	1/47 (2.1%)
6	Mastoid	43	Male	8/77 (10.4%)	4/107 (0.9%)

reported values obtained with different assays are poorly correlated.[24] In the current study, we used a highly sensitive assay with a very low limit of detection, and we assessed for the first time its specificity in detecting sclerostin. Our findings suggest that this assay detects the whole sclerostin molecule rather than circulating fragments, which may be active. The lower values measured with this assay compared with those reported with other assays may be attributed to lower detection of protein-bound forms of the protein.

Previous studies have shown that sclerostin is not expressed by osteocytes of patients with sclerosteosis,[5] and our data on serum sclerostin are in agreement with these findings and the pathogenesis of the disease. We could not detect sclerostin in the serum of any patient with sclerosteosis, which provides a powerful negative control for the assay we used, supporting its specificity and suggesting that measured values can be of biological significance. Contrary to patients with sclerosteosis, sclerostin was detectable in the serum of all carriers of the disease, being, however, on average 60% lower than values measured in healthy controls. These lower circulating levels of sclerostin most likely mirror a decreased synthesis of the protein by the osteocytes of these individuals as a result of the affected *SOST* allele. The reduced synthesis of sclerostin in disease carriers affects bone metabolism but is not associated with any of the clinical manifestations or complications of sclerosteosis. The clinical presentation of our patients with sclerosteosis is in accordance with earlier descriptions of the disease and confirms the high frequency of serious complications resulting from entrapment of cranial nerves attributable to increased bone formation in the skull. Disease carriers, however, were symptom-free, and there were no clinical signs suggestive of any of these complications. This may be because of the lower rates of bone formation of carriers compared with patients as evidenced by serum P1NP values. On the other hand, when compared with healthy individuals, disease carriers had higher P1NP values, and there was a significant negative correlation between serum sclerostin and P1NP values. These results help to explain the previously reported high BMD values of carriers[11] and minor changes apparent on skull radiographs.[25] In addition, they suggest that nonexcessive inhibition of sclerostin production may have a positive effect on the skeleton without causing any of the complications associated with the absence of the protein. Currently, inhibitors of sclerostin are being developed as potential bone-forming treatment for patients with osteoporosis, but values of circulating sclerostin and P1NP were not reported.[26,27] Our data indicate that decreasing the synthesis of sclerostin can have a beneficial effect on the strength of the skeleton, as already shown in animal studies.[28–30]

An important finding, as also previously noted by Beighton and colleagues,[10] was the stabilization of the disease and the dramatic decrease in the frequency of complications after the third decade of life, suggesting that the rate of bone formation slows down in patients with aging. This hypothesis is supported by the negative correlation between age and serum P1NP values and by the histological findings. It is also of interest to note that serum P1NP changes follow a normal pattern during growth, being high in childhood and adolescence and reaching a plateau after completion of growth. In addition, the highest bone-formation rate, as assessed by histomorphometry, was found in a 14-year-old patient. In a previous longitudinal study of a patient with the closely related bone dysplasia van Buchem disease, we showed that biochemical markers of bone turnover were always increased for age but followed a normal pattern during growth with the highest levels observed during the growth spurt with a decline thereafter.[31] Taken together, these observations strongly suggest that the skeleton of patients lacking sclerostin responds normally to local and systemic signals, as also evidenced by the significant relation between serum P1NP and CTX values. The lack of sclerostin may be also responsible for the patients' tall stature. Although this has been mentioned as a clinical feature of the disease, our study is the first to our knowledge to examine this in detail. Sclerostin is expressed by terminally differentiated chondrocytes,[32,33] which can be regarded as the equivalent to osteocytes in the chondrocyte lineage, and canonical Wnt signaling promotes differentiation and maturation of chondrocytes.[34] It might, therefore, well be that sclerostin has a similar inhibiting role on Wnt signaling in chondrocytes in the growth plate. In sclerosteosis, the sclerostin deficiency would lead to increased differentiation toward hypertrophic chondrocytes, resulting in a larger hypertrophic zone in the growth plate and, therefore, more new bone accrual and more longitudinal growth.

Whereas serum P1NP levels were higher in patients with sclerosteosis compared with disease carriers, CTX levels, in nonfasting blood samples taken at the same time of the day, did not differ between these two groups. In a previous study, another biochemical marker of bone resorption, hydroxyproline, was found to be within the normal range in 3 patients with sclerosteosis.[35] These findings are in line with the histomorphometric results of the current study as well as with those in a murine model of the disease.[36] In the latter, bone resorption as assessed biochemically and histologically did not differ from that of wild-type mice. Wnt signaling in osteoblasts decreases bone resorption by downregulating the expression of receptor activator of NF-κB ligand (RANKL) and upregulating that of osteoprotegerin (OPG).[37–39] In addition to stimulating bone formation, the lack of sclerostin leading to stimulation of Wnt signaling may, thus, also decrease the rate of bone resorption. This premise is supported by the changes in serum P1NP and CTX in animals and humans treated with an antibody to sclerostin.[27,29]

The results of our study provide further strong evidence for the paradigm that inhibition of sclerostin activity has an anabolic effect on bone. Although patients with sclerosteosis and disease carriers form a proper model to study the effect of decreased sclerostin activity on bone in humans, this model differs from treatment with an inhibitor of sclerostin because in these individuals the decreased or absent sclerostin production is continuous and permanent, whereas treatment with an inhibitor results in an immediate but reversible inhibition of sclerostin. The negative relation between serum P1NP and age we found in patients and disease carriers suggests that in sclerosteosis bone formation is not elevated to the same extent throughout life and that the absence of sclerostin alone does not act as a constant stimulus for bone formation. This raises the question whether prolonged treatment with a sclerostin inhibitor will be associated with a sustained anabolic effect on bone or whether the beneficial effect on bone formation may become blunted after a certain period of time. It should be noted that sclerostin does not stimulate osteoblastogenesis but rather acts at later stages of osteoblast development and inhibits their activity and reduces their life span.[5,32,40] In the young, osteoblastogenesis is increased as required for skeletal growth, whereas it decreases after skeletal maturity. It may, therefore, be that in the presence of an increased pool of osteoblasts, as occurs in the young, the lack of sclerostin leads to excessive bone formation, which, however, decreases considerably when this pool is reduced, as occurs in adults. The potential contribution to these responses of other inhibitors of the Wnt signaling pathway, such as Dkk1, warrants further investigation. This hypothesis can explain the changes of bone formation with age in the studied individuals and suggests that the response to exogenously administered inhibitors of sclerostin may be more complex than that illustrated by short-term studies in animals and humans.

In conclusion, our findings provide compelling in vivo evidence of how the absence or decreased synthesis of sclerostin leads to increased bone formation in humans. Furthermore, inhibition of sclerostin can be titrated because the decreased sclerostin levels in disease carriers did not elevate bone formation to the same extent as in patients with sclerosteosis and did not lead to any of the symptoms or complications of the disease but had a positive effect on bone mass. Further studies are needed to clarify the role of sclerostin on bone resorption.

Disclosures

All the authors state that they have no conflicts of interest.

Acknowledgments

We thank Dr Jaap Willem Back and Dr Peter Timmerman, Pepscan Therapeutics, for performing the epitope mapping of the antibodies and for providing the sclerostin fragments. We also thank Prof Thomas Mueller, University of Würzburg, Germany, for the recombinant human sclerostin, and Dr Jonathan Reeve, University of Cambridge, United Kingdom, for his helpful comments. This work was supported by a grant from the European Commission-FP7 (HEALTH-F2-2008-201099, TALOS).

Authors' roles: AvL, NH, and SP: contributed to the conception, design, analysis, and interpretation of the data. AvL, HH, JP, and NL: contributed to the acquisition, analysis, and interpretation of the data. RvB: contributed to the conception and design of the data. All authors participated in drafting and/or revising of the manuscript, and all authors approved the final version of the manuscript. AvL and SP take responsibility for the integrity of the analysis of the data.

References

1. Moester MJ, Papapoulos SE, Lowik CW, van Bezooijen RL. Sclerostin: current knowledge and future perspectives. Calcif Tissue Int. 2010; 87:99–107.
2. Balemans W, Ebeling M, Patel N, Van Hul E, Olson P, Dioszegi M, Lacza C, Wuyts W, Van Den EJ, Willems P, Paes-Alves AF, Hill S, Bueno M, Ramos FJ, Tacconi P, Dikkers FG, Stratakis C, Lindpaintner K, Vickery B, Foernzler D, Van Hul W. Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). Hum Mol Genet. 2001;10:537–43.
3. Brunkow ME, Gardner JC, Van Ness J, Paeper BW, Kovacevich BR, Proll S, Skonier JE, Zhao L, Sabo PJ, Fu Y, Alisch RS, Gillett L, Colbert T, Tacconi P, Galas D, Hamersma H, Beighton P, Mulligan J. Bone dysplasia sclerosteosis results from loss of the SOST gene product, a novel cystine knot-containing protein. Am J Hum Genet. 2001; 68:577–89.
4. Piters E, Culha C, Moester M, Van Bezooijen R, Adriaensen D, Mueller T, Weidauer S, Jennes K, de Freitas F, Lowik C, Timmermans JP, Van Hul W, Papapoulos S. First missense mutation in the SOST gene causing sclerosteosis by loss of sclerostin function. Hum Mutat. 2010;31:E1526–43.
5. van Bezooijen RL, Roelen BA, Visser A, van der Wee-Pals L, de Wilt E, Karperien M, Hamersma H, Papapoulos SE, ten Dijke P, Lowik CW. Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. J Exp Med. 2004; 199:805–14.
6. Li X, Zhang Y, Kang H, Liu W, Liu P, Zhang J, Harris SE, Wu D. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. J Biol Chem. 2005;280:19883–7.
7. van Bezooijen RL, Svensson JP, Eefting D, Visser A, van der Horst G, Karperien M, Quax PH, Vrieling H, Papapoulos SE, ten Dijke P, Lowik CW. Wnt but not BMP signaling is involved in the inhibitory action of

sclerostin on BMP-stimulated bone formation. J Bone Miner Res. 2007;22:19–28.

8. Truswell AS. Osteopetrosis with syndactyly; a morphological variant of Albers-Schonberg's disease. J Bone Joint Surg Br. 1958;40-B: 209–18.
9. Hamersma H, Gardner J, Beighton P. The natural history of sclerosteosis. Clin Genet. 2003;63:192–7.
10. Beighton P, Durr L, Hamersma H. The clinical features of sclerosteosis. A review of the manifestations in twenty-five affected individuals. Ann Intern Med. 1976;84:393–7.
11. Gardner JC, van Bezooijen RL, Mervis B, Hamdy NA, Lowik CW, Hamersma H, Beighton P, Papapoulos SE. Bone mineral density in sclerosteosis; affected individuals and gene carriers. J Clin Endocrinol Metab. 2005;90:6392–5.
12. van Lierop AH, Witteveen JE, Hamdy NA, Papapoulos SE. Patients with primary hyperparathyroidism have lower circulating sclerostin levels than euparathyroid controls. Eur J Endocrinol. 2010;163:833–7.
13. Weidauer SE, Schmieder P, Beerbaum M, Schmitz W, Oschkinat H, Mueller TD. NMR structure of the Wnt modulator protein Sclerostin. Biochem Biophys Res Commun. 2009;380:160–5.
14. Zanelli JM, Pearson J, Moyes ST, Green J, Reeve J, Garrahan NJ, Stanton MR, Roux JP, Arlot ME, Meunier PJ. Methods for the histological study of femoral neck bone remodelling in patients with fractured neck of femur. Bone. 1993;14:249–55.
15. Bell KL, Loveridge N, Power J, Rushton N, Reeve J. Intracapsular hip fracture: increased cortical remodeling in the thinned and porous anterior region of the femoral neck. Osteoporos Int. 1999;10:248–57.
16. du Plessis JJ. Sclerosteosis: neurosurgical experience with 14 cases. J Neurosurg. 1993;78:388–92.
17. Cejka D, Herberth J, Branscum AJ, Fardo DW, Monier-Faugere MC, Diarra D, Haas M, Malluche HH. Sclerostin and Dickkopf-1 in renal osteodystrophy. Clin J Am Soc Nephrol. 2011;6:877–82.
18. Appel H, Ruiz-Heiland G, Listing J, Zwerina J, Herrmann M, Mueller R, Haibel H, Baraliakos X, Hempfing A, Rudwaleit M, Sieper J, Schett G. Altered skeletal expression of sclerostin and its link to radiographic progression in ankylosing spondylitis. Arthritis Rheum. 2009;60: 3257–62.
19. Gaudio A, Pennisi P, Bratengeier C, Torrisi V, Lindner B, Mangiafico RA, Pulvirenti I, Hawa G, Tringali G, Fiore CE. Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. J Clin Endocrinol Metab. 2010;95:2248–53.
20. Mirza FS, Padhi ID, Raisz LG, Lorenzo JA. Serum sclerostin levels negatively correlate with parathyroid hormone levels and free estrogen index in postmenopausal women. J Clin Endocrinol Metab. 2010;95:1991–7.
21. Modder UI, Hoey KA, Amin S, McCready LK, Achenbach SJ, Riggs BL, Melton LJ III, Khosla S. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res. 2011;26:373–9.
22. Modder UI, Clowes JA, Hoey K, Peterson JM, McCready L, Oursler MJ, Riggs BL, Khosla S. Regulation of circulating sclerostin levels by sex steroids in women and in men. J Bone Miner Res. 2011;26:27–34.
23. Polyzos SA, Anastasilakis AD, Bratengeier C, Woloszczuk W, Papatheodorou A, Terpos E. Serum sclerostin levels positively correlate with lumbar spinal bone mineral density in postmenopausal women—the six-month effect of risedronate and teriparatide. Osteoporos Int. 2011 Jan 11 [Epub ahead of print].
24. McNulty M, Singh RJ, Li X, Bergstralh EJ, Kumar R. Determination of serum and plasma sclerostin concentrations by enzyme-linked immunoassays. J Clin Endocrinol Metab. 2011 May 4 [Epub ahead of print].
25. Beighton P. Sclerosteosis. J Med Genet. 1988;25:200–3.
26. Lewiecki EM. Sclerostin monoclonal antibody therapy with AMG 785: a potential treatment for osteoporosis. Expert Opin Biol Ther. 2011; 11:117–27.
27. Padhi D, Jang G, Stouch B, Fang L, Posvar E. Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. J Bone Miner Res. 2011;26:19–26.
28. Li X, Ominsky MS, Warmington KS, Morony S, Gong J, Cao J, Gao Y, Shalhoub V, Tipton B, Haldankar R, Chen Q, Winters A, Boone T, Geng Z, Niu QT, Ke HZ, Kostenuik PJ, Simonet WS, Lacey DL, Paszty C. Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. J Bone Miner Res. 2009;24:578–88.
29. Ominsky MS, Vlasseros F, Jolette J, Smith SY, Stouch B, Doellgast G, Gong J, Gao Y, Cao J, Graham K, Tipton B, Cai J, Deshpande R, Zhou L, Hale MD, Lightwood DJ, Henry AJ, Popplewell AG, Moore AR, Robinson MK, Lacey DL, Simonet WS, Paszty C. Two doses of sclerostin antibody in cynomolgus monkeys increases bone formation, bone mineral density, and bone strength. J Bone Miner Res. 2010;25: 948–59.
30. Tian X, Jee WS, Li X, Paszty C, Ke HZ. Sclerostin antibody increases bone mass by stimulating bone formation and inhibiting bone resorption in a hindlimb-immobilization rat model. Bone. 2011; 48:197–201.
31. van Lierop AH, Hamdy NA, Papapoulos SE. Glucocorticoids are not always deleterious for bone. J Bone Miner Res. 2010;25:2796–800.
32. Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, Shpektor D, Jonas M, Kovacevich BR, Staehling-Hampton K, Appleby M, Brunkow ME, Latham JA. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J. 2003; 22:6267–76.
33. van Bezooijen RL, Bronckers AL, Gortzak RA, Hogendoorn PC, Wee-Pals L, Balemans W, Oostenbroek HJ, Van Hul W, Hamersma H, Dikkers FG, Hamdy NA, Papapoulos SE, Lowik CW. Sclerostin in mineralized matrices and van Buchem disease. J Dent Res. 2009; 88:569–74.
34. Chun JS, Oh H, Yang S, Park M. Wnt signaling in cartilage development and degeneration. BMB Rep. 2008;41:485–94.
35. Epstein S, Hamersma H, Beighton P. Endocrine function in sclerosteosis. S Afr Med J. 1979;55:1105–10.
36. Li X, Ominsky MS, Niu QT, Sun N, Daugherty B, D'Agostin D, Kurahara C, Gao Y, Cao J, Gong J, Asuncion F, Barrero M, Warmington K, Dwyer D, Stolina M, Morony S, Sarosi I, Kostenuik PJ, Lacey DL, Simonet WS, Ke HZ, Paszty C. Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. J Bone Miner Res. 2008;23:860–9.
37. Fujita K, Janz S. Attenuation of WNT signaling by DKK-1 and -2 regulates BMP2-induced osteoblast differentiation and expression of OPG, RANKL and M-CSF. Mol Cancer. 2007;6:71.
38. Glass DA, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, Taketo MM, Long F, McMahon AP, Lang RA, Karsenty G. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell. 2005;8:751–64.
39. Spencer GJ, Utting JC, Etheridge SL, Arnett TR, Genever PG. Wnt signalling in osteoblasts regulates expression of the receptor activator of NFkappaB ligand and inhibits osteoclastogenesis in vitro. J Cell Sci. 2006;119:1283–96.
40. Sutherland MK, Geoghegan JC, Yu C, Turcott E, Skonier JE, Winkler DG, Latham JA. Sclerostin promotes the apoptosis of human osteoblastic cells: a novel regulation of bone formation. Bone. 2004;35: 828–35.