

Patients with Van Buchem Disease, an Osteosclerotic Genetic Disease, Have Elevated Bone Formation Markers, Higher Bone Density, and Greater Derived Polar Moment of Inertia than Normal

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Van Buchem disease is an autosomal recessive disease characterized by overgrowth of the skeleton. In a group of Dutch patients the disease is thought to be due to a 52-kb deletion that results in decreased expression of the SOST gene. To further characterize the disease, the morphology of the metacarpals of six adult subjects and two juveniles with Van Buchem disease were measured on hand x-rays along with nine normal adults and nine adult carriers of the disease. Serum bone formation markers, alkaline phosphatase, type I procollagen peptide, and osteocalcin, and the urinary bone resorption marker, cross-linked N-telopeptide, were determined. Van Buchem patients had increased metacarpal outer

diameter, inner diameter, cortical thickness, and bone mineral density. Calculated bone volume and derived polar moment of inertia were markedly elevated (elevations of $158 \pm 33\%$ and $497 \pm 95\%$, respectively) consistent with increased bone strength. Serum procollagen peptide and osteocalcin were significantly higher in Van Buchem patients. Urinary cross-linked N-telopeptide was significantly elevated in Van Buchem patients. None of these changes was found in Van Buchem carriers. These observations indicate that decreased expression of the SOST gene can lead to increased bone formation and to stronger bones. (*J Clin Endocrinol Metab* 88: 5778–5783, 2003)

MUTATIONS IN THE region of the SOST gene on chromosome 17 have been identified with two similar diseases, sclerosteosis (1, 2) and Van Buchem disease or hyperostosis corticalis generalisata (3, 4). Both of these diseases are characterized by overgrowth of the skeleton. Patients with sclerosteosis have mutations of the SOST coding region resulting in absence of the gene product, sclerostin. A group of Dutch patients with Van Buchem disease have a 52-kb deletion downstream of the SOST gene that is thought to decrease gene expression (3). These findings suggest that sclerostin may be an important regulator of bone homeostasis. We have further characterized the bone abnormalities in the group of Dutch patients with Van Buchem disease to help establish the role of sclerostin in bone regulation.

First documented in 1955 by Van Buchem (5), the skeletal condition that carries his name was categorized as hyperostosis corticalis generalisata. Van Buchem disease is an autosomal recessive bone dysplasia linked to a genetic locus on chromosome 17q12–21 (6). This disease is characterized by a symmetrically increased thickness of bones, most frequently found as an enlarged jawbone, but also an enlargement of the skull, ribs, diaphysis of long bones, as well as tubular bones of hands and feet, resulting in increased cortical bone den-

sity. The clinical consequence of increased thickness of the skull include facial nerve palsy causing hearing loss, visual problems, neurological pain, and, very rarely, blindness as a consequence of optic atrophy (7). The most common radiological features are massive hyperostosis of the calvarium and mandible, resulting in increased weight of skull and sclerosis of the diaphyses of the long bones, clavicles, ribs, and pelvis with disruption of bone contours resulting in a very rough bone surface (8). Bone anomalies are symmetric, appearing in the first decade of life and becoming more prominent among the oldest patients, suggesting progression of disease with aging.

In sclerosteosis, increased formation of normal bone by osteoblast, but not osteoclast, abnormalities (9) has been implicated as the underlying pathological mechanism responsible for the skeletal changes. Accordingly, increased skeletal alkaline phosphatase, in adults as well as in children, has been reported in this condition (10). Bony changes in Van Buchem disease are consistent with increased bone formation in this disease also, but clear evidence of increased bone formation has not been obtained.

Characterization of the effects of the genetic changes in patients with Van Buchem and other similar osteosclerotic diseases can provide valuable insights into potential therapeutic modalities for low bone mass diseases. To further identify the nature of the bone changes occurring in Van Buchem subjects, we carefully examined the changes in bone

Abbreviations: ALP, Alkaline phosphatase; BMP, bone morphogenic protein; CED, Camurati-Englemann disease; NTx, type I collagen cross-linked N-telopeptide; PINP, type I procollagen.

density and bone size in hand x-rays of these Van Buchem patients and examined bone biochemical markers in samples of their serum. In addition, we examined a group of subjects who have been identified as carriers of the disease by genetic analysis to determine whether there are any manifestations of the disease in subjects with only one disease allele.

Subjects and Methods

Subjects

The affected individuals include six subjects, three women and three men (age range, 32–69 yr). Six- and 14-yr-old males were also studied, but were not included in the comparison tables. These data were compared with data from nine normal subjects and nine subjects who were carriers for Van Buchem disease. In the carrier group there were five men and four women (age range, 27–68 yr); in normal group, there were three men and six women (age range, 32–69). The Van Buchem carriers were diagnosed based on the haplotypes obtained after analyzing chromosome 17 markers surrounding the Van Buchem linkage region and the presence of the deletion (6). This study was approved by the institutional review board.

Metacarpal bone size measurements

Hand x-rays for the Van Buchem patients were quantitated for changes in bone morphology. Metacarpal bone size measurements, including total width, diaphyseal length, and medullary diameter, were made on the second, third, fourth, and fifth metacarpals. Measurements were made with calipers (accurate to 0.01 mm). Total width and medullary diameter were measured at the one third, one half, and two third lengths of the diaphysis. The three values for the diameter of each metacarpal were averaged. The coefficient of variation for metacarpal measurements (<1%) was determined by repeat measurements on all subjects. The data used for the group means were the average of the four metacarpals. The measurements were not corrected for magnification of the specimens by x-rays.

Calculated parameters

Metacarpal cortical volume and polar moment of inertia (bone tension strength) were calculated by applying the following formulas: cortical volume, $CV = \pi (D/2)^2 L - \pi (d/2)^2 L$, where $\pi = 3.142$, D is metacarpal total diameter (width), d is medullary diameter, and L is metacarpal length; polar moment of inertia, $I = \pi (D^4 - d^4)/64$; and epidiaphyseal index, calculated by dividing the middiaphyseal diameter by the diameter of the distal epiphysis of the metacarpal.

Bone density

The x-rays were suitable for determination of bone density in five affected subjects and nine carriers. Phalangeal bone mineral density was measured using standard radiographic absorptiometry of hand x-ray (OsteoGram Analysis Center, El Segundo, CA). This technique measures the bone density within the periosteal perimeter. Bone density data were adjusted for changes in bone width.

Biochemical bone markers

Blood and urine samples were available for bone marker analysis in seven affected individuals and nine carriers. Serum and urine samples were frozen at -70 C after collection and transported to the J. L. Pettis Veterans Affairs Medical Center for bone markers analysis. All samples were analyzed in single batch to minimize the assay variation. Samples were analyzed for type I procollagen (PINP), skeletal alkaline phosphatase (ALP), serum osteocalcin, and urinary cross-linked N-telopeptide (NTx).

Serum procollagen peptide

PINP was identified by a type I procollagen intact PINP RIA (Orion Diagnostica, Espoo, Finland). This assay is a quantitative test designed for *in vitro* measurements of the concentration of amino-terminal

propeptide of type I procollagen in serum and other biological fluids (Orion Diagnostica).

Serum osteocalcin

Osteocalcin levels were measured using an immunoradiometric assay (ELSA-Osteo, CIS BioInternational, Gif-sur-Yvette, France) that recognizes a large N-terminal midfragment in addition to the intact osteocalcin molecule. The sensitivity of the assay was 0.7 ng/ml midfragment osteocalcin calibrator and the linear range of the standard curve was 2.85–270 ng/ml. Samples with an osteocalcin concentration higher than 270 ng/ml were measured after dilution with zero standard. The averaged intraassay ($n = 20$) and interassay ($n = 30$) coefficients of variation were less than 7%.

Serum ALP

Bone-specific ALP was measured by a kinetic assay (11). Bone ALP was determined by the heat inactivation of one aliquot of serum sample, standards, and controls at 54 C for 12 min. The second aliquot was assayed without heating and represents the total activity. The sensitivity of the total ALP assay was 0.6 U/liter, with a linear assay range of 6.0–136.8 U/ml. The average intraassay ($n = 20$) and interassay ($n = 70$) coefficients of variation were less than 12%.

Urinary NTx

Urinary NTx was measured with an ELISA (Osteomark, Ostex International, Inc., Seattle, WA) according to the package insert provided with the kit. The sensitivity of the ELISA was 20 nM bone collagen equivalent, and the linear range of the assay was 20–3000 nM bone collagen equivalent. The intraassay ($n = 20$) and interassay ($n = 30$) coefficients of variation were less than 14%.

Creatinine

Creatinine was measured by the colorimetric Jaffe method (Sigma-Aldrich Corp., St. Louis, MO). The sensitivity of the colorimetric assay was 0.43 mg/dl, and the linear range of the assay was -20 mg/dl. The intraassay ($n = 20$) and interassay ($n = 30$) coefficients of variation were less than 3.0%.

Statistical analysis

For the metacarpal measurements, a three-way ANOVA (digit, sex, and group) was used for determination of statistical significance, so that the variance between subject groups could be separated from the variance among the four metacarpals and the variance due to sex. For the analysis of serum marker data, a two-way ANOVA (sex and group) was used. The significance of differences between groups was determined by Tukey's highest significant difference *post hoc* test.

Results

Quantitative analysis of hand x-rays

To characterize the disease, careful measurements were obtained from hand x-rays. In addition to Van Buchem patients and normal controls, a group of nine subjects who were carriers of the disease were included. The mean ages of the three groups were not significantly different (Table 1). Both male and female subjects were included in the study. None of the measured parameters differed significantly according to sex in the ANOVA. However, if all subjects were analyzed together, there were significant effects of sex on metacarpal length and epiphyseal diameter ($P < 0.01$). Therefore, sex was still included as a variable in the ANOVA.

Metacarpal bone size measurements

Metacarpal bone size measurements were made on the second, third, fourth, and fifth metacarpals, and the averages

TABLE 1. Measured values from the metacarpals in adult Van Buchem-affected, Van Buchem carriers, and normal subjects

	Van Buchem affected (n = 6)	Van Buchem carriers (n = 9)	Normal subjects (n = 9)
Age (yr)	51.0 ± 5.8	45.8 ± 5.2	51.7 ± 5.0
Length (mm)	68.4 ± 3.1	71.4 ± 3.1	62.5 ± 2.3
Epiphysis diameter (mm)	16.3 ± 0.7	18.0 ± 0.6 ^a	14.9 ± 0.9
Diaphyseal outer diameter (mm)	12.83 ± .69 ^b	9.43 ± .33	8.20 ± .37
Diaphyseal inner diameter (mm)	6.45 ± .76 ^c	4.52 ± .36	3.85 ± .39
Thickness (mm)	3.19 ± 0.31 ^c	2.46 ± 0.12	2.18 ± .13

Values are the mean ± SE of combined digits and sexes. Data were analyzed by three-way ANOVA and *post hoc* testing with Tukey's highest significant difference test.

^a $P < 0.05$ vs. normal subjects.

^b $P < 0.001$ vs. normal subjects and carriers.

^c $P < 0.01$ vs. normal subjects; $P < 0.05$ vs. carriers.

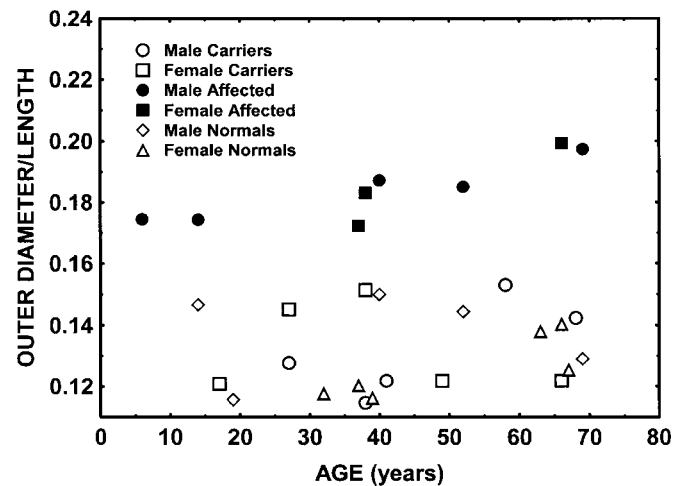
of these four sites are shown in Table 1. As expected, the Van Buchem-affected metacarpals did not differ significantly from Van Buchem carriers or controls in metacarpal length or in width at the epiphysis. However, cortical bone in the diaphysis showed striking increases in the affected group. The outer diameter of the diaphysis of metacarpal bones in the nondominant hand, as measured on hand radiographs, was significantly greater in the Van Buchem patients (by 56 ± 8%) than in the control subjects ($P < 0.001$; Table 1). All digits showed the increase in outer diameter (data not shown). The medullary cavity diameter was also significantly increased (by 67 ± 20%) in the Van Buchem patients compared with the controls. Despite the increase in inner diameter, the cortical thickness was also increased (Table 1). In addition to the data in Table 1, a 14-yr-old Van Buchem patient had values close to the mean values for the Van Buchem-affected group.

When we analyzed the metacarpal differences as a function of age, we initially did not find a significant correlation. However, metacarpal dimensions are partly dependent on body size. Division of midshaft diameter by metacarpal length to correct for differences in body size resulted in highly significant ($P < 0.001$) differences between the Van Buchem-affected group and the other two groups in midshaft outer diameter, but not in epiphyseal diameter. The corrected midshaft outer diameter was significantly correlated with age ($P < 0.05$). A plot of midshaft diameter/length with age is given in Fig. 1. No significant correlations with age were found in the normal control or carrier groups.

As a further test of body size correction we divided outer diameter by epiphyseal width to yield an epidiaphyseal index. We used epiphyseal width because it is not affected by Van Buchem disease. The ratio was not only elevated (Table 2), but also was significantly correlated with age (metacarpal; $r = 0.71$; $P < 0.05$). A similar calculation for the phalanges also showed a significant correlation with age ($r = 0.88$; $P < 0.01$).

Metacarpal bone volume and polar moment of inertia

The diameter and length data were further used to calculate cortical bone volume and the polar moment of inertia. Polar moment of inertia is a figure of merit that engineers

**FIG. 1.** Effects of age and sex on metacarpal outer diameter/length in Van Buchem carriers, Van Buchem-affected patients, and normal subjects.**TABLE 2.** Calculated metacarpal parameters from adult Van Buchem-affected, Van Buchem carriers, and normal subjects

	Van Buchem affected (n = 6)	Van Buchem carriers (n = 9)	Normal subjects (n = 9)
Bone volume (mm ³)	6960 ± 909 ^a	3990 ± 376	2694 ± 328
P. moment of inertia (mm ⁴)	1452 ± 231 ^a	416 ± 50	243 ± 45
Epidiaphyseal index	1.020 ± 0.025 ^a	0.619 ± 0.011	0.562 ± .024

Values are the mean ± SE of combined digits and both sexes. Data were analyzed by three-way ANOVA and *post hoc* testing with Tukey's highest significant difference test.

^a $P < 0.001$ vs. normal subjects and carriers.

used to describe the effect of architectural parameters on bone strength. Cortical volume and polar moment of inertia were markedly increased (158 ± 33% and 497 ± 95%, respectively) in Van Buchem patients compared with normal subjects (Table 2). The polar moment of inertia is particularly influenced by the large increase in periosteal size.

Van Buchem carriers

Van Buchem disease is an autosomal recessive trait. Genetic studies have identified a group of subjects who are heterozygous for the chromosome 17 haplotype and the deletion and who are thus carriers of the disease. These subjects were studied to determine whether they showed any manifestations of the disease. For the carrier group, there was a trend toward higher values than the control group (Tables 1 and 2). For epiphyseal width, the value was significantly different from the control group. To determine whether there could be some significant effect of the disease in the carrier group, the data were examined further. The carrier group had a 14% greater metacarpal length than the control group. Because the disease does not appear to affect growth in the length of the long bones, this difference in length suggests that the carrier group, by chance, had a slightly greater average size. The differences between the carrier group and the control group for outer and inner diameters were 15% and

17%, respectively. Thus, there were proportionate increases in all parameters, rather than specific changes in diaphyseal parameters. As noted above, correction for length eliminated the differences in diameters between the carrier and control groups. Thus, the differences appear to be due to a difference in body size rather than an effect of the gene mutation.

Bone mineral density

Some of the hand x-rays were available for radiodensity determination. A subgroup of four affected individuals and a carrier group of nine subjects were analyzed. Bone density was measured in the phalanges using standard radiographic absorptiometry of the hand x-rays. This technique measures bone density within the periosteal perimeter. There was a highly significant increase in bone density in Van Buchem patients compared with the carrier group (153 ± 6 vs. 111 ± 4 ; $P < 0.01$). The affected subjects had a significantly higher T-score than the carrier group (3.2 ± 1.7 vs. 0.3 ± 0.3 ; $P < 0.05$). These bone density data were adjusted for changes in bone width. Thus, in the Van Buchem patients, not only is there more bone because of the increase in periosteal perimeter and the increase in periosteal volume, but the data suggest an increase in the amount of bone within a unit of periosteal volume, based on the assumption that the adjusted areal bone density is an estimate of volumetric bone density. This is consistent with increased cortical bone thickness in the affected patients (Table 1). Visual inspection of the hand radiographs indicated that the number and width of trabeculae in the metacarpal bones were greater in Van Buchem patients than in control patients.

Serum bone markers

Because of previously published work on ALP in sclerosteosis (10), which appears to be a related disease, we anticipated an increase in bone formation markers in Van Buchem's patients. When we examined data from the Van Buchem-affected and carrier groups, serum ALP was higher in the Van Buchem group, but the difference did not reach statistical significance ($P = 0.12$; Table 3). However, increases in serum PINP and osteocalcin in the Van Buchem group over the carrier were statistically significant (Table 3), and mean values were in the high normal range. The data are plotted as a function of age and sex in Figs. 2 and 3. In

TABLE 3. Bone marker data for Van Buchem affected (n = 6) and Van Buchem carriers (n = 9)

Marker	Van Buchem (n)	Carriers (n)
Serum alkaline phosphatase	13.6 ± 1.5 (6) ^a	10.0 ± 1.6 (8)
Serum PINP	75.6 ± 13.7 (6) ^b	29.4 ± 5.0 (8)
Serum osteocalcin	25.1 ± 3.8 (5) ^c	13.6 ± 2.0 (7)
Urinary NTx	73.0 ± 19.6 (4) ^c	36.9 ± 2.8 (8)
Bone mineral density (arbitrary units)	153 ± 6 (4) ^b	111 ± 4 (9)

Values are the mean \pm SE of both males and females. Data were analyzed by two-way ANOVA and *post hoc* testing with Tukey's highest significant difference test. Analyses were not available in all subjects due to limited serum sample size or urine collections.

^a $P = 0.12$.

^b $P < 0.01$.

^c $P < 0.05$.

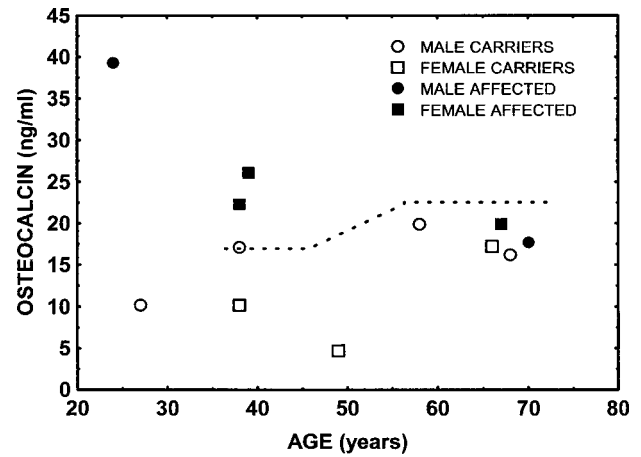


FIG. 2. Effects of age and sex on serum osteocalcin in Van Buchem carriers and Van Buchem-affected subjects. The dotted line represents the upper limit of normal (mean \pm 2 SD) (18).

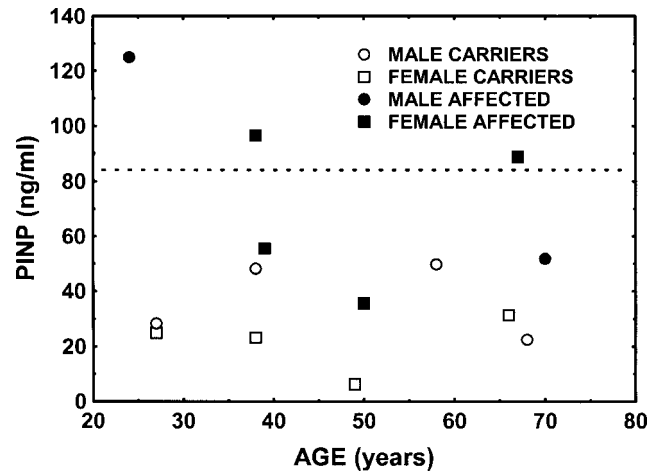


FIG. 3. Effects of age and sex on serum PINP in Van Buchem carriers and Van Buchem-affected subjects. The dotted line represents the upper limit of normal (mean \pm 2 SD) (19).

addition, a 15-yr-old Van Buchem patient had z-scores (compared with age-matched controls) for ALP and PINP of 18.9 and 6.9. These data are all consistent with increased bone formation in Van Buchem patients.

Urinary bone resorption marker

Urinary NTx is a bone resorption marker. Although there were only limited data (four Van Buchem-affected subjects), this marker was also increased significantly in Van Buchem patients over the carrier group (Table 3). This evidence for increased resorption would be consistent with an increase in medullary cavity size. Thus, there is evidence for increased bone turnover in Van Buchem disease.

Discussion

The osteosclerosis found in Van Buchem disease had previously suggested that bone formation is increased in this disease. Our study provided evidence that there is an increase in bone formation markers in Van Buchem disease in both young and adult subjects. In adults, serum markers of

bone formation, PINP and osteocalcin, were significantly elevated over those in the carrier group, and the elevation in serum ALP approached statistical significance. Although the serum marker data for Van Buchem-affected subjects were only compared with the Van Buchem carrier group and not with normal subjects, the mean values for the affected group were in the high normal range, whereas the mean values for the carrier group were in the normal range. Furthermore, the metacarpal measurements from the carrier group were not different from those in normal subjects, suggesting that the carrier group is normal and is not affected by the genetic disease. The serum marker evidence for increased bone formation is consistent with evidence for increased bone formation in the related disease, sclerosteosis (9, 10). It is unlikely that the increase in metacarpal periosteal diameter was due to a change in resorption, because the resorption marker NTx was increased, not decreased. Thus, the increased metacarpal size and cortical thickness in Van Buchem disease appear to be due to elevated periosteal bone formation. Confirmation of increased bone formation will require histomorphological evidence. The elevated periosteal bone formation appears to manifest itself in the teens (at least this was the case in one of our patients) and continues to progress throughout the aging process, as shown by the positive slope for the relationship between periosteal perimeter and chronological age in Fig. 1.

A further surprising finding in this study is that the inner diameter of the metacarpal increased rather than decreased as might be expected for sclerosteosis. This increased marrow cavity size could be due to decreased formation or increased bone resorption. Because both formation and resorption markers were increased, the increased marrow size is probably due to increased resorption. These results lead to the surprising conclusion that this disease may be an increased turnover disease rather than simply an increased bone formation disease. Alternatively, the increase in resorption might not be a direct consequence of Van Buchem disease, but, instead, is a manifestation of a normal skeletal response to an increasing periosteal perimeter. This would be analogous to the growth process, where the endosteal perimeter increases as does the periosteal perimeter.

Van Buchem disease, like sclerosteosis, has increased bone formation and overgrowth of the skeleton. Both diseases are associated with reduction in sclerostin gene (SOST) expression (1–4). Thus, sclerostin has an important role in the regulation of bone formation. Sclerostin has a cysteine-rich region and has homology with the DAN family of secreted glycoproteins. This family includes *dan*, *cerberus/cer 1*, *gremlin/drm*, *prdc*, *caronte*, and *dante*. On the basis of their sequence similarity to the cysteine knot structures of TGF β 2 (determined by crystallography) and Norrie disease protein (characterized by molecular modeling) (2), the *dan* family has also been proposed to include a cysteine knot. Members of the *dan* family share the ability to antagonize bone morphogenic protein (BMP) signaling. Sclerostin preferentially binds and inhibits BMP-5 and BMP-6 (12). Thus, the increase in bone formation in Van Buchem disease may be the result of reduced BMP antagonism by sclerostin and increased BMP activity.

In this regard, it is interesting that the gene that has been

identified for the Mendelian disease, Camurati-Englemann disease (CED), which is another example of osteosclerosis, has been attributed to mutations in the inhibitory binding protein of TGF β (13, 14). CED and Van Buchem disease are two of the classical diseases that are osteosclerotic in nature. Osteosclerotic diseases are thought to be diseases of increased bone formation, whereas osteopetrotic diseases are thought to be diseases of decreased bone resorption (15). One of the big differences between CED and Van Buchem disease is that in Van Buchem disease the periosteal perimeter is increased, whereas in CED the periosteal perimeter is not increased, and the increase in bone apposition is on the endosteum, particularly around neural foramina, a change that leads to severe neurological changes.

These two diseases together provide critically important information about genes that might be considered in the future for gene therapy for bone-wasting diseases, such as osteoporosis. We do not know why CED has more bone formation on the endosteum and Van Buchem disease has more bone formation on the periosteum. In any case, the simplest explanation for these data is that the increase in BMP expression in the adult skeleton could lead to an increase in periosteal bone formation, whereas an increase in TGF β expression in the adult skeleton could lead to an increase in endosteal bone formation. In both of these cases, there are neurological deficits because of narrowing of neural foramina. Therefore, these two members of the TGF β superfamily, although candidates for gene therapy, perhaps on a short-term basis with highly localized application, could not be used chronically because of the severe debilitating neurological complication of excess bone formation in sites that compromises nerve function.

Van Buchem disease and also CED, if eventually confirmed to be due to excess expression of genes of the TGF β superfamily, would provide a unique clinical lesson on the problems that confront the gene therapy laboratories using corresponding genes to stimulate bone formation on a generalized basis throughout the skeleton. These results emphasize the importance of targeting the increase in gene expression to specific sites within the skeleton and avoiding other sites.

Our data provide strong evidence for increases in bone density and bone size, both of which act to increase bone strength (16, 17). Assuming there is no change in bone quality, the increase in polar moment of inertia means that the bones are considerably stronger in Van Buchem patients. Accordingly, because of the increases in cortical volume and periosteal perimeter and the lack of change in the length of the metacarpals, there was a large increase in the polar moment of inertia, which suggests that these bones would be markedly stronger than the control bones under standard loading conditions. The serum marker data are consistent with increased bone turnover. This might lead to a decrease in bone quality, which would tend to decrease bone strength. Nevertheless, the large increase in polar moment of inertia suggests that bone strength is increased.

The possibility that bone strength is actually increased in these patients is consistent with the history of several patients who have sustained substantial trauma without evidence of fracture. Increased bone strength is, of course, a very

desirable characteristic for bone therapy in osteoporosis and other low bone mass diseases. In conclusion, patients with this disease have a mutation that most likely leads to an increase in bone strength.

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