Modulation of the canonical Wnt signaling pathway in bone and cartilage

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Chapter 5

APC mutations are associated with increased bone mineral density in patients with familial adenomatous polyposis

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ABSTRACT

The canonical Wnt pathway plays a key regulatory role in osteoblastogenesis and bone mass acquisition through its main effector, β-catenin. Adenomatous polyposis coli (APC) represents the key intracellular gate-keeper of β-catenin turnover and heterozygous germline mutations in the *APC* gene cause familial adenomatous polyposis (FAP). Whether *APC* mutations affect bone mass has not been previously investigated. We conducted a cross-sectional study evaluating skeletal status in FAP patients with a documented *APC* mutation. Twenty-two FAP patients with a mean age of 42 years (54.5% women) were included in this study. Mean BMD Z-scores were significantly increased above normal at all measured sites: lumbar spine ($p<0.01$), total hip ($p<0.01$), femoral neck ($p<0.05$) and trochanter ($p<0.01$). Z-scores were ≥ +1 in 14 patients (63.6%) and ≥ +2 in 5 patients (22.7%). Mean values of bone turnover markers were within normal ranges. There was a significant positive correlation between Procollagen type I N-terminal propeptide (P1NP) and β-crosslaps (β-CTX) ($r = 0.70$; $p<0.001$). Total hip BMD was positively correlated with P1NP ($r = 0.37$; $p = 0.084$), β-CTX ($r = 0.59$; $p < 0.01$) and sclerostin ($r = 0.56$; $p < 0.01$). We demonstrate that FAP patients display a significantly higher than normal mean BMD compared to age- and sex-matched healthy controls in the presence of a balanced bone turnover. Our data suggest a state of "controlled" activation of the Wnt signalling pathway in heterozygous carriers of *APC* mutations, most likely due to upregulation of β-catenin.

INTRODUCTION

Familial adenomatous polyposis (FAP) is an autosomal dominant syndrome caused by heterozygous germline mutations in the *APC* gene. Occurring in approximately 1:10,000 live births, FAP is characterized by the development of hundreds to thousands of colorectal adenomatous polyps during childhood and adolescence (1). The adenomas inevitably progress to colorectal carcinoma (CRC) at a mean age of 40-50 years, if colectomy is not performed (2). FAP is also associated with an increased risk of extracolonic malignancies (e.g., duodenal, pancreatic, and thyroid cancer) and other pathologic conditions, such as osteomas, dental anomalies, epidermoid cysts, lipomas, desmoid tumors, and congenital hypertrophy of the retinal pigment epithelium (CHRPE) (3). Whereas the majority of FAP patients have a family history of the disease, up to 30% of cases are due to *de novo* mutations in the *APC* gene (4). Genetic analysis identifies gene inactivating mutations within the *APC* gene in more than 70% of cases (1).

The *APC* tumor suppressor gene is located on chromosome 5q21-q22 and consists of 15 exons that encode a large multifunctional protein product (312kDa, 2843 codons), known to be involved in a broad spectrum of cellular processes such as cell cycle regulation, apoptosis, cell adhesion and migration, microtubule assembly, cell fate determination, and chromosomal stability (5). However, APC's main tumor suppressor function is to bind to and to down-regulate the key transducer of the canonical Wnt signaling pathway, β-catenin, thereby acting as a strong negative regulator of the Wnt signaling cascade (6;7). The efficiency of mutant APC to downregulate β-catenin is compromised according to the site of the mutation (8). As a result, β-catenin accumulates in the cytoplasm and subsequently translocates into the nucleus, where it stimulates transcription of Wnt target genes leading to increased cell proliferation and decreased apoptosis (6).

Over the past decade, evidence has accumulated about the important role of β-catenin in the regulation of bone mass and in the pathophysiology of a number of skeletal disorders (9). Since APC represents a critical regulator of β-catenin levels, it is not surprising that FAP patients carrying mutations in the *APC* gene commonly develop diverse skeletal pathology including osteomas and/or dental anomalies such as supernumerary and impacted teeth (10). Several *in vivo* and *in vitro* studies further support the role of APC in both skeletal development and metabolism. Inactivation of *Apc* in skeletal progenitor cells has been shown to lead to an osteosclerotic bone phenotype, due to the formation of highly active osteoblasts (11), whereas inactivation of *Apc* in mature osteoblasts results in an osteopetrotic bone phenotype, due to impaired osteoclast differentiation (12). Interestingly, it has been reported that mice carrying a heterozygous loss-of-function mutation in *Apc* (*Apc*$^{min/+}$) display a significantly increased BMD of the distal femur (13). Taken together, these data strongly imply that APC might be involved in the regulation of bone mass by regulating the cytoplasmic levels of β-catenin.

FAP patients carry heterozygous *APC* mutations that result in a constitutively active transduction of β-catenin. We hypothesized, therefore, that FAP may represent a valuable human model for studying the role of the canonical Wnt signaling pathway in bone mass acquisition and maintenance. In this study we addressed the question whether heterozygous mutations in the *APC* gene are associated with alterations in bone mass and/or bone turnover.

MATERIALS AND METHODS

Study population

Thirty consecutive FAP patients attending the Outpatient Clinic of the Department of Gastroenterology of the Leiden University Medical Centre (LUMC) for routine follow-up visits over a 6-month calendar period were invited to take part in the study. The inclusion criteria were a clinically established, histologically documented, and genetically confirmed diagnosis of FAP and the willingness of the patient to participate in the study. The only exclusion criterion was the current or past use of any agent known to affect bone metabolism such as bisphosphonates or parathyroid hormone (PTH). The study was approved by the Ethics Committee of the LUMC, and written informed consent was obtained from all patients.

Demographic and clinical characteristics

Demographic characteristics including age, gender, ethnicity, body weight, height, body mass index (BMI), menopausal status, smoking habits, and alcohol use were documented in all patients. Other data collected were family history of FAP, age at diagnosis, history of colectomy, age at colectomy, extra-colonic manifestations, dental and fracture history, and use of medication. To allow for comparison between different ages and genders, height was expressed as standard deviation score (SDS) corrected for shrinking and secular trend (14).

BMD measurements

BMD was measured in all patients at the lumbar spine (L1-L4) and at different sites of both hips (total hip, femoral neck, and trochanter) using dual X-ray absorptiometry (DXA) (Hologic QDR-4500, Hologic Inc.). Mean BMD of the left and right hip was used for analysis. Data were expressed as T-scores (number of SDs from the mean value of the sex-matched reference population) and Z-scores (number of SDs from the mean value of the age- and sex-matched reference population) using the NHANES reference values, which are compatible with those of Dutch control populations (15-17). Standard WHO reference values established for BMD measurements were used to define osteopenia ($-2.5 < \text{T-score} \leq -1$) and osteoporosis ($\text{T-score} \leq -2.5$) (18). In our analysis, we considered “low” BMD if $\text{Z-score} \leq -1$, “normal” BMD if $-1 < \text{Z-score} < +1$ and “higher than normal” BMD if $\text{Z-score} \geq +1$. The coefficient of variation (CV) of BMD measurements was 1% and the machine was cross-calibrated at regular intervals using a validated phantom.

Vertebral fracture assessment (VFA)

Since vertebral compression fractures may increase lumbar BMD and since these may be clinically silent, we additionally performed vertebral fracture assessment (VFA) to assess the morphology of vertebrae at the lumbar spine (L1–L5). Paired anteroposterior and lateral VFA images were examined by a nuclear medicine physician who was blinded to the DXA results. The Genant's semiquantitative method was used to visually assess abnormal vertebral morphology (AVM) (19). Using this method, grade 1 vertebral deformity (mild) was judged to be present when an anterior, posterior or middle reduction in vertebral height of 20–25% was observed, grade 2 (moderate) when a reduction of 26–40% was observed and grade 3 (severe) when a reduction greater than 40% was observed. Standard paired anteroposterior and lateral radiographs of the thoracic and lumbar spine were performed when AVM was suggested by VFA.

Skeletal scintigraphy

Since scintigraphy is more sensitive than conventional radiographs in the diagnosis of osteomas (20-22) and since the area of uptake on scintigraphy may extend beyond the limits of any radiographic change (22), we used radionuclide imaging to detect this focal bone pathologic condition in our study. Whole body scintigraphy was performed using a standard protocol of images on a dual-headed Toshiba gammacamera (Toshiba CGA 7200, Japan). Anterior and posterior views were acquired 3 hours after injection of 500 MBq (13.5 mCi) of Tc-99m-HDP. Osteomas were considered to be present when a pattern of focally intense tracer uptake surrounded by less prominent increase uptake was visualized. All scintigraphy scans were visually analysed by an independent experienced nuclear medicine physician.

Laboratory investigations

Blood was collected from all patients between 8.00 and 10.00 a.m. after an overnight fast. Serum was used to measure calcium (corrected for an albumin of 42 g/l), phosphate (P), and total alkaline phosphatase (ALP) activity using semi-automated techniques. Serum 25-hydroxyvitamin D (25-OH-D), 1,25-dihydroxyvitamin D (1,25-$(OH)_2$-D3), PTH, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen (E), testosterone (T), procollagen type I N-terminal propeptide (P1NP), and β-crosslaps (β-CTX) concentrations (respectively markers of bone formation and resorption) were measured using standard radio-immuno-assays (RIA). Vitamin D levels were not corrected for seasonal variation. Vitamin D insufficiency was diagnosed when 25-OH-D concentration was < 50 nmol/l (23). Hypogonadism was defined in men by a serum T concentration < 10 nmol/l and in women by a serum E concentration < 70 pmol/l in the absence of menstrual cycles, associated in both men and women with FSH and LH concentrations > 30 U/l and > 60 U/l, respectively. Additional blood samples were collected at the same time, immediately centrifuged and serum was separated and stored at -80°C for later measurement of sclerostin. This was performed using a solid phase sandwich immunoassay following an in-house protocol on a Sector Imager 2400 platform (Meso Scale Discovery) with a minimum detection limit of 1 pg/ml.

Statistical analysis

Statistical analysis was performed using the SPSS 16.0 for Windows software package. The number of patients needed to demonstrate a significant effect of *APC* mutations on BMD was statistically determined using a power (1-beta) of 80% and an alpha level of 5%. Under the assumption that the FAP group may demonstrate a 10% increase in BMD, the number of patients needed to be studied to show this effect was calculated to be 20 patients in this single-centre observational cross-sectional study.

All descriptive parameters are expressed as mean ± standard deviation (SD) or as numbers and percentages of patients within groups, as appropriate. Categorical variables are presented as frequency and percentage and compared using the Chi-square test. One-sample t-tests were used to compare BMD T- and Z-scores with normal scores. One way ANOVA was used to compare the mean values of several variables between groups. Pearson scores (*r*) were calculated to determine correlation between BMD and different clinical and biochemical variables. A p value < 0.05 was considered to be statistically significant.

RESULTS

Patients

Of the 30 consecutive FAP patients invited to take part in the study, 4 were not included because a mutation in the *APC* gene could not be identified on DNA analysis, 3 declined to participate in the study and 1 was excluded because of bisphosphonate use. All remaining 22 patients fulfilled the study inclusion criteria and were included in the study.

Demographic, clinical and genetic characteristics

The demographic, clinical and genetic characteristics of the 22 patients (10 men and 12 women) included in the study are shown in Table 1. Mean age was 42 ± 12 (SD) years. Twenty patients (90.9%) were Caucasian. Four of the 12 women (33.3%) were postmenopausal not using hormone replacement therapy. Mean height SDS was -0.50 ± 1.33. Mean BMI was 27.0 ± 5.0 kg/m^2. None of the patients used excessive alcohol, 7 (31.8%) were former smokers and 6 (27.2%) were smoking an average of 12 cigarettes per day at the time of the study. All patients reported a positive family history for FAP, indicating that none of their mutations were *de novo* mutations. Mean age at diagnosis was 21 years, ranging from 6 to 47 years. All patients but one (95.5%) had undergone prophylactic colectomy at a mean age of 23 ± 11 years. Typical of the FAP syndrome, we documented non-skeletal extra-colonic manifestations in several patients: single or multiple fibroma(s) in 3 patients (13.6%), epidermoid cysts in 4 patients (18.2%), and desmoid tumors in 4 patients (18.2%). Seven patients (31.8%) reported dental anomalies such as impacted teeth (other than third molars), congenitally missing teeth, and/or supernumerary teeth. None of the patients reported a history of low-energy trauma fracture or a clinically manifest vertebral fracture.

Parameters	Values
Demographic characteristics	
Age (years)*	42 ± 12 (21-60)
Ethnicity (Caucasian/non-Caucasian) ‡	20 (90.9) / 2 (9.0)
Female gender‡	12 (54.5)
Menopause#	4 (33.3)
Height (SDS)*	-0.50 ± 1.33 (-2.94-1.71)
Weight (kg)*	79.4 ± 18.3 (42.4-120.9)
Body mass index (kg/m^2)*	27.0 ± 5.0 (17.9-37.7)
Smoking status (never/former/current)‡	9 (40.9) / 7 (31.8) / 6 (27.2)
Clinical characteristics	
Family history of FAP‡	22 (100)
Age at diagnosis (years)*	21 ± 10 (6-47)
Colectomy‡	21 (95.5)
Age at colectomy (years)*	23 ± 11 (13-51)
Fibromas‡	3 (13.6)
Epidermoid cysts‡	4 (18.2)
Desmoid tumor‡	4 (18.2)
Teeth anomalies (current or in the past)‡	7 (31.8)
Clinical fractures‡	0 (0)
APC mutation	
c.609 insA‡	1 (4.5)
c.697C>T‡	1 (4.5)
c.1548G>C‡	1 (4.5)
c.1744-2A>G splice acceptor defect‡	3 (13.6)
c.1958+1_1958+2dupGT‡	2 (9.0)
c.1972_1975delGAGA‡	1 (4.5)
c.2805C>A‡	3 (13.6)
c.2864_2865delAA‡	2 (9.0)
c.3164_3168delTAATA‡	1 (4.5)
c.3183_3187delACAAA‡	1 (4.5)
c.3927_3931delAAAGA‡	1 (4.5)
c.4069G>T‡	1 (4.5)
c.4348C>T‡	2 (9.0)
c.4786delC‡	2 (9.0)

Table 1. Demographic, clinical and genetic characteristics of the 22 FAP patients included. Results are expressed as *mean ± SD (min-max), ‡n (%) or #n (valid %).

The 14 different heterozygous mutations in the *APC* gene identified in our study population included 6 deletions, 6 base substitutions, one insertion, and one duplication. Although none of the identified *APC* mutations were located in the same functional domain, most were clustered in exon 15 of the *APC* gene (Figure 1).

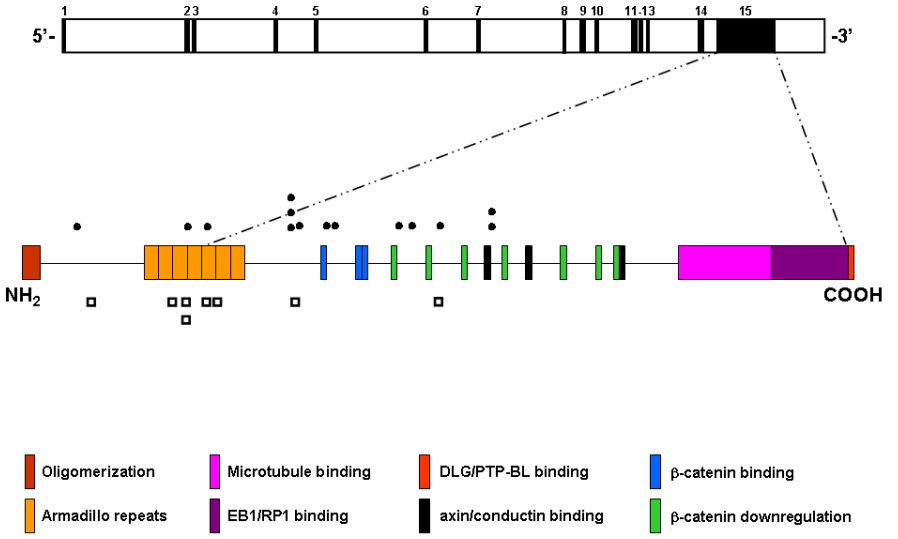

Figure 1. Diagram of the *APC* gene (upper bar) comprising 15 exons and the location of the mutations found in our study population (black dots = patients with BMD Z-scores ≥ +1, black squares = patients with BMD Z-scores < +1) in relation to the functional domains of the APC protein (lower bar). Of note is the clustering of *APC* mutations found in patients with BMD Z-score ≥ +1 among the β-catenin binding/downregulating domains.

Bone mineral density measurements

BMD data are shown in Table 2 and Figure 2. None of the patients had osteoporosis at any of the sites measured. Osteopenia was present at the lumbar spine in a 58-year-old man (T-score = -1.2) and in a 52-year-old postmenopausal woman (T-score = -2.4), and at the total hip in 2 premenopausal women (T-score = -1.1). None of the patients included in the study had osteopenia at more than one site.

Mean BMD Z-scores were significantly increased above normal (Z-score > 0) at all sites measured: +0.8 ± 1.2 in the lumbar spine ($p < 0.01$), +0.7 ± 1.1 in the total hip ($p < 0.01$), +0.5 ± 1.0 in the femoral neck ($p < 0.05$), and +1.1 ± 1.3 in the trochanter ($p < 0.01$). From the total of 22 patients investigated, 14 patients (63.6%) had BMD Z-scores ≥ +1 and 5 patients had a Z-score ≥ +2 at one or more sites measured.

Assessment of vertebral morphology of the lumbar spine

On visual analysis of VFA images of the lumbar spine (L1-L5), mild abnormal vertebral morphology (AVM) was observed in 3 male patients. The AVM observed by VFA was confirmed by standard radiographs in only 2 of the patients in whom it was attributed to vertebral osteochondrosis and degenerative changes respectively. Both these patients had a history of back pain and a normal lumbar BMD. AVM observed on VFA was not confirmed by standard radiography in the 3rd patient who had a lumbar BMD Z-score > +2. The finding of higher than normal Z-scores on lumbar BMD assessment

was thus deemed to be reliable. There was no statistically significant demographic, clinical and laboratory difference between patients with and without AVM.

Bone mineral density	Mean ± SD (min-max)
Lumbar spine (L1-L4)	
g/cm^2	1.123 ± 0.168 (0.790-1.460)
T-score	+0.5 ± 1.4 (-2.4-3.4)
Z-score	+0.8 ± 1.2 (-1.5-3.6)**
Total hip	
g/cm^2	1.051 ± 0.194 (0.811-1.508)
T-score	+0.4 ± 1.2 (-1.0-3.2)
Z-score	+0.7 ± 1.1 (-1.0-3.2)**
Femoral neck	
g/cm^2	0.899 ± 0.161 (0.695-1.280)
T-score	+0.0 ± 1.2 (-1.4-2.6)
Z-score	+0.5 ± 1.0 (-1.3-2.6)*
Trochanter	
g/cm^2	0.841 ± 0.177 (0.629-1.242)
T-score	+0.9 ± 1.3 (-0.7-3.7)**
Z-score	+1.1 ± 1.3 (-0.7-3.7)**

Table 2. BMD measurements of the 22 FAP patients included. *p<0.05, **p<0.01 one sample t-test compared to normal scores

Skeletal scintigraphy

Single or multiple foci of increased radioisotope tracer uptake, consistent with the presence of an osteoma, were observed in 13 patients (59.0%). Mean number of foci was 1.85 ± 0.55 per patient. The most common localization of osteomas was craniofacial, where 10 patients (76.9%) demonstrated single or multiple foci of increased tracer uptake, particularly in the mandible or maxilla. One patient displayed scintigraphic signs of osteoma in the knees, 1 in both the mandible and the clavicle and 1 in a cervical vertebra. There was no statistically significant difference in mean age between patients with or without osteoma(s) (39 ± 13 years *vs*. 45 ± 8 years; p = 0.238). There was a trend of an association between the presence of teeth anomalies and scintigraphic evidence for osteoma(s) (odds ratio = 4.1; 95%CI, 0.5 to 28.8; p = 0.083).

Laboratory investigations

Mean laboratory values of all parameters investigated were within the normal laboratory reference ranges (Table 3). Of the 12 patients (60%) with 25-OH-D insufficiency, 2 had increased PTH concentrations in the presence of normal BMD in both the lumbar spine and the hips. None of the patients studied had evidence for hypogonadism, except for the 4 postmenopausal women.

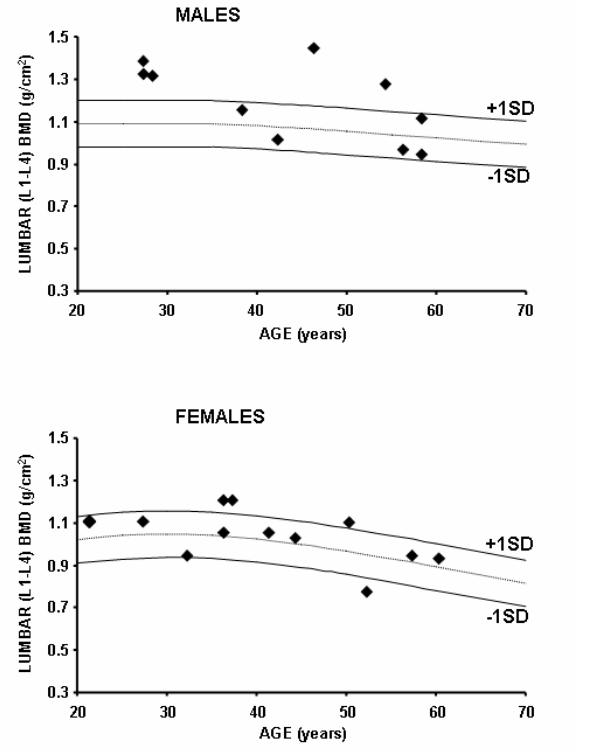

Figure 2. Lumbar BMD values in all FAP patients included in the study plotted against the NHANES normative data (upper graph - males, lower graph - females).

None of the patients included in the study had serum ALP activity above normal. Since both markers of bone turnover P1NP and β-CTX display different reference values according to gender and menopausal status, we expressed the measured values of these 2 bone turnover markers as a multiple of the upper limit of the normal reference (X ULN) (24-27). While mean P1NP was normal for the whole population (0.72 ± 0.28 X ULN), 5 patients (22.7%) had a P1NP value above the ULN. Two of these patients exhibited normal BMD, while the other 3 had higher than normal BMD. Similarly, mean β-CTX was normal for the whole population (0.60 ± 0.37 X ULN), with only 2 patients (9%) having β-CTX values above the ULN in the presence of higher than normal BMD. Interestingly, these 2 patients also had P1NP values above the ULN. Mean sclerostin (n = 20) was 38.32 ± 23.33 pg/ml, with 1 patient (5%) showing a concentration above the upper limit of the reference range (10.5-71.3 pg/ml). This patient showed higher than normal BMD at the lumbar spine and total hip, as well as levels of P1NP and β-CTX levels above the ULN.

Parameters	Mean ± SD (min-max)	Reference values
Corrected Ca for Alb	2.23 ± 0.09 (2.12-2.57)	2.15-2.55
P	1.14 ± 0.16 (0.83-1.49)	0.9-1.5
25-OH-D	52.5 ± 16.3 (27-78)	50-120
1,25-$(OH)_2$-D3	94.5± 36.0 (30-174)	40-140
PTH	4.8 ± 2.0 (0.4-8.9)	1.5-8
ALP	73.6 ± 23.1 (36.2-118.3)	40-120
P1NP		
whole group (*ULN)	0.72 ± 0.28 (0.26-1.29)	
men + premenop. women	46.5 ± 15.7 (19.3-76.3)	<59
postmenop. women	31.4 ± 9.8 (19.8-42.7)	<76
β-CTX		
whole group (*ULN)	0.60 ± 0.37 (0.16-1.81)	
men < 50 years	0.545 ± 0.294 (0.27-1.06)	<0.584
men 50 - 70 years	0.312 ± 0.087 (0.18-0.37)	<0.704
men > 70 years	NA	<0.854
women < 50 years	0.335 ± 0.142 (0.16-0.56)	<0.573
women > 50 years	0.294 ± 0.150 (0.17-0.51)	<1.008
Sclerostin (n=20)	38.32 ± 23.33 (23.1-129.3)	10.5-71.3

Table 3. Laboratory investigations of the 22 FAP patients included. Key: Ca, Calcium (mmol/l); Alb, Albumin; P, Phosphate (mmol/l); 25-OH-D, 25-hydroxyvitamin D (nmol/l); 1,25-$(OH)_2$-D3, 1,25-dihydroxyvitamin D3 (pmol/l); PTH, parathyroid hormone (pmol/l); ALP, Alkaline Phosphatase (U/l); P1NP, procollagen type 1 amino-terminal propeptide (ng/ml); ULN, upper limit of normal; β-CTX, beta-crosslaps (ng/ml); Sclerostin (pg/ml).

Correlations between markers of bone turnover and BMD

P1NP was significantly correlated with β-CTX ($r^2 = 0.49$; $p < 0.001$), and lumbar BMD was significantly correlated with total hip BMD ($r^2 = 0.53$; $p < 0.001$). Total hip BMD further correlated with β-CTX ($r^2 = 0.34$; $p < 0.01$) and with sclerostin ($r^2 = 0.31$; $p < 0.01$) and lumbar BMD correlated with sclerostin ($r^2 = 0.24$; $p < 0.05$). Although not reaching statistical significance we also found a trend towards a correlation of P1NP with total hip BMD ($r^2 = 0.13$; $p = 0.084$) and with sclerostin ($r^2 = 0.16$, $p = 0.068$).

Potential determinants of increased BMD in FAP patients

To find possible explanations for the increased mean BMD observed in our study population, we took a closer look at the subgroup of patients characterized by BMD Z-scores ≥ +1 at one or more sites measured (n = 14). None of the patients had any particular anthropometric, physical activity, lifestyle, dietary or pharmacological factors that might have contributed to an increase in BMD. Patients with a Z-score ≥ +1 showed a significant correlation between lumbar BMD and total hip BMD ($r^2 = 0.36$; $p < 0.05$), and between P1NP and β-CTX concentrations ($r^2 = 0.58$; $p < 0.01$). Lumbar BMD significantly correlated with sclerostin concentrations ($r^2 = 0.37$; $p < 0.05$), and total hip BMD correlated with P1NP ($r^2 = 0.41$; $p < 0.05$), β-CTX ($r^2 = 0.60$; $p < 0.01$) and sclerostin concentrations ($r^2 = 0.47$; $p < 0.05$).

There was no significant difference between patients with Z-scores ≥ +1 and the remainder of the patients included in our study in any demographic, clinical or laboratory characteristics (Table 4). There was also no significant difference between the P1NP-β-CTX regression lines when comparing these 2 groups (Figure 3).

Characteristic	**Z-score ≥ +1**	**Z-score < +1**	***P* value**
Demographic			
Number of patients	14	8	
Age (years)*	40 ± 11	45 ± 12	0.377
Caucasian ethnicity‡	13 (92.8)	7 (87.5)	0.674
Female gender‡	6 (42.8)	6 (75)	0.145
Menopause#	2 (33.3)	2 (33.3)	0.346
Height (SDS)*	-0.2 ± 1.0	-1.0 ± 1.7	0.196
Body mass index (kg/m^2)*	27.5 ± 4.5	26.0 ± 6.0	0.513
Clinical			
Family history of FAP‡	14 (100)	8 (100)	-
Age at diagnosis (years)*	20 ± 9	24 ± 12	0.462
Colectomy‡	13 (92.8)	8 (100)	0.439
Age at colectomy (years)*	22 ± 11	26 ± 10	0.477
Fibromas‡	2 (14.2)	1 (12.5)	0.907
Epidermoid cysts‡	3 (21.4)	1 (12.5)	0.601
Desmoid tumor‡	4 (28.5)	0 (0)	0.095
Teeth anomalies‡	3 (21.4)	4 (50)	0.166
Osteomas by scintigraphy‡	8 (57.1)	5 (62.5)	0.806
Abnormal vertebral morphology‡	2 (14.2)	0 (0)	0.262
Serum biochemistry			
Corrected Ca for Alb*	2.25 ± 0.10	2.21 ± 0.04	0.368
P*	1.13 ± 0.17	1.15 ± 0.16	0.836
25-OH-D*	54.6 ± 15.5	48.8 ± 18.2	0.440
PTH*	4.83 ± 2.12	4.96 ± 1.96	0.890
ALP*	74.5 ± 26.0	72.0 ± 18.4	0.808
P1NP*	0.73 ± 0.30	0.70 ± 0.26	0.835
β-CTX*	0.65 ± 0.44	0.51 ± 0.20	0.425
Sclerostin*	40.8 ± 29.3	34.4 ± 9.6	0.561

Table 4. Demographic, clinical and biochemical characteristics of patients grouped according to BMD Z-score values. Results are expressed as *mean ± SD (min-max), ‡n (%), #n (valid %). Key: Ca, Calcium (mmol/l); Alb, Albumin; P, Phosphate (mmol/l); 25-OH-D, 25-hydroxyvitamin D (nmol/l); PTH, parathyroid hormone (pmol/l); ALP, Alkaline Phosphatase (U/l); P1NP, procollagen type 1 amino-terminal propeptide (*ULN); β-CTX, beta-crosslaps (*ULN); ULN, upper limit of normal; Sclerostin (pg/ml).

Interestingly, mutations found in patients with BMD Z-score ≥ +1 were predominantly located among the β-catenin binding/downregulating domains, while the majority of the mutations found in patients with BMD Z-score < +1 were found among the Armadillo repeats of the APC protein.

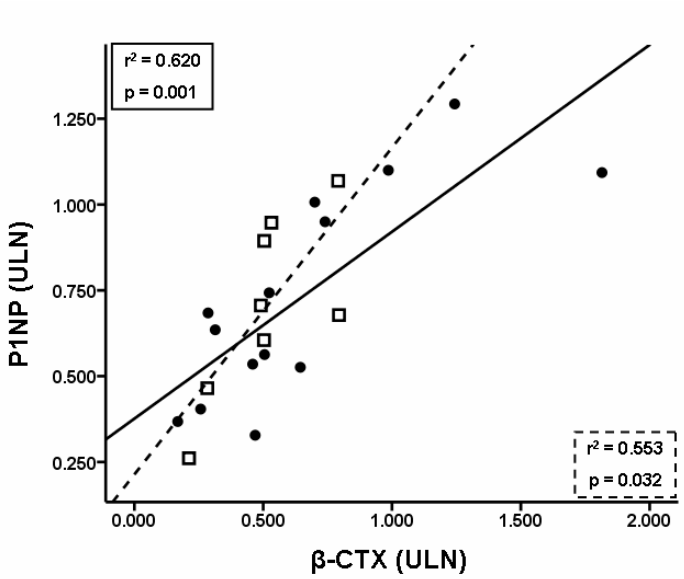

Figure 3. Correlations between P1NP (ULN) and β-CTX (ULN) fitted to the linear model. Black dots indicate patients (n = 14; 63.6%) with BMD Z-scores ≥ +1 at one or more sites measured, and empty black squares indicate the rest of the patients (n = 8; 36.3%). The continuous fit line indicates correlation for the "higher than normal" BMD group (BMD Z-scores ≥ +1 at one or more sites measured), with its correlation coefficient and *p* value in the upper left corner of the graph. The dotted fit line indicates correlation for the other patients, with their correlation coefficient and *p* value in the lower right corner of the graph.

DISCUSSION

To our knowledge this is the first systematic evaluation of bone and mineral metabolism in FAP patients carrying heterozygous mutations in the *APC* gene. Our data show that these patients display a statistically significant higher mean BMD than age- and sex-matched controls. Seventeen of the 22 patients studied (77.3%) had consistently higher BMD values than the mean for age and sex-matched controls (Z-score > 0), 14 patients (63.6%) had Z-scores ≥ +1 and 5 patients (27.2%) had Z-scores ≥ +2 at one or more sites measured. Our only inclusion criterion for the study was a clinical, histological and genetically confirmed FAP, and our sole exclusion criterion was the use of bone modulating agents that may positively influence bone mass. FAP patients were thus included in the study regardless of age, gender or severity of disease manifesta-

tions. Although heterogeneous, our study population was representative of FAP patients, since all clinical findings (colonic and extracolonic manifestations) were present in proportions previously reported in other FAP cohorts (1;28). In our study population, mean P1NP and β-CTX concentrations were within the normal ranges and were significantly positively correlated. Both these markers were also positively correlated with BMD. These results suggest that heterozygous inactivating *APC* mutations have a moderately positive effect on bone mass accrual, by a mechanism probably involving decreased β-catenin degradation. In keeping with published literature (reviewed in 1), we also observed a high prevalence of focal bone pathology, such as osteomas and teeth anomalies, in our study population. Whereas a positive correlation was found between the prevalence of these two focal pathologies, we detected no correlation between these anomalies and increased BMD. This finding suggests that the two phenotypes observed, the increased BMD on one hand, and the focal bone pathology on the other hand, are likely to occur as a result of independent molecular mechanisms.

It is well established that the canonical Wnt signaling pathway plays a key regulatory role in osteoblastogenesis and in bone mass accrual (29). Activation of this signaling cascade promotes osteoblast differentiation from progenitor cells and enhances bone mass acquisition via β-catenin, while suppression of this pathway results in bone loss. Alteration of several intracellular and extracellular controllers of the levels of transduced Wnt/β-catenin signaling has indeed been linked to disturbed skeletal homeostasis (30-34). Using murine conditional genetic models, we and others have shown that Apc is involved in the regulation of both prenatal and postnatal bone mass accrual by regulating the levels of Wnt/β-catenin signaling (11;12). Our present study confirms findings from these *in vivo* animal studies by demonstrating that heterozygous mutations in the *APC* gene are associated with a higher than normal bone mass in a majority of FAP patients.

However, not all FAP patients included in our study displayed an increased BMD, implying that different mutations in the *APC* gene may have distinct effects on bone mass acquisition. Interestingly, in our study population, the *APC* mutations found in patients with increased BMD were mostly located among the β-catenin binding/downregulating domains, suggesting that the heterozygous loss of β-catenin-regulating activity is likely to have a positive effect on BMD. Alternatively, an effect of APC on BMD may be balanced by patient-specific environmental and/or (epi)genetic factors. It is likely that due to the relative small size of our study we were unable to detect specific effects of different *APC* mutations on BMD. Which particular *APC* mutation has the most beneficial effect on BMD remains thus to be elucidated. As observed in conditional heterozygous *Apc* mutant mice (11;12), FAP patients display an average height similar to that of the general population, suggesting that one functional *APC* allele is sufficient for normal longitudinal bone growth.

In our study population, increased BMD was associated with coupled increases in the concentrations of biochemical markers of bone formation and resorption, albeit the mean remaining within the normal laboratory reference ranges. Higher BMD than normal could thus not be explained by an imbalance between bone resorption and bone formation. Inhibition of sclerostin by mechanical stimulation or following treatment of osteoporosis with intermittent PTH has been demonstrated to have positive

effects on bone mass (35;36). However, mean serum sclerostin was increased in the FAP patients we studied, and was significantly positively correlated with BMD. Sclerostin normally antagonizes the canonical Wnt signaling pathway by binding to the Wnt co-receptors LRP5 and LRP6 (37). The increase in sclerostin we observed might therefore be interpreted as a negative feedback mechanism to prevent further bone mass accrual. This notion is in agreement with *in vivo* data indicating that heterozygous *Apc*$^{min/+}$ mice display high sclerostin transcript levels in the tibia associated with increased BMD (13).

Osteomas are benign, slow-growing osteogenic lesions, usually affecting the jaws and flat bones of the calvaria. They are present in 46–93% of FAP patients, an incidence 4 to 20 times higher than in control groups (3–16%) (38-40). In keeping with previous studies (2), osteomas were predominantly located in the upper and/or lower mandible in our patients, and their presence was positively correlated with the presence of teeth anomalies (41;42). Patients with scintigraphic evidence of osteoma(s) were on average younger than those without osteomas, albeit non-statistically significantly. This observation is in keeping with previous reports indicating that osteomas are more prevalent in young FAP patients (1). However, it is well documented that osteomas may resolve spontaneously (43;44), so that due to the cross-sectional design of our study we cannot exclude the possibility that some of the patients without any present sign of osteoma(s) may have had this pathology in the past.

In the present study, we found no difference in BMD between patients with and without focal bone pathology, and no correlation between increased BMD and focal bone pathology. These findings suggest that, in FAP patients, osteomas and teeth anomalies are likely to develop due to local mechanisms, whereas the positive effect of the *APC* mutations on BMD is likely to be systemic. A local mechanism, such as loss of heterozygosity (LOH), has indeed been described in colonic, duodenal and desmoid tumors in FAP patients (45). To our knowledge, there are no available data on genetic analysis of osteomas from FAP patients. We hypothesize that somatic inactivation of the second *APC* allele may be responsible for the occurrence of focal bone pathology, while the systemic BMD increase may be attributed to the β-catenin activation secondary to heterozygous *APC* mutations.

In recent years, the Wnt/β-catenin signaling pathway has undoubtedly emerged as a pivotal regulator of bone formation prenatally, during growth and throughout adulthood. We document here for the first time that FAP individuals carrying heterozygous *APC* mutations, resulting in activation of the canonical Wnt signaling pathway, display significantly higher mean BMD compared to age- and sex-matched healthy controls in the presence of a balanced bone turnover. Whether increased bone mass accrual is sustained over the years, and whether this higher BMD than normal may reduce age-related fracture risk remains to be established by long-term follow-up studies in FAP patients. Whereas our data may not thus have direct clinical implications to FAP patients, they do add to the volume of evidence regarding the important role of the canonical Wnt signaling pathway in the regulation of bone mass. That our findings in FAP patients may be relevant is indeed supported by their analogy to similar data reported in relatives of patients with sclerosteosis, who carry heterozygous mutations in the *SOST* gene, another negative regulator of the canonical Wnt signaling

pathway (46). These individuals are asymptomatic, lack the characteristic “high bone mass phenotype” of their homozygous relatives, but do display consistently increased BMD values than the mean of age and sex-matched controls, either within the high normal range or clearly above it, but reaching Z-scores ≥ +2 in only a minority of cases. Findings from these two human genetic models suggest a state of “controlled” activation of the Wnt signalling pathway in heterozygous carriers of *SOST* or *APC* mutations that may be exploited in the identification of potentially attractive therapeutic targets in the treatment of osteoporosis.

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