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Copy number variation of the *APC* gene is associated with regulation of bone mineral density ☆

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ABSTRACT

Introduction: Genetic studies of osteoporosis have commonly examined SNPs in candidate genes or whole genome analyses, but insertions and deletions of DNA, collectively called copy number variations (CNVs), also comprise a large amount of the genetic variability between individuals. Previously, SNPs in the *APC* gene have been strongly associated with femoral neck and lumbar spine volumetric bone mineral density in older men. In addition, familial adenomatous polyposis patients carrying heterozygous mutations in the *APC* gene have been shown to have significantly higher mean bone mineral density than age- and sex-matched controls suggesting the importance of this gene in regulating bone mineral density. We examined CNV within the *APC* gene region to test for association with bone mineral density.
Methods: DNA was extracted from venous blood, genotyped using the Human Hap610 arrays and CNV determined from the fluorescence intensity data in 2070 Caucasian men and women aged 47.0 ± 13.0 (mean ± SD) years, to assess the effects of the CNV on bone mineral density at the forearm, spine and total hip sites.
Results: Data for covariate adjusted bone mineral density from subjects grouped by *APC* CNV genotype showed significant difference ($P = 0.02–0.002$). Subjects with a single copy loss of *APC* had a 7.95%, 13.10% and 13.36% increase in bone mineral density at the forearm, spine and total hip sites respectively, compared to subjects with two copies of the *APC* gene.
Conclusions: These data support previous findings of *APC* regulating bone mineral density and demonstrate that a novel CNV of the *APC* gene is significantly associated with bone mineral density in Caucasian men and women.

Introduction

Osteoporosis is characterized by low bone mineral density (BMD) and deterioration in bone microarchitecture which results in fragility and increased risk for fracture [1]. Several large genome-wide association studies (GWAS) have recently identified single nucleotide polymorphisms (SNPs) associated with BMD and fracture risk [2,3]. However combinations of these SNPs only contribute approximately 5% of the risk of developing osteoporosis [4]. Alternative structural genomic mechanisms, including copy number variations (CNVs), are being investigated as a source of phenotypic variation in diseases such as asthma, autoimmune disease and psychiatric disease [5–13] and recent data suggests CNVs may also be relevant to osteoporosis [4,14].

In 2009, Yerges et al. screened for associations between SNPs in 383 candidate genes and femoral neck and lumbar spine volumetric BMD and found three SNPs in the *APC* gene to be strongly associated [15]. In addition, Miclea et al. conducted a cross-sectional study and reported familial adenomatous polyposis (FAP) patients with heterozygous mutations within the *APC* gene displayed significantly higher mean BMD than age- and sex-matched controls [16]. The *APC* tumor suppressor gene encodes for a protein known to be involved in a broad spectrum of cellular processes including apoptosis, adhesion, cell cycle control, cell migration and chromosomal stability [17–19]. However, its main role is to bind β-catenin, a key transducer of the Wnt signaling pathway, and negatively regulate the Wnt signaling cascade [20,21]. There is emerging evidence of the important role of β-catenin in bone regulation and the pathophysiology of skeletal

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Table 1
Demographic data and bone density parameters of the TwinsUK cohort.

Variables	Females (n = 1815)	Males (n = 255)
Age (years)	46.8 ± 12.8	48.3 ± 14.0
Height (m)	1.62 ± 0.07	1.75 ± 0.07
Weight (kg)	67.0 ± 12.6	80.7 ± 12.4
BMI (kg/m^2)	25.5 ± 6.7	26.3 ± 3.9
Forearm BMD (g/cm^2)	0.555 ± 0.058	0.658 ± 0.062
Spine BMD (g/cm^2)	0.997 ± 0.142	1.026 ± 0.151
Total hip BMD (g/cm^2)	0.930 ± 0.129	1.042 ± 0.144

Data are mean ± SD.

disorders [22–24]. Interestingly, mice carrying a heterozygous loss of function mutation in *Apc* display significantly increased BMD of the distal femur [25]. Collectively, these data strongly imply that *APC* may have a role in BMD through β-catenin regulation. In this study, we performed a candidate gene study of *APC* CNV to further examine the effect of genetic variation in the *APC* gene on BMD.

Material and methods

Subjects and clinical assessment

Subjects who participate in the study were identified from the St Thomas' UK adult twin registry (TwinsUK) and included both males and females between 16 and 81 years of age. Measurement of the anterior–posterior projection of forearm, lumbar spine (L1–4) and total hip BMD was performed using DEXA (QDR 4500, Hologic) as described previously [26]. Clinical data which included age, height and weight were collected at interview and lifestyle questionnaires were also completed. Body mass index (BMI) was derived by computation: weight (kg)/height (m)^2. All subjects from the TwinsUK cohort provided written informed consent, and the institutional ethics committees of participating institutions approved the experiment protocols.

Genotyping and CNV calling

Genotyping was performed on genomic DNA extracted from venous blood and analyzed using the Human Hap610 Quad array (Illumina, San Diego, USA) according to the manufacturer's instructions. PennCNV software was used for the calling of CNVs, which uses the combined values of log R ratio and B allele frequency values and an integrated hidden Markov model [27]. Quality control threshold was performed as per PennCNV guidelines [27].

Linkage disequilibrium

To assess the linkage disequilibrium of the *APC* CNV with surrounding SNPs using available software, we recoded the CNV call as a bi-allelic variable ("one copy loss" = TA and "two copy" = TT) and combined this with the SNP genotype data obtained from the Human Hap610 Quad array. We used SNP genotype data from all SNPs in the gene region including approximately 45 kb before and after the *APC* gene, and perform linkage disequilibrium analysis using Haploview 4.2 [28].

Statistical analyses

Statistical analysis was performed using SPSS for Windows v17.0 (SPSS Inc., Chicago, IL, USA). All individuals in the study were from independent families with only a single sib from each pedigree participating in the study. We first tested whether the following variables: age, age^2, height, BMI and sex were significantly associated with BMD phenotypes using multiple linear regression — all variables were retained as covariates in subsequent analyses. BMD standardized residuals were generated after adjustment for covariates and the differences in mean BMD for each genotype group were examined using independent t-test. Two-tailed *P* values are reported throughout, with values ≤ 0.05 considered statistically significant.

Results

The demographic data and bone density parameters for the study subjects are detailed in Table 1.

Among the 2070 participants, eight subjects (0.4%) had a heterozygous deletion encompassing a portion of the *APC* gene, with the remaining 2062 (99.6%) subjects having two copies of the *APC* gene. No subjects were detected with homozygous deletion or copy gain in this region. Out of the eight subjects with a single copy deletion, there were seven females and one male individual. Interestingly, the eight different heterozygous deletions of the *APC* gene share a common deletion region (chr5:112144628–112152268 (hg18)), clustered near the 3′ end of exon 5 of the *APC* gene (Fig. 1). However it is unclear whether a specific region within the CNV is responsible for the effect on BMD or whether any of the observed combination of deletions of exons is sufficient to elicit the effect. In support of the PennCNV genotypes for this region, a distinct decrease in log R ratio values for the eight single copy subjects encompassing only the *APC* gene, compared to 100 kb before and after the gene, was evident on examination of the genotyping array data (Fig. 2).

The pairwise linkage disequilibrium (r^2) of SNPs in the region approximately 45 kb before and after the *APC* gene (chr5:112055101–112254554 (hg18)) and the *APC* CNV is depicted in Fig. 3. This analysis showed that the common one-copy deletion region, shared by the eight affected subjects, is not in strong linkage disequilibrium with any of the surrounding SNPs.

There was evidence of significant association between *APC* CNV genotype and BMD at all sites studied: forearm, spine and total hip ($P = 0.023$, $P = 0.004$ and $P = 0.002$ respectively; Table 2) after adjustment for age, age^2, height, BMI and sex. Subjects with heterozygous deletion of the *APC* gene showed 7.95%, 13.10% and 13.36% higher mean forearm, spine and total hip sites respectively compared to subjects with two copies of the *APC* gene.

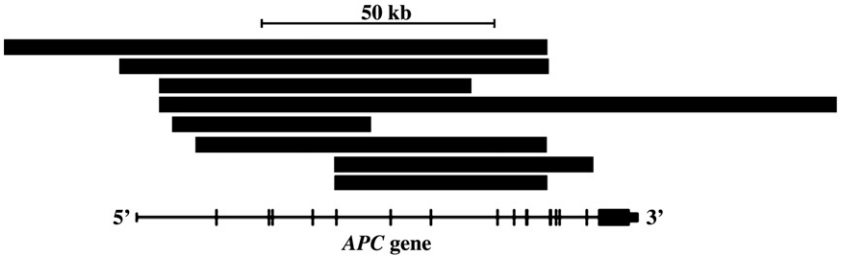

Fig. 1. Location of the *APC* gene copy number variations in the eight individuals from the TwinsUK cohort. The *APC* gene transcript spans 108 kb at chromosome 5q22.2 (NCBI Genome Build 36/hg18).

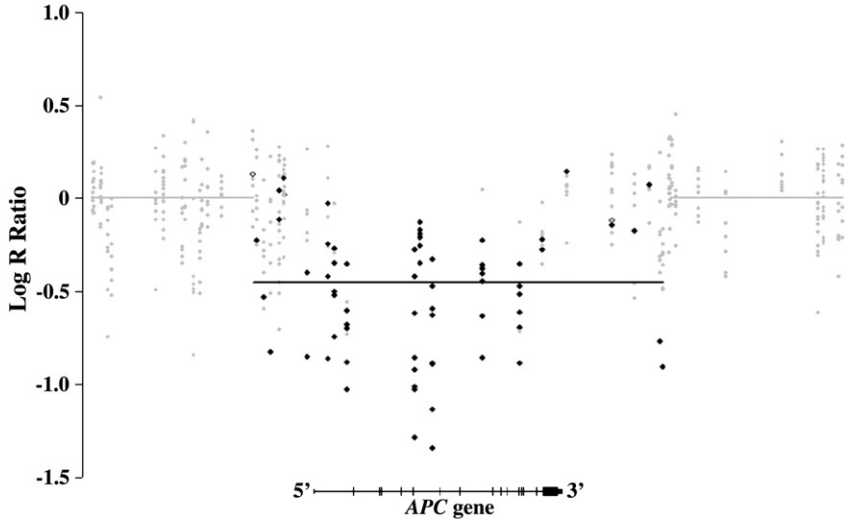

Fig. 2. Graph of log R ratio by chromosomal position for the eight subjects with a single copy loss of the *APC* CNV in the *APC* gene region. Gray symbols represent log R ratio for SNPs upstream/downstream of the CNV region and gray lines represent expected value for two copies. Black symbols represent log R ratio for SNPs within the CNV region and the black line indicates the expected value of a loss in copy number (NCBI Genome Build 36/hg18).

Discussion

The data presented in this study provides evidence that CNV within the *APC* gene is associated with increased BMD in a Caucasian cohort of 2070 individuals. We found strong evidence that subjects with single copy loss of *APC* display a significantly higher mean BMD than subjects with two copies of *APC*. These finding suggest that the *APC* gene may be an important negative regulator of bone mineral density in humans. Our results are in agreement with a recently published study reporting that FAP patients with heterozygous mutation in the *APC* gene also displayed increased mean BMD [16]. In addition, our findings in humans are supported by the study of Holmen et al. which demonstrated that mice with osteoblast-specific deletion of the *Apc* gene revealed significant accumulation of bone matrix in the femur and dramatically increased bone deposition associated with disturbances in bone architecture and composition in the tibia [25].

A proportion of CNVs in the genome are in strong linkage disequilibrium with common SNPs and potential association of those CNVs can be assessed by association testing with the SNPs acting as surrogate markers. However this is not the case for all CNVs and in this study we have shown that the CNV in the *APC* gene is not in strong linkage disequilibrium with any of the surrounding SNPs. This lack of linkage disequilibrium may account for the *APC* gene not being documented to be associated with the regulation of BMD in recent GWAS. In addition to identifying a novel CNV association with BMD, our study also shows the importance of direct analysis of CNVs and their role in diseases as it is not always possible to draw appropriate conclusions from tagSNP studies alone.

We and others have previously examined the role of candidate CNVs in osteoporosis and regulation of BMD [4,14,29] and this study further adds to the growing body of literature on the role of CNVs in bone metabolism. Our findings on the effect of *APC* CNV on BMD compliment and extend the findings of Yerges et al. [15]. Data from

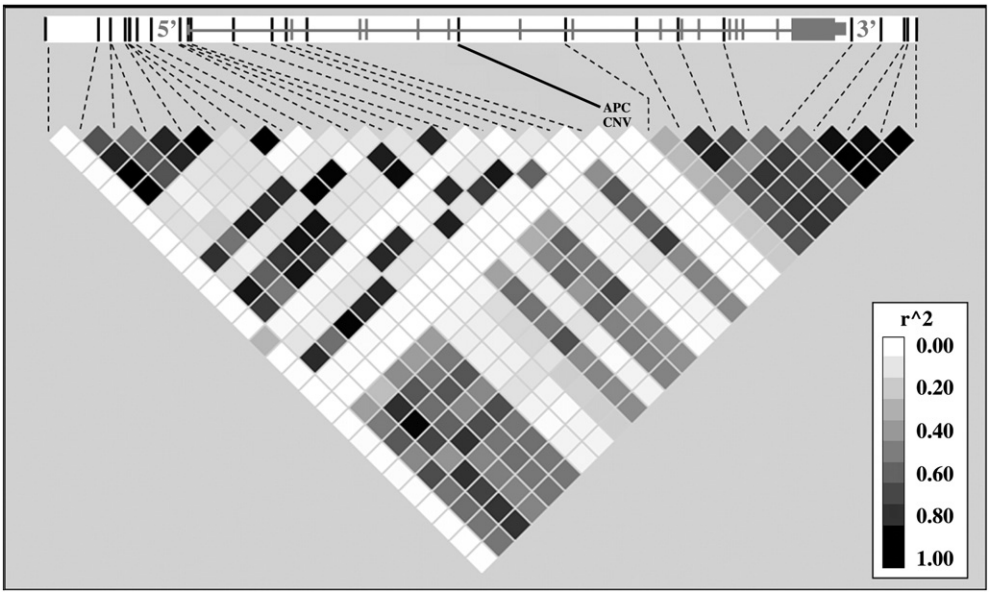

Fig. 3. Linkage disequilibrium plot of the *APC* gene region for the TwinsUK cohort. The region of one copy loss shared among the eight affected individuals is titled APC CNV. Pairwise linkage disequilibrium, measured as r^2, was calculated from genotyping data using Haploview 4.2.

Table 2
Mean bone mineral density parameters by body site in relation to the *APC* CNV genotype.

Variable	1 copy	2 copies	*P* value
Forearm BMD (g/cm^2)	0.607 ± 0.018	0.566 ± 0.001	0.023
Spine BMD(g/cm^2)	1.137 ± 0.047	1.000 ± 0.003	0.004
Total hip BMD (g/cm^2)	1.077 ± 0.042	0.943 ± 0.003	0.002

Results are given as mean ± SEM. *P*-values were determined using independent t-test after adjustment for age, age^2, height, BMI and sex.

studies of human missense mutations and that from the conditional knockout mouse are similarly in accord with the findings of our research. However, this is the first study that examined the effects of CNV within the *APC* gene in BMD regulation in humans.

The tumor suppressor *APC* is an intracellular cytosolic Wnt signaling inhibitor which forms a 'multi-protein destruction complex' with axin and glycogen synthase kinase 3 (GSK3) [30]. Together with axin, *APC* acts as a scaffold protein, increasing the affinity of GSK3 to bind and phosphorylate β-catenin, leading to its degradation by the β-TrCP-mediated ubiquitination/proteasome pathway [31]. This results in the deactivation of the canonical Wnt signaling pathway. However, loss of *APC* disrupts the destruction complex, thereby preventing the phosphorylation and degradation of β-catenin, leading to its cytoplasmic stabilization and proliferation [32]. In the murine models presented by Holmen et al., mice carrying osteoblast-specific deletions of both *Apc* and the β-catenin genes are phenotypically similar to those lacking only the β-catenin gene. This confirms that the phenotype induced by loss of *Apc* is due to dysregulation of β-catenin signaling [25].

The most common target for both germ line and somatic mutation within the *APC* gene lies mainly in exon 15, where more than 75% of the coding sequence is located [33,34]. In our study, only one affected individual had a single copy deletion encompassing all exons, including exon 15, however the phenotype of that individual was not significantly different in comparison to other individuals with a 1 copy loss. Although the common deletion region that the eight affected individuals share is located within intron 6, this region does cover regulatory elements, including enhancer elements [35]. In addition, the single copy loss for every individual encompasses at least 4 exons, which suggests that each deletion would result in an abnormal or non-functional APC protein. As there is strong evidence of the pivotal role of Wnt signaling in bone regulation, this would imply that haploinsufficiency of *APC* results in over-activation of the canonical Wnt signaling pathway via the stabilization of more β-catenin, increasing bone mass through diverse range of mechanisms such as osteoblastogenesis induction, apoptosis inhibition of osteoblast and osteocyte, stimulation of preosteoblast replication and the renewal of stem cells [36–39].

Conclusions

Our data add to the accumulating evidence regarding the importance of CNV in the regulation of BMD and the role of the *APC* gene as an antagonist to the canonical Wnt signaling pathway in the regulation of bone mass. This study also demonstrates that as for some other complex genetic diseases, CNVs may play a role in the regulation of key clinically relevant traits and that this effect is not always defined by attempting to exploit linkage disequilibrium of CNVs with common tagSNPs.

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References

[1] Kanis JA, Melton III LJ, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res 1994;9:1137-41.
[2] Richards J, Rivadeneira F, Inouye M, Pastinen TM, Soranzo N, Wilson SG, et al. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. Lancet 2008;371:1505-12.
[3] Rivadeneira F, Styrkársdottir U, Estrada K, Halldórsson BV, Hsu YH, Richards JB, et al. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nat Genet 2009;41:1199-206.
[4] Yang TL, Chen XD, Guo Y, Lei SF, Wang JT, Zhou Q, et al. Genome-wide copy-number-variation study identified a susceptibility gene, *UGT2B17*, for osteoporosis. Am J Hum Genet 2008;83:663-74.
[5] Brasch-Andersen C, Christiansen L, Tan Q, Haagerup A, Vestbo J, Kruse TA. Possible gene dosage effect of glutathione-S-transferase on atopic asthma: using real-time PCR for quantification of GSTM1 and GSTT1 gene copy numbers. Hum Mutat 2004;24:208-14.
[6] Ivaschenko TE, Sideleva OG, Baranov VS. Glutathione-S-transferase micro and theta gene polymorphisms as new risk factors of atopic bronchial asthma. J Mol Med (Berl) 2002;80:39-43.
[7] Fanciulli M, Norsworthy PJ, Petretto E, Dong R, Harper L, Kamesh L, et al. *FCGR3B* copy number variation is associated with susceptibility to systemic, but not organ-specific, autoimmunity. Nat Genet 2007;39:721-3.
[8] McKinney C, Merriman ME, Chapman PT, Gow PJ, Harrison AA, Highton J, et al. Evidence for an influence of chemokine ligand 3-like 1 (*CCL3L1*) gene copy number on susceptibility to rheumatoid arthritis. Ann Rheum Dis 2008;67:409-13.
[9] Yang Y, Chung EK, Wu YL, Savelli SL, Nagaraja HN, Zhou B, et al. Gene copy-number variation and associated polymorphisms of complement component C4 in human systemic lupus erythematosus (SLE): low copy number is a risk factor for and high copy number is a protective factor against SLE susceptibility in Europeans Americans. Am J Hum Genet 2007;80:1037-54.
[10] Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, et al. Rare structural variants disrupt multiple genes in neurodevelopment pathways in schizophrenia. Science 2008;320:539-43.
[11] Wilson GM, Flibotte S, Chopra V, Melnyk BL, Honer WG, Holt RA. DNA copy-number analysis in bipolar and schizophrenia reveals aberrations in genes involved in glutamate signalling. Hum Mol Genet 2006;15:743-9.
[12] Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, et al. Strong association of de novo copy number mutations with autism. Science 2007;316:445-99.
[13] Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, et al. Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med 2008;358:667-75.
[14] Deng FY, Zhao LJ, Pei YF, Sha BY, Liu XG, Yan H, et al. Genome-wide copy number variation association study suggested VPS13B gene for osteoporosis in Caucasians. Osteoporos Int 2010;21:579-87.
[15] Yerges LM, Klei L, Cauley JA, Roeder K, Kammerer CM, Moffett SP, et al. High-density association study of 383 candidate genes for volumetric BMD at the femoral neck and lumbar spine among older men. J Bone Miner Res 2009;24:2039-49.
[16] Miclea RL, Karperien M, Langers AM, Robanus-Maandaq EC, van Lierop A, van der Hiel B, et al. *APC* mutations are associated with increased bone mineral density in patients with familial adenomatous polyposis. J Bone Miner Res 2010;25:2624-32.
[17] Bienz M, Clevers H. Linking colorectal cancer to Wnt signalling. Cell 2000;103:311-20.
[18] Näthke IS, Adams CL, Polakis P, Sellin JH, Nelson WJ. The adenomatous polyposis coli tumor suppressor protein localizes to plasma membrane sites involved in active cell migration. J Cell Biol 1996;134:165-79.
[19] Fodde R, Kuipers J, Rosenberg C, Smits R, Kielman M, Gaspar C, et al. Mutations in the APC tumor suppressor gene cause chromosomal instability. Nat Cell Biol 2001;3:433-8.
[20] Korinek V, Barker N, Morin PJ, van Wichen D, de Weger R, Kinzler KW, et al. Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC−/− colon carcinoma. Science 1997;275:1784-7.
[21] Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, et al. Activation of beta-catenin-Tcf signalling in colon cancer by mutations in beta-catenin or APC. Science 1997;275:1787-90.

[22] Monaghan H, Bubb VJ, Sirimujalin R, Milward-Sadler SJ, Salter DM. Adenomatous polyposis coli (APC), beta-catenin, and cadherin are expressed in human bone and cartilage. Histopathology 2001;39:611-9.
[23] Baron R, Rawadi G. Targeting the Wnt/beta-catenin pathway to regulate bone formation in the adult skeleton. Endocrinology 2007;148:2635-43.
[24] Kubota T, Michigami T, Ozono K. Wnt signaling in bone metabolism. J Bone Miner Metab 2009;27:265-71.
[25] Holmen SL, Zylstra CR, Mukherjee A, Sigler RE, Faugere MC, Bouxsein ML, et al. Essential role of beta-catenin in postnatal bone acquisition. J Biol Chem 2005;280: 21162-8.
[26] Hunter DJ, de Lange M, Andrew T, Snieder H, MacGregor AJ, Spector TD. Genetic variation in bone mineral density and calcaneal ultrasound: a study of the influence of menopause using female twins. Osteoporos Int 2001;12:406-11.
[27] Wang K, Li M, Hadley D, Liu R, Glessner J, Grant SF, et al. PennCNV: an integrated hidden Markov model designed for high-resolution copy number variation detection in whole-genome SNP genotyping data. Genome Res 2007;17:1665-74.
[28] Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-5.
[29] Chew S, Mullin BH, Lewis JR, Spector TD, Prince RL, Wilson SG. Homozygous deletion of the UGT2B17 gene is not associated with osteoporosis risk in elderly Caucasian women. Osteoporos Int 2011;22:1981-6.
[30] Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. Axin, a negative regulator of the Wnt signalling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. EMBO J 1998;17:1371-84.
[31] Spiegelman VS, Slaga TJ, Pagano M, Minamoto T, Ronai Z, Fuchs SY. Wnt/beta-catenin signalling induces the expression and activity of beta TrCP ubiquitin ligase receptor. Mol Cell 2000;5:877-82.
[32] Polakis P. The oncogenic activation of beta-catenin. Curr Opin Genet Dev 1999;9: 15-21.
[33] Powell SM, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN, et al. APC mutations occur early during colorectal tumorigenesis. Nature 1992;359:235-7.
[34] Béroud C, Soussi T. APC gene: database of germline and somatic mutations in human tumors and cell lines. Nucleic Acids Res 1996;24:121-4.
[35] Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, et al. The human genome browser at UCSC. Genome Res 2002;12:996–1006.
[36] Krishnan V, Bryant HU, MacDouglad OA. Regulation of bone mass by Wnt signalling. J Clin Invest 2006;116:1202-9.
[37] Kato M, Patel MS, Levasseur R, Lobov I, Chang BH, Glass II DA, et al. Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. J Cell Biol 2002;157:303-14.
[38] Bodine PV, Zhao W, Kharode YP, Bex FJ, Lambert AJ, Goad MB, et al. The Wnt antagonist secreted frizzled-related protein-1 is a negative regulator of trabecular bone formation in adult mice. Mol Endocrinol 2004;18:1222-37.
[39] Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature 2005;434:843-50.