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

Shelby Chew a,*, Zari Dastani b, c, Suzanne J. Brown a, Joshua R. Lewis a, d, Frank Dudbridge e, Nicole Soranzo f, Gabriela L. Surdulescu g, J. Brent Richards b, c, g, Tim D. Spector g, Scott G. Wilson a, d, g

a Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands 6009, Australia
b Lady Davis Institute, McGill University, Montreal H3T 1E2, Canada
c School of Medicine and Pharmacology, University of Western Australia, Nedlands 6009, Australia
d Department of Human Genetics, McGill University, Montreal H3A 1B1, Canada
e School of Hygiene and Tropical Medicine, London WC1E 7HT, UK
f Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton CB10 1SA, UK
g Department of Twin Research & Genetic Epidemiology, King’s College London, London SE1 7EH, UK

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A B S T R A C T
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 7.05%, 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.

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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 prescribed previously [26]. Clinical data which included age, height and weight were collected at interview and lifestyle questionnaires were prescribed previously [26]. Clinical data which included age, height and weight were collected at interview and lifestyle questionnaires were prescribed previously [26]. Clinical data which included age, height and weight were collected at interview and lifestyle questionnaires were prescribed previously [26]. Clinical data which included age, height and weight were collected at interview and lifestyle questionnaires were prescribed previously [26]. Clinical data which included age, height and weight were collected at interview and lifestyle questionnaires were prescribed previously [26]. Clinical data which included age, height and weight were collected at interview and lifestyle questionnaires were prescribed previously [26]. Clinical data which included age, height and weight were collected at interview and lifestyle questionnaires were prescribed previously [26].

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 for the eight single copy subjects encompassing only the PennCNV genotypes for this region, a distinct decrease in log R ratio values for the eight single copy subjects. Genotyping and CNV calling

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, age², 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 show 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 (r²) 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, age², 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).](image)
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 complement and extend the findings of Yerges et al. [15]. Data from
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.

Acknowledgments

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References

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Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>1 copy</th>
<th>2 copies</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forearm BMD (g/cm²)</td>
<td>0.607±0.018</td>
<td>0.566±0.001</td>
<td>0.023</td>
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<td>Spine BMD (g/cm²)</td>
<td>1.137±0.047</td>
<td>1.000±0.003</td>
<td>0.004</td>
</tr>
<tr>
<td>Total hip BMD (g/cm²)</td>
<td>1.077±0.042</td>
<td>0.943±0.003</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Results are given as mean±SEM. P-values were determined using independent t-test after adjustment for age, age², height, BMI and sex.


