



Published in final edited form as:

*Bone*. 2019 April ; 121: 221–226. doi:10.1016/j.bone.2019.01.026.

## Postmenopausal osteoporotic fracture-associated *COL1A1* variant impacts bone accretion in girls

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### Abstract

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Author Contributions

D.L.C., S.E.M., and B.S.Z. performed the analyses. D.L.C., S.E.M., B.S.Z., and S.F.A.G. devised, designed the experiments, interpreted the results and wrote the paper. All additional contributors reviewed and approved of the final submission.

Over the past two decades, a low frequency variant (rs1800012) within the first intron of the type I collagen alpha 1 (*COL1A1*) gene has been implicated in lower areal BMD (aBMD) and increased risk of osteoporotic fracture. This association is particularly strong in postmenopausal women, in whom net bone loss arises in the context of high bone turnover. High bone turnover also accompanies childhood linear growth; however, the role of rs1800012 in this stage of net bone accretion is less well understood. Thus, we assessed the association between rs1800012 and aBMD and bone mineral content (BMC) Z-scores in the Bone Mineral Density in Childhood Study, a mixed-longitudinal cohort of children and adolescents (total n=804 girls and 771 boys; n=19 girls and 22 boys with the TT genotype). Mixed effects modeling, stratified by sex, was used to test for associations between rs1800012 and bone Z-scores and for pubertal stage interactions. Separately, SITAR growth modeling of aBMD and BMC reduced the complex longitudinal bone accrual curves into three parameters representing *a-size*, *b-timing*, and *c-velocity*. We tested for differences in these three parameters by rs1800012 genotype using t-tests. Homozygous TT girls had significantly lower bone Z-scores prior to puberty completion (e.g. spine aBMD-Z *P-interaction*= $1.0 \times 10^{-6}$ ), but this association was attenuated post-puberty. SITAR models revealed that homozygous TT girls began pubertal bone accrual later (*b-timing*; e.g. total hip BMC, *P*=0.03). BMC and aBMD Z-scores also increased across puberty in TT homozygous boys. Our data, along with previous findings in post-menopausal women, suggest that rs1800012 principally affects female bone density during periods of high turnover. Insights into the genetics of bone gain and loss may be masked during the relatively quiescent state in mid-adulthood, and discovery efforts should focus on early and late life.

## Keywords

bone mineral density; alpha1 type 1 collagen; longitudinal study; bone development; puberty

## Introduction

Twenty years ago, a variant (rs1800012) within an Sp1 binding site embedded in the first intron of the type I collagen alpha 1 (*COL1A1*) gene was implicated in accelerated bone loss and increased rate of fracture in post-menopausal women<sup>(1)</sup>. In contrast to its marked effect after menopause, this variant has only a modest impact on peak BMD in pre-menopausal women (with TT homozygous minor allele carriers having an average of 25 mg/cm<sup>2</sup> lower BMD at the femoral neck)<sup>(2,3)</sup>, suggesting a stronger effect during high bone turnover periods.

Similar to post-menopause, childhood and adolescence are also periods of high bone turnover, resulting in net bone gain. Bone mass acquisition during this critical developmental window has consequences for lifelong skeletal health<sup>(4)</sup>. While the implications of rs1800012 in later life are well-established<sup>(1,5-7)</sup>, the effect of this variant during bone development has not been clearly delineated. Three studies have tested the association between this variant and BMD among children and adolescents, but these studies were hampered by small sample sizes, particularly of carriers of the minor homozygous genotype, and did not account for changes across puberty<sup>(8-10)</sup>. Furthermore, these studies arrived at

conflicting conclusions, with two reporting an association of this variant with lower childhood BMD<sup>(8,10)</sup> and one finding no evidence of association<sup>(9)</sup>.

Therefore, to address the limitations of previous studies and clarify the role of rs1800012 during growth, we assessed the association of this variant with areal-BMD (aBMD) and BMC and tested for puberty interactions in the Bone Mineral Density in Childhood Study (BMDCS), a mixed-longitudinal cohort of 1,575 healthy children and adolescents with annual DXA and puberty measurements.

## Materials and Methods

### Study subjects

The BMDCS was a prospective, longitudinal study of healthy children and adolescents aimed at establishing norms for BMC and aBMD for children 5 to 20 years of age in the United States. Subjects were recruited in 2002–2003 at 5 US study centers (Los Angeles, CA; Cincinnati, OH; Omaha, NE; Philadelphia, PA; and New York, NY). Children were recruited in two phases, including a longitudinal and cross-sectional cohort. In the longitudinal cohort, girls (aged 6–15 yr) and boys (aged 6–16 yr) were followed annually for up to 6 yr, with a maximum of 7 study visits per individual. In 2006–2007, individuals aged 5 and 19 yr were also enrolled, and were subsequently followed for 2 years annually. In the cross-sectional cohort, intended as a replication dataset, children of European descent aged 5–18 yr were enrolled at two of the study centers (Cincinnati, OH and Omaha, NE). The enrollment criteria were term birth (> 37 weeks gestation), birth weight > 2.3 kg, no evidence of precocious or delayed puberty, and height and weight or BMI in the 3<sup>rd</sup> to 97<sup>th</sup> percentiles for age and sex. Subjects were excluded if they had been exposed to medications or medical conditions known to affect bone accrual, had been on extended bed rest, or had a history of multiple fractures. At the final visit, a saliva or blood sample was taken for DNA extraction. In this study of rs1800012, only participants of non-African American ancestry were included (n=804 girls and 771 boys), as the homozygous recessive genotype for rs1800012 was not present among African Americans.

### Phenotypes

aBMD was measured by DXA bone densitometers (Hologic, Bedford, MA; QDR4500A, QDR4500W, Delphi A, and Discovery models). Scans were performed at the lumbar spine, proximal femur, forearm, and total body by trained research technicians using standardized protocols that followed the manufacturer's guidelines. The scans were centrally analyzed by the DXA Core Laboratory (University of California, San Francisco, CA) with Hologic software v.12.3 for baseline scans and Apex 2.1 for follow-up scans, as previously reported<sup>(11)</sup>. Scan results were adjusted for clinical center differences and longitudinal drift. Age, sex and ancestry (African American vs. non-African American) specific Z-scores for the spine, total hip, femoral neck, and distal 1/3 radius, and BMC of the total body less head (TBLH) were calculated and adjusted for height-for-age Z-scores<sup>(12)</sup>.

Height was measured using a stadiometer, and height Z-scores were calculated using CDC 2000 growth charts<sup>(12)</sup>. Pubertal stage was assessed by trained physicians or nurses, and

study subjects were designated as pre-pubertal (Tanner stage I), pubertal (Tanner II-IV), or post-pubertal (Tanner V) based on breast development in girls and testicular volume in boys<sup>(13,14)</sup>. Dietary calcium was assessed with a semi-quantitative food frequency questionnaire (Block Dietary Data Systems, Berkeley, CA). Physical activity was estimated using a modified version of the Slemenda questionnaire that queried over 40 different weight and non-weight bearing physical activities. The time spent in each activity was summed and expressed as hours of physical activity per week.

### Genotyping

High-throughput genome-wide genotyping was performed using the Illumina Infinium II OMNI Express plus Exome BeadChip (Illumina, San Diego, CA) at the Children's Hospital of Philadelphia, Center for Applied Genomics (Philadelphia, PA). Quality control included filtering out SNPs with call rate <95%, minor allele frequency <1%, missing genotype rate >2%, and Hardy-Weinberg  $P < 1 \times 10^{-5}$ , as previously described<sup>(15)</sup>.

### Mixed-Effects Modeling

We tested the effect of homozygous (TT) minor allele groups for rs1800012 against the common homozygotes (GG) and the heterozygous (TG) allele carriers on height-for-age adjusted aBMD and BMC at the hip neck, total hip, lumbar spine, distal 1/3 radius, and total body less head (TBLH) using linear mixed effects models, with random intercepts, accounting for multiple measurements per individual. Given the established association between skeletal maturation and linear growth, we also tested the effect of genotype on height Z-score. We assessed males and females separately. The models included age, cohort ("discovery" or "replication"), recruitment site, BMI Z-score, dietary calcium, and physical activity level as covariates. STATA v 14 or 15 (StataCorp, College Station, TX) were used to perform all analyses. We further tested for pubertal stage interactions between the three pubertal stage groups (Group I, pre-pubertal (Tanner stage 1); Group II, pubertal (Tanner stages 2–4); and Group III, post-pubertal (Tanner stage 5)) and genotype in an additive model.

### SITAR modeling

To further assess the impact of carrying the rare homozygous genotype on longitudinal bone accrual, growth modeling was performed with SITAR (Shape Invariant Translation And Rotation), as previously described<sup>(16,17)</sup>, using the R package 'sitar'. Here, we extracted the bone accrual curves for the TT homozygous minor allele carriers vs. TG and GG carriers for aBMD and BMC at the hip neck, total hip, lumbar spine, distal 1/3 radius, and TBLH, as well as height gain. We tested the differences between carrier groups (GG vs. TT and GT vs. TT) for *a-size*, *b-tempo*, and *c-velocity* with unpaired t-tests in R.

### Results

In our cohort, there were 19 girls with up to 63 observations per skeletal site and 22 boys with up to 92 observations per skeletal site who carried the TT genotype (Supplementary Table 1). Overall, aBMD was slightly lower in female T carriers at the femoral neck (per copy of T, beta (SE)=-0.126 (0.059),  $P=0.033$ ) and total hip (per T, beta (SE)=-0.134

(0.062),  $P=0.029$ ) (Fig. 1A, Supplementary Fig. 1). However, we found significant ( $P$ -interaction  $< 0.05$ ) variant-pubertal status interactions at all skeletal sites (except for distal 1/3 radius) for girls (Fig. 1B). Specifically, TT homozygotes had significantly lower predicted aBMD and BMC Z-scores prior to puberty completion at many skeletal sites (e.g. spine aBMD-Z, Tanner group 3 vs 2,  $-0.44$  [95% CI:  $-0.69, -0.20$ ],  $P<0.001$ ). While male carriers of the TT genotype did not have lower aBMD or BMC in the main interactions (Supplementary Fig. 2), we observed similar variant-Tanner interactions in boys (all  $P$ -interaction  $< 0.05$ ; Supplementary Fig. 3).

SITAR longitudinal modeling of aBMD and BMC gain revealed that girls who were TT genotype carriers had lower aBMD and BMC prior to puberty (femoral neck and spine, shown in Fig. 2; all skeletal sites shown in Supplementary Fig. 4), began pubertal bone accrual ( $b$ -timing; e.g. total hip BMC  $P=0.03$ ) and their growth spurt (height  $P=0.007$ ) later (Supplementary Fig. 5). These girls on average demonstrated partial “catch up” in BMC and aBMD at most skeletal sites post-puberty. In boys, the aBMD and BMC SITAR curves of TT carriers were similar to individuals with the TG or GG genotypes (Supplementary Fig. 4). However, boys had reduced height, even post-puberty (Supplementary Fig. 5). At the same time, the stature of TT girls caught up to GG and GT carriers (Supplementary Fig. 5).

## Discussion

Genetic studies to date have implicated dozens of variants impacting BMD at various times throughout the lifespan<sup>(18,19)</sup>. Among the first genetic variants to be described was a polymorphism upstream of the *COL1A1* gene that affects a binding site for the transcription factor Sp1<sup>(1,5)</sup>. While the implications of this variant are clear in later life, its impact during bone development has not been clearly delineated. The current study helps fill this gap in knowledge regarding the role of this *COL1A1* variant in childhood by utilizing a larger sample size than previous studies, adopting a longitudinal design, and testing for sex differences and puberty effects. We observed that individuals homozygous for the minor genotype (i.e., TT carriers) had delayed bone accrual during pubertal development. In contrast to previous pediatric studies<sup>(8–10)</sup>, we analyzed boys and girls separately, and found that TT girls had lower aBMD before puberty and began pubertal bone accrual later. These girls attained some degree of “catch up” in BMC and aBMD at most skeletal sites post-puberty, consistent with the modest association of this genotype with pre-menopausal BMD<sup>(2,3)</sup>. While previous reports on the effect of this variant focused on the femoral neck and lumbar spine, we observed similar reductions across puberty at the other skeletal sites assessed.

In boys, while the same pattern of increasing aBMD and BMC was evident across puberty only in the minor allele homozygotes, we observed that predicted post-pubertal height-adjusted values were actually quite high. This may be partly explained by the reduced height seen in TT boys<sup>(10)</sup>, even post-puberty (Supplementary Fig. 5). Since our aBMD and BMC phenotypes were adjusted for height-for-age Z-scores, shorter height may result in inflated bone strength surrogates (i.e., Z-scores). Although our study contains the largest number of TT homozygotes in any pediatric study to date by an order of magnitude, these numbers are still small ( $n=19$  girls and 22 boys) and could impact the results.

Given the observed effects of this variant on both female pubertal bone acquisition and postmenopausal bone loss, effects that are partly masked during the relative quiescence in pre-menopausal adulthood, our findings suggest that high bone turnover states may be important windows into bone biology that provide valuable insight beyond peak bone density. Investigating the genetic determinants of bone health during high bone turnover states may reveal biological factors that impact the balance between osteoblasts and osteoclasts and represent candidate targets for osteoporosis prevention and therapy<sup>(20)</sup>.

## Conclusions

The genetic regulation of bone gains and losses may be masked during the relative quiescence of adulthood. Our findings strongly support investigating the interaction between genetics, maturational phase, and the hormonal milieu during periods of high bone turnover, particularly in childhood and adolescence, to fully understand the pathogenesis of osteoporosis.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

This work was funded by R01 HD58886. D.L.C. is supported by American Diabetes Association grant #1-17-PDF-077. J.M. is supported by NIH/NHLBI grant K01HL123612. S.F.A.G. is supported by the Daniel B. Burke Endowed Chair for Diabetes Research

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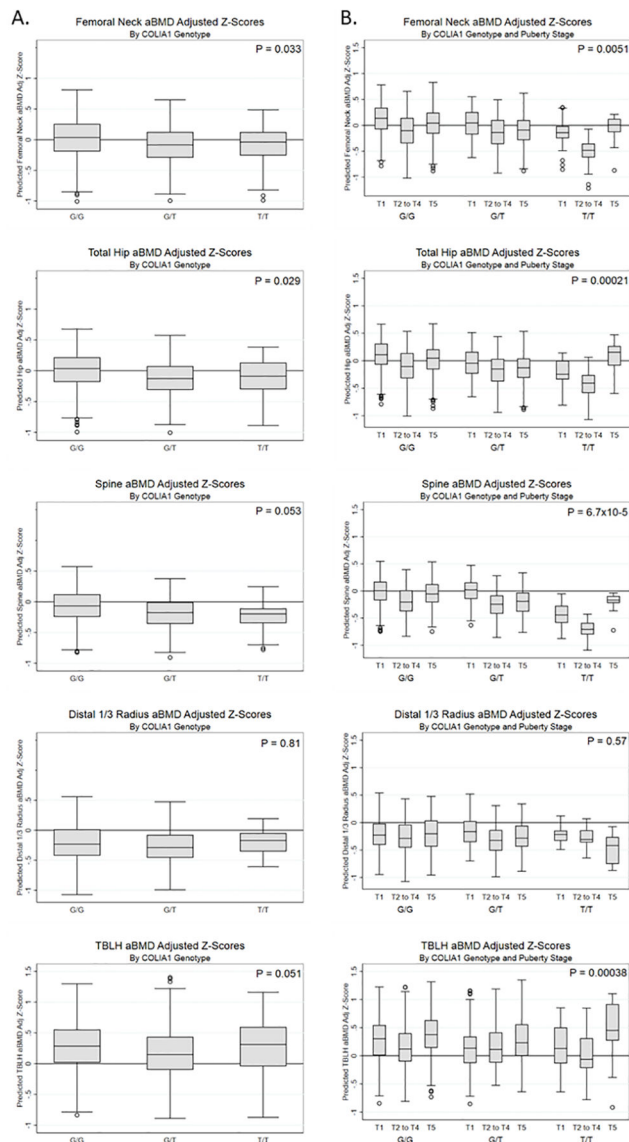
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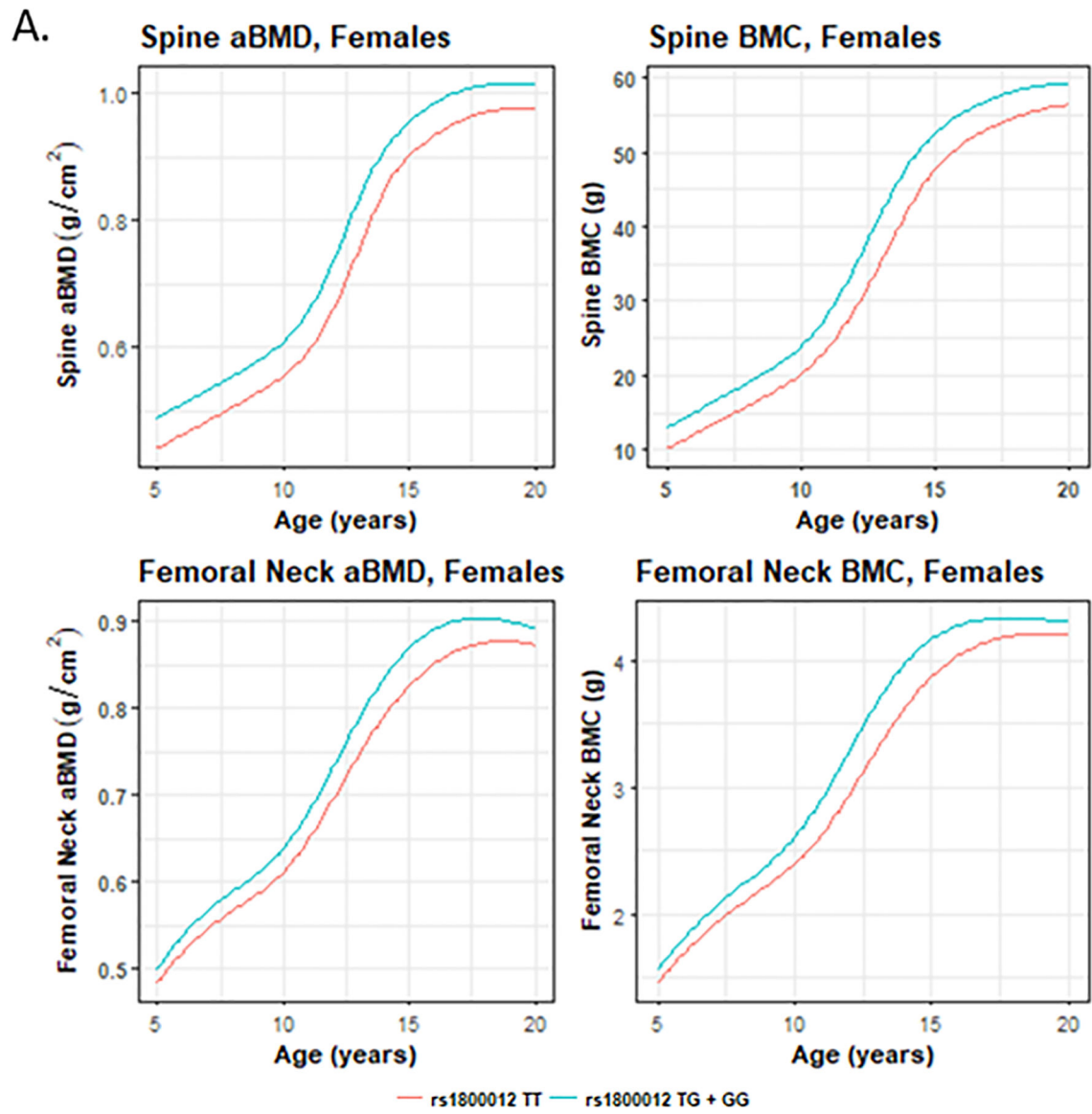
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**Figure 1.**

Sp1 variant in *COL1A1* affects bone density prior to and during puberty in females. aBMD and BMC age, sex, and population ancestry-specific Z-scores were calculated and further adjusted for height-for-age Z-scores. A. Main effects by genotype for lumbar spine and femoral neck aBMD Z-scores. B. Puberty interactions by genotype for lumbar spine and femoral neck aBMD Z-scores. T1, Tanner stage 1 (pre-pubertal); T2-T4, Tanner stages 2 to 4 (pubertal); T5, Tanner stage 5 (post-pubertal). *P*-values in column A are for the main variant associations; *P*-values in column B are for the variant-puberty stage interactions. TBLH, total body less head.



**Figure 2.**  
SITAR curves for lumbar spine and femoral neck aBMD and BMC for homozygotes for the minor T effect allele (red) versus TG and GG genotype carriers (blue).