**Level and change in bone microarchitectural parameters and their relationship with previous fracture and established bone mineral density loci**

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**Abstract**

**Background**

Osteoporosis is characterised by a reduction of bone mineral density (BMD) and predisposition to fracture. Bone microarchitecture, measured by high resolution peripheral quantitative computed tomography (HR-pQCT), has been related to fragility fractures and BMD and has been the subject of large-scale genome-wide analysis. We investigated whether fracture was related to baseline values and longitudinal changes in bone microarchitecture and whether bone microarchitecture was associated with established BMD loci.

**Methods**

115 males and 99 females (aged 72-81 at baseline) from the Hertfordshire Cohort Study (HCS) were analysed. Fracture history was determined in 2011-2012 by self-report and vertebral fracture assessment. Participants underwent HR-pQCT scans of the distal radius and tibia in 2011-2012 and 2017. Previous fracture in relation to baseline values and changes in tibial HR-pQCT parameters was examined using sex-adjusted logistic regression with and without adjustment for age, sociodemographic, lifestyle and clinical characteristics; baseline values and changes in parameters associated with previous fracture were then examined in relation to four established BMD loci after adjustment for sex and age.

**Results**

Previous fracture was related to: higher trabecular area (fully-adjusted odds ratio [95% CI] per SD greater baseline value: 2.18 [1.27,3.73], p=0.005); lower total volumetric BMD (0.53 [0.34,0.84], p=0.007), cortical area (0.53 [0.30,0.95], p=0.032), cortical BMD (0.56 [0.36,0.88], p=0.011) and cortical thickness (0.45 [0.27,0.77], p=0.004); and greater declines in trabecular BMD (p=0.001). Associations were robust in sex- and fully-adjusted analysis. Relationships between BMD loci and these HR-pQCT parameters were weak: rs3801387 (*WNT16*) was related to decline in trabecular BMD (p=0.011) but no other associations were significant (p>0.05).

**Conclusion**

Baseline values of HR-pQCT parameters and greater decline in trabecular BMD were associated with fracture. Change in trabecular BMD was associated with *WNT16* which has been demonstrated to influence bone health in murine models and human genome-wide association studies (GWAS).

**Keywords:** osteoporosis, epidemiology, fracture, quantitative computed tomography, loci

**1.0 Introduction**

Fragility fractures are a cause of widespread global concern with a fracture occurring (on average) every 3 seconds (1). There is a strong genetic component to fracture risk, with a history of parental fracture conferring an increased risk of fracture which is independent of bone mineral density (BMD) as measured by dual-energy x-ray absorptiometry (DXA) (2). BMD itself is highly heritable (with an h2 = 50-80%) as shown in twin studies (3) and, in 2012, Estrada and colleagues complied a genome-wide meta-analysis which identified 56 genetic loci which were associated with femoral neck and lumbar spine BMD in individuals with a European or East Asian ancestry (4).

There are fewer data investigating the genetic determinants of bone microarchitecture. In previous studies, other genetic variants in RANK/OPG have been associated with cortical volumetric BMD (5, 6) and SNPs in *FMN2*/*GREM2* genes were associated with trabecular volumetric BMD and fracture risk (6). Furthermore, regions mapped to *WNT4*, *ZBTB40*, *TNFRSF11B*, *AKAP11*, and *TNFSF11* have been associated with lumbar spine vBMD (7).

Compared to DXA, high resolution peripheral quantitative computed tomography (HR-pQCT) provides a more detailed analysis of bone microarchitecture with in-depth measurement of parameters within the trabecular and cortical compartments at the peripheral skeleton. Associations between HR-pQCT parameters and fracture have previously been observed in populations of post-menopausal females in the GERICO (8), CaMOS (9) and OFELY (10) cohorts. However, the relationship between longitudinal change in bone microarchitecture and fracture has not been investigated in a group of community-dwelling older adults comprising males, such as the Hertfordshire Cohort Study (HCS), and neither has an examination of HR-pQCT parameters in relation to established BMD loci been performed.

The aims of this study were: to describe baseline values and 5-year changes in HR-pQCT parameters; examine baseline values and changes in HR-pQCT parameters in relation to previous fracture; and examine those HR-pQCT parameters associated with previous fracture in relation to established BMD loci.

**2.0 Methods**

*2.1 The Hertfordshire Cohort Study*

The Hertfordshire Cohort Study (HCS) comprises 2997 individuals born in Hertfordshire from 1931-1939 and who still lived there in 1998-2004 where they completed a home interview and clinic visit for a detailed assessment of their health. In 2004, of the 966 participants from East Hertfordshire who had a dual-energy X-ray absorptiometry (DXA) scan at the start of the study, 642 were recruited for a musculoskeletal follow-up study. In 2011-2012, 376/642 participated in a further bone follow-up study and 224/376 took part in a 2017 bone follow-up study. The background of the HCS and further details of the follow-up studies have been described previously (11, 12).

*2.2 Ascertainment of participant characteristics in 1998-2004*

Dietary calcium intake was determined using a food-frequency questionnaire (13). Current or most recent full-time occupation (husband's for ever-married females) was ascertained; social class was coded from the 1990 OPCS Standard Occupational Classification (SOC90) unit group for occupation (14). Current use of bisphosphonates at the study commencement (1998-2004), at the musculoskeletal follow-up (2004-2005) and in 2011-2012 was ascertained from details of all currently used over the counter or prescription medications.

*2.3 Ascertainment of participant characteristics in 2011-2012*

Smoking status, alcohol consumption (in units per week), average daily outdoor physical activity in minutes (Longitudinal Aging Study Amsterdam Physical Activity Questionnaire (LAPAQ)((15))) and any previous self-reported fractures since aged 45 years were ascertained at the home interview through nurse-administered questionnaires. Height was measured (wall-mounted SECA stadiometer) along with weight (calibrated SECA 770 digital floor scales, SECA Ltd, Hamburg) and used to derive BMI (kg/m2). Morphometric vertebral fractures were diagnosed from a lateral spine view imaged using a Lunar Prodigy Advance DXA scanner (GE Medical Systems) and graded based on the Genant semi-quantitative method of vertebral fracture assessment (16) using a single reader. Participants with a vertebral fracture or a self-reported fracture since age 45 years were regarded as having had a previous fracture.

HR-pQCT scans (XtremeCTi, Scanco Medical AG, Switzerland) of the non-dominant distal radius and tibia were performed; dominant limbs were scanned if the non-dominant limb had previously fractured. In total, 110 parallel CT slices were obtained, representing a volume of bone 9mm in axial length with a nominal resolution (voxel size) of 82μm. The scan protocol was in accordance with manufacturer’s guidelines and as described by Boutroy *et al* (17). Using the method of Pauchard *et al* (18), scans exhibiting excessive motion artefact (grade 5 scans) were excluded; scans of quality 4 and above were included in the analysis. Manufacturer standard evaluation and cortical porosity scripts were used for image analysis (8, 19-23). Extended cortical analysis was performed for all scans (24).

*2.4 Ascertainment of participant characteristics at follow-up (2017)*

HR-pQCT scans of the distal radius and tibia were repeated using the same devices and protocol as in 2011-2012. Quality assurance and quality control were performed as per manufacturer guidance and no significant changes were made to the machines over follow-up. Overlap between baseline and follow-up HR-pQCT scans was derived using the manufacturer’s slice-match method. Change measures for HR-pQCT parameters were set to missing if the overlap between the baseline and follow-up scan regions was less than 75% as recommended(25); median (lower quartile, upper quartile) overlap between the remaining scans were 93% (87%, 96%) for the radius and 95% (92%, 97%) for the tibia. Short term precision values (percentage root mean squared coefficient of variation) for cortical and trabecular BMD have been shown to range from 0.3 to 1.2 (26).

*2.5 Selection of loci for analysis*

SNP genotyping was carried out using Infinium Global Screening Array v1. Raw IDAT files were loaded into Illumina Genome Studio 2.0.0 and analysed using genotyping Module 2.0.0. Data was processed using Illumina hard cut-off technical specifications (27). *bcftools* and PLINK 1.9 beta (28) were used to prepare the QC genotype data ready for imputation according to SANGER specifications. Data were uploaded in VCF format to SANGER servers, pre-phased using EAGLE2 pipeline and imputed using UK10K + 1000 Genome Phase 3 (29).

Estrada et al performed a genome-wide meta-analysis and identified 64 SNPs that were associated with BMD (femoral neck or lumbar spine) or fracture (4). In total, 61/64 of these SNPs were available in HCS and were examined in relation to femoral neck and lumbar spine BMD (acquired via DXA) at HCS commencement (1998-2004). The following loci were associated with both femoral neck and lumbar spine BMD in HCS (data not shown) and, therefore, were used in this study: rs1053051 (*TMEM263* (alias name: *C12orf23*)); rs7812088 (*ABCF2*); rs10226308 (*TXNDC3* (also known as *NME8*)); and rs3801387 (*WNT16*).

*2.6 Ethical approval and informed consent*

The initial phase of the HCS (1998-2004) had ethical approval from the Hertfordshire and Bedfordshire Local Research Ethics Committee, and the 2011-2012 bone follow-up had ethical approval from the East of England - Cambridgeshire and Hertfordshire Research Ethics Committee. All participants provided written informed consent. Ethical approval was obtained for all follow-ups of the study at the time they were conducted. Investigations were conducted in accordance with the principles expressed in the Declaration of Helsinki.

*2.7 Statistical analysis*

In the statistical description and results, ‘baseline’ refers to the baseline HR-pQCT scan visit (2011-12). Participant characteristics, including baseline values and annual percentage changes in HR-pQCT parameters, were described using summary statistics. Standard deviation (SD) scores were coded for baseline values and annual percentage changes in HR-pQCT parameters for use in models to enable the comparison of effect sizes. Odds ratios for previous fracture per SD difference in baseline values and changes in HR-pQCT parameters were derived using logistic regression after adjustment for sex and after additional adjustment for age, height, BMI, dietary calcium, physical activity, smoking history (ever vs never), alcohol consumption and social class. Baseline values and changes in HR-pQCT parameters were then examined in relation to the four selected loci using linear regression after adjustment for sex and age.

To maintain sample size, males and females were pooled (sex-interaction effects were not statistically significant) and analyses were adjusted for sex; p<0.05 was regarded as statistically significant. Analyses were conducted using Stata, release 15.1. The analysis sample comprised 214 participants with both baseline values and change in at least one HR-pQCT parameter (tibial or radial) and had data on previous fracture or the loci of interest.

**3.0 Results**

*3.1 Participant characteristics*

The characteristics of the study population are presented in Table 1. Mean (SD) age of the 214 participants at the time of the baseline HR-pQCT scans (2011-2012) was 76.0 (2.5) years. Median (lower quartile, upper quartile) duration between the baseline and follow-up HR-pQCT scans was 5.2 (4.8, 5.4) years. Overall 27 (25.2%) males and 32 (33.3%) females had a previous fracture (vertebral or self-reported since age 45 years). The sites of fractures in this cohort have been described previously (30).

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| **Table 1: Descriptive statistics for participant characteristics in 2011-2012** |
|  |  |  |
| **Participant characteristic** | **Mean (standard deviation), n(%) or median (lower quartile, upper quartile)** |
| **Males (n=115)** | **Females (n=99)** |
| Age (years) | 75.8 (2.5) | 76.2 (2.6) |
| Height (cm) | 173.3 (6.5) | 160.4 (5.7) |
| Weight (kg) | 83.1 (13.0) | 72.4 (12.5) |
| BMI (kg/m2) | 27.7 (4.0) | 28.1 (4.5) |
| Weekly dietary calcium (g)\* | 8.3 (2.2) | 8.0 (2.9) |
| Physical activity in last 2 weeks (min/day) \*\* | 195.0 (128.6, 291.4) | 207.9 (150.0, 287.1) |
| Ever smoked | 61 (56.5%) | 39 (40.2%) |
|  |  |  |
| Weekly alcohol units (M: Males; F: Females) |  |  |
| Very low (0/<1 M&F) | 24 (22.2%) | 48 (49.5%) |
| Low (1-10M,1-7F) | 48 (44.4%) | 39 (40.2%) |
| Moderate (11-21M,8-14F) | 17 (15.7%) | 8 (8.2%) |
| High (>21M, >14F) | 19 (17.6%) | 2 (2.1%) |
|  |  |  |
| Social class (manual)\* | 59 (53.6%) | 53 (53.5%) |
| Bisphosphonate use (1998-2004 to 2012)\* | 4 (3.7%) | 20 (20.6%) |
| Self-reported fracture since aged 45 years | 24 (22.4%) | 30 (30.9%) |
| Vertebral fracture | 6 (5.3%) | 6 (6.1%) |
| Any fracture (self-reported or vertebral) | 27 (25.2%) | 32 (33.3%) |
|  |  |  |
| rs1053051\*: CC | 26 (24.3%) | 29 (31.5%) |
|  CT | 47 (43.9%) | 33 (35.9%) |
|  TT | 34 (31.8%) | 30 (32.6%) |
|  |  |  |
| rs7812088\*: GG | 82 (76.6%) | 70 (76.1%) |
|  AG | 24 (22.4%) | 22 (23.9%) |
|  AA  | 1 (0.9%) | 0 (0.0%) |
|  |  |  |
| rs10226308\*: AA | 78 (72.9%) | 47 (51.1%) |
|  GA | 26 (24.3%) | 40 (43.5%) |
|  GG | 3 (2.8%) | 5 (5.4%) |
|  |  |  |
| rs3801387\*: AA | 60 (56.1%) | 45 (48.9%) |
|  GA | 39 (36.4%) | 40 (43.5%) |
|  GG | 8 (7.5%) | 7 (7.6%) |
| \*Ascertained using information at initial phase of the Hertfordshire Cohort Study (1998-2004)\*\*Derived using the Longitudinal Ageing Study Amsterdam Physical Activity Questionnaire |

Associations between tibial parameters and previous fracture were substantially stronger than those observed for radial parameters; however, the patterns of association were similar for each. Therefore, we confined the remainder of the results section for tibial measures.

Median (lower quartile, upper quartile) values for baseline values and annual percentage changes in tibial HR-pQCT parameters are presented in Table 2. Although annual percentage changes in parameters were small, many median values for these longitudinal changes were significantly different from zero (p<0.05). For example, there were decreases in trabecular density (females only), cortical area, density and thickness, and total volumetric bone density; increases in cortical pore diameter (males only), trabecular area and cortical porosity were also observed. Equivalent information for the radial HR-pQCT parameters is presented in Supplementary Table A.

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| **Table 2: Descriptive statistics for tibial HR-pQCT parameters at baseline (2011-2012) and for changes in parameters from 2011-2012 to 2017**  |
|  |  |  |  |  |  |  |
| **Parameter [Median (lower quartile, upper quartile) values shown]** | **Males (n=115)** | **Females (n=99)** |
| **Baseline** | **Annual change (%)** | **P-value** | **Baseline** | **Annual change (%)** | **P-value** |
|  |  |  |  |  |  |  |
| Trabecular area (mm2) | 743 (648, 838) | **0.1 (0.0, 0.2)** | **<0.001** | 620 (542, 707) | **0.2 (0.0, 0.3)** | **<0.001** |
| Total volumetric bone density (mg/cm3) | 294 (262, 343) | ***-0.5 (-1.0, -0.2)*** | ***<0.001*** | 252 (219, 276) | ***-0.8 (-1.5, -0.3)*** | ***<0.001*** |
| Trabecular density (mg/cm3) | 196 (170, 215) | -0.1 (-0.3, 0.2) | 0.180 | 167 (149, 199) | ***-0.3 (-0.8, 0.2)*** | ***0.012*** |
| Trabecular number (mm-1) | 2.5 (2.2, 2.7) | -0.1 (-1.0, 1.1) | 0.444 | 2.3 (2.0, 2.5) | -0.2 (-1.7, 1.4) | 0.213 |
| Trabecular thickness (mm) | 0.066 (0.058, 0.072) | 0.0 (-1.1, 1.0) | 1.000 | 0.064 (0.054, 0.071) | 0.0 (-1.3, 1.2) | 1.000 |
| Trabecular separation (mm) | 0.34 (0.30, 0.38) | 0.1 (-1.1, 1.1) | 0.501 | 0.37 (0.34, 0.43) | 0.3 (-1.3, 2.0) | 0.300 |
| Cortical area (mm2) | 129 (116, 153) | ***-0.7 (-1.5, 0.0)*** | ***<0.001*** | 83 (74, 98) | ***-1.3 (-2.4, -0.4)*** | ***<0.001*** |
| Cortical density (mg/cm3) | 836 (803, 873) | ***-0.6 (-1.0, -0.3)*** | ***<0.001*** | 763 (721, 807) | ***-0.7 (-1.2, -0.2)*** | ***<0.001*** |
| Cortical porosity (%) | 8.8 (7.3, 10.6) | **2.5 (0.5, 4.8)** | **<0.001** | 9.9 (7.9, 12.2) | **1.5 (-0.1, 3.3)** | **<0.001** |
| Cortical thickness (mm) | 1.2 (1.1, 1.4) | ***-0.4 (-1.1, 0.1)*** | ***<0.001*** | 0.9 (0.8, 1.1) | ***-1.1 (-2.0, -0.1)*** | ***<0.001*** |
| Cortical pore diameter (mm) | 0.17 (0.16, 0.18) | **0.2 (-0.7, 1.3)** | **0.031** | 0.18 (0.17, 0.19) | 0.1 (-0.9, 0.9) | 0.602 |
|  |  |  |  |  |  |  |
| P-values correspond to tests that median annual percentage changes were zero and were calculated from sign tests |
| Median annual percentage changes that were significantly different from zero (p<0.05) are highlighted in bold (underlined for increases and italic for decreases) |

*3.2 Relationships between baseline values and changes in tibial HR-pQCT parameters and previous fracture*

Associations between baseline values and annual percentage changes in tibial HR-pQCT parameters in relation to previous fracture are presented in Table 3 and Figure 1. Increased odds of previous fracture were observed for; higher trabecular area (p<0.01); lower total volumetric bone density (p<0.01); lower cortical area (p<0.04), lower cortical density (p<0.02) and lower cortical thickness (p<0.01); and greater declines in trabecular density (p<0.002). These relationships were robust in sex-adjusted and fully-adjusted models. Associations between baseline values and annual percentage changes in radial HR-pQCT parameters in relation to previous fracture are presented in Supplementary Table B.

**Figure 1: Fully-adjusted odds ratios for previous fracture per standard deviation difference in baseline values and changes in key tibial HR-pQCT parameters**



Odds ratios were adjusted for sex, age, height, BMI, dietary calcium, physical activity, smoking history (ever vs never), alcohol consumption and social class

Odds ratios greater than one: higher baseline values in 2011-2012 or reduced declines from 2011-2012 to 2017 were associated with greater risk of previous fracture

Odds ratios less than one: higher baseline values in 2011-2012 or reduced declines from 2011-2012 to 2017 were associated with lower risk of previous fracture

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| **Table 3: Odds ratios for previous fracture per standard deviation difference in both baseline values and changes in parameters** |
|  |  |  |  |  |  |  |  |  |
| **HR-pQCT tibia parameter (SD)** | **Baseline values in 2011-2012** | **Annual percentage change from 2011-2012 to 2017** |
| **Sex-adjusted** | **Fully-adjusted\*** | **Sex-adjusted** | **Fully-adjusted\*** |
| **Odds ratio** **(95% CI)** | **P-value** | **Odds ratio** **(95% CI)** | **P-value** | **Odds ratio** **(95% CI)** | **P-value** | **Odds ratio** **(95% CI)** | **P-value** |
| Trabecular area | ***1.70 (1.15,2.51)*** | ***0.007*** | ***2.18 (1.27,3.73)*** | ***0.005*** | 1.11 (0.80,1.56) | 0.525 | 1.01 (0.71,1.44) | 0.945 |
| Total volumetric bone density | ***0.58 (0.39,0.84)*** | ***0.004*** | ***0.53 (0.34,0.84)*** | ***0.007*** | 0.72 (0.52,1.01) | 0.059 | 0.75 (0.53,1.07) | 0.117 |
| Trabecular density | 0.79 (0.56,1.10) | 0.168 | 0.72 (0.49,1.06) | 0.099 | ***0.56 (0.40,0.80)*** | ***0.001*** | ***0.50 (0.34,0.75)*** | ***0.001*** |
| Trabecular number | 0.92 (0.66,1.27) | 0.602 | 0.96 (0.64,1.43) | 0.836 | 0.99 (0.72,1.36) | 0.951 | 0.91 (0.64,1.29) | 0.584 |
| Trabecular thickness | 0.80 (0.58,1.10) | 0.168 | 0.69 (0.48,1.00) | 0.052 | 0.83 (0.60,1.15) | 0.256 | 0.88 (0.62,1.27) | 0.502 |
| Trabecular separation | 1.12 (0.81,1.55) | 0.504 | 1.10 (0.74,1.64) | 0.626 | 1.03 (0.75,1.41) | 0.872 | 1.13 (0.79,1.60) | 0.515 |
| Cortical area | ***0.52 (0.32,0.84)*** | ***0.008*** | ***0.53 (0.30,0.95)*** | ***0.032*** | 0.86 (0.62,1.20) | 0.377 | 0.98 (0.68,1.40) | 0.908 |
| Cortical density | ***0.51 (0.34,0.76)*** | ***0.001*** | ***0.56 (0.36,0.88)*** | ***0.011*** | 0.93 (0.67,1.28) | 0.641 | 1.00 (0.70,1.42) | 0.987 |
| Cortical porosity | 1.09 (0.79,1.50) | 0.618 | 0.96 (0.67,1.37) | 0.808 | ***0.71 (0.51,1.00)*** | ***0.048*** | 0.73 (0.51,1.05) | 0.090 |
| Cortical thickness | ***0.48 (0.31,0.74)*** | ***0.001*** | ***0.45 (0.27,0.77)*** | ***0.004*** | 0.82 (0.59,1.15) | 0.255 | 0.91 (0.64,1.30) | 0.597 |
| Cortical pore diameter | 0.85 (0.61,1.17) | 0.317 | 0.71 (0.49,1.03) | 0.070 | 0.82 (0.59,1.15) | 0.253 | 0.82 (0.56,1.20) | 0.309 |
|  |  |  |  |  |  |  |  |  |
| HR-pQCT: High resolution peripheral quantitative computed tomography; CI: Confidence interval |
| \*Adjusted for sex, age, height, BMI, dietary calcium, physical activity, smoking history (ever vs never), alcohol consumption and social class  |
| Odds ratio of less than one for annual percentage change in parameter shows that reduced declines are related to lower risk of previous fracture |
| Significant associations (p<0.05) are given in italics |

*3.3 Selected loci in relation to tibial HR-pQCT parameters that were associated with previous fracture*

Relationships between selected loci and baseline values and annual percentage changes in tibial HR-pQCT parameters that were associated with previous fracture are presented in Table 4. Few loci were associated with these measures; rs3801387 (*WNT16*) was related to change in trabecular density (p=0.011) and rs7812088 (ABCF2) was related to baseline values of trabecular area (p=0.072) but the remaining associations were weak (p>0.09).

Pearson correlations between the tibial HR-pQCT parameters associated with previous fracture are presented in Supplementary Table C.

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| **Table 4: Selected loci in relation to tibial HR-pQCT parameters that were associated with previous fracture** |
|  |  |  |  |  |  |  |  |  |
| **HR-pQCT tibia parameter****(z-scores)** | **rs1053051 (*TMEM263*):** **per extra T allele** | **rs7812088 (*ABCF2*):** **AG compared to GG** | **rs10226308 (*TXNDC3*):** **per extra G allele** | **rs3801387 *(WNT16)*:** **per extra G allele** |
|
| **Estimate (95% CI)** | **P-value** | **Estimate (95% CI)** | **P-value** | **Estimate (95% CI)** | **P-value** | **Estimate (95% CI)** | **P-value** |
| **Baseline values** |  |  |  |  |  |  |  |  |
| Trabecular area | -0.07 (-0.24,0.10) | 0.426 | -0.28 (-0.59,0.03) | 0.072 | 0.07 (-0.17,0.30) | 0.584 | -0.02 (-0.23,0.18) | 0.810 |
| Total volumetric bone density | 0.13 (-0.03,0.30) | 0.112 | 0.18 (-0.13,0.48) | 0.251 | 0.02 (-0.21,0.25) | 0.856 | 0.03 (-0.17,0.23) | 0.767 |
| Cortical area | 0.06 (-0.07,0.18) | 0.385 | 0.10 (-0.13,0.33) | 0.384 | -0.03 (-0.21,0.15) | 0.734 | 0.01 (-0.14,0.16) | 0.879 |
| Cortical density | 0.04 (-0.12,0.20) | 0.636 | 0.00 (-0.28,0.29) | 0.974 | 0.06 (-0.15,0.28) | 0.560 | 0.03 (-0.16,0.21) | 0.784 |
| Cortical thickness | 0.09 (-0.06,0.24) | 0.244 | 0.21 (-0.07,0.49) | 0.136 | -0.06 (-0.27,0.15) | 0.571 | 0.01 (-0.17,0.20) | 0.874 |
|  |  |  |  |  |  |  |  |  |
| **Annual percentage changes** |  |  |  |  |  |  |  |  |
| Trabecular density | 0.08 (-0.11,0.26) | 0.397 | -0.10 (-0.43,0.23) | 0.543 | 0.22 (-0.04,0.47) | 0.092 | ***-0.28 (-0.50,-0.07)*** | ***0.011*** |
|  |  |  |  |  |  |  |  |  |
| Estimates shown are standard deviation differences in HR-pQCT parameters according to each loci |
| Associations were adjusted for age and sex |
| Significant associations (p<0.05) are given in italics |

*3.4 Selected loci in relation to tibial HR-pQCT parameters that were not associated with previous fracture*

Of the list of tibial HR-pQCT parameters that were not associated with previous fracture, rs10226308 (*TXNDC3*) was related to baseline values of trabecular number and separation; rs1053051 (*TMEM263*) was related to change in trabecular thickness and cortical porosity; and rs3801387 (WNT16) was related to baseline values of cortical pore diameter (Supplementary Table D). For completeness, Supplementary Table D presents associations for the selected loci in relation to baseline values and annual percentage changes in both tibial and radial HR-pQCT parameters.

**4.0 Discussion**

Through this study, we have described the longitudinal changes in tibial bone microarchitecture which occur in older adults including decreased trabecular density (females only) and increased trabecular area, cortical porosity and pore diameter (males only) and decreased cortical area, density and thickness and total volumetric bone density. We have shown that the odds of fracture increase with larger trabecular area and decrease with higher volumetric BMD and cortical area, density and thickness. Additionally we have demonstrated that the odds of fracture increase with greater loss in trabecular density. We have also shown that a SNP within *ABCF2* has a borderline association with trabecular area and a SNP within *WNT16* is associated with change in trabecular density.

The description of tibial bone microarchitectural changes over 5 years in our study is defined by decreased trabecular density (in females only) and increased trabecular area, cortical porosity and pore diameter (males only) and decreased cortical area, density and thickness. These characteristics are aligned to the current theory of bone aging with trabecular bone loss and decreased cortical bone. The reason for increasing trabecular area is likely due to the process of endocortical resorption with increasing trabecular area being at the cost of cortical thinning (31).

When we consider our findings with regard to fracture prediction it is important to bear in mind the current literature regarding HR-pQCT outcomes and fracture associations. It is, of course, vital to recognise that in the studies below, the fractures described are incident whereas in this current study the fractures are prevalent. This may have a bearing on the associations observed.

Lower baseline volumetric BMD was predictive of future fracture over 5 years in the GERICO cohort of post-menopausal females (8), as was radial total BMD (Odds ratio (OR) per standard deviation (SD) lower baseline values: 2.1) and trabecular BMD (OR: 2.0) in the CaMOS cohort(9). Lower baseline cortical thickness at the tibia was also predictive of fracture in the CaMOS study (OR: 2.2) though trabecular parameters at the radius had greater predictive capacity over 10 years of follow-up in the OFELY cohort(10) and trabecular parameters had a greater association with future fracture in a group taking denosumab (32). Interestingly an additional finding from the CaMOS study was that baseline values rather than rate of change were more associated with future fractures in post-menopausal females(9) and this was certainly echoed in our study, with only change in trabecular density being associated with fracture odds. Associations between previous fracture and HR-pQCT parameters in our study were unaltered after additional adjustment for bisphosphonate use and relationships between previous fracture and changes in HR-pQCT parameters were also unaltered when adjusted for baseline values of the corresponding parameters. The reason why associations with fracture were stronger regarding baseline values of HR-pQCT parameters, compared to longitudinal changes in parameters, is potentially because genes and variants involved in building the skeleton up (to peak bone mass) are more relevant to fracture than those associated with the degradation of bone microarchitecture that is observed with ageing.

A SNP near *ABCF2*, the gene for a member of the ATP-binding cassette (ABC) transporter superfamily, was borderline associated with trabecular area. *ABCF2* plays a role in transmembrane transportation and previous work has uncovered associations with cancer progression(33, 34), the molecular pathogenesis of Duchenne Muscular Dystrophy(35), and is down-regulated in ulcerative colitis(36). It is possible that the association between trabecular area is mediated via stem cell pathways or inflammatory mechanisms which have previously been described in this cohort (37).

We also saw that a SNP at *WNT16* was associated with change in trabecular density. Wnt16 is thought to be largely secreted by osteoblasts (38) and directly suppresses osteoclastogenesis via the non-canonical JNK MAPK pathway with upregulation of osteoprotegerin. In murine models Wnt16 has been shown to be reduced by steroid administration (39) and the particular SNP rs3801387 has previously been associated with BMD, fracture and cortical bone thickness (4, 40, 41). It is surprising that we should have only observed effects on density in the trabecular compartment and also that the direction of association suggests that a greater number of G alleles was associated with a disadvantageous greater reduction of trabecular density. In the Estrada GWAS, from which the *WNT16* SNP was identified, each additional A allele was disadvantageous to bone and associated with lower femoral neck BMD (SD difference per additional A allele: -0.08), lower spinal BMD (β= -0.10) and higher risk of low-trauma fracture (OR: 1.06) (4). Interestingly, we observed the same direction of association between rs3801387 and cross-sectional DXA BMD in our study at the HCS commencement (1998-2004) as was observed by Estrada et al. which therefore suggests that our finding related to change in trabecular density could be due to the different parameter of bone microarchitecture being measured. Similar deleterious effects of this SNP (rs3801387) were observed in cohort of pre-menopausal females with each additional T allele associated with lower BMD at the lumbar spine (β= -0.16) and femoral neck (-0.12) (40). It is important, therefore, that our finding is investigated in other cohorts which have HR-pQCT and bone microarchitecture available. Other GWAS have demonstrated associations between *WNT16* and BMD but not at rs3801387 (42, 43).

Our study has many strengths. Firstly, the longitudinal HR-pQCT dataset in a group of older adults is a novel contribution to the literature as many previous studies have focused on post-menopausal females or included participants from a broad age range from adolescence to older age. Secondly, the opportunity to examine genotypic data in conjunction with longitudinal HR-pQCT is unique. Thirdly, the HCS has been phenotyped according to strict protocols by highly-trained fieldworkers and managed by an experienced multi-disciplinary team. The limitations of our study are that it was exploratory and used a relatively small sample size which does not allow for site-specific fracture analyses, and the fact that we have a prevalent (rather than incident) fracture history. Additionally, as the SNPs we analysed were taken from the work by Estrada and colleagues(4), these were based initially on a meta-analysis of BMD GWAS before Estrada also examined them in relation to fracture.

In conclusion, we have described the cortical and trabecular bone deterioration and endocortical bone loss associated with ageing and demonstrated that baseline cortical and BMD levels held greater associations with fracture than changes in bone microarchitecture. We have also shown borderline associations between *ABCF2*, a SNP previously associated with BMD,and trabecular area, and *WNT16* and change in trabecular density.

Our findings contribute to the literature describing the effects of ageing on bone microarchitecture but also add to previous work teasing out the genetic determinants of trabecular and cortical characteristics.

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**Compliance with Ethical Standards**

**Declaration of interest:** CC reports personal fees (outside the submitted work) from Amgen, Danone, Eli Lilly, GSK, Kyowa Kirin, Medtronic, Merck, Nestle, Novartis, Pfizer, Roche, Servier, Shire, Takeda and UCB. NCH reports consultancy, lecture fees and honoraria (outside the submitted work) from Alliance for Better Bone Health, AMGEN, MSD, Eli Lilly, Servier, Shire, UCB, Kyowa Kirin, Consilient Healthcare, Radius Health and Internis Pharma. EMD reports personal fees (outside the submitted work) from Pfizer Healthcare and from the UCB Discussion panel. NRF has received travel bursaries from Pfizer and Eli Lilly. LDW, GB, PT, MB and KAW declare that they have no conflicts of interest.

**Ethical Approval:** The initial phase of the Hertfordshire Cohort Study (1998-2004) had ethical approval from the Hertfordshire and Bedfordshire Local Research Ethics Committee and the 2011-2012 follow-up had ethical approval from the East of England - Cambridgeshire and Hertfordshire Research Ethics Committee.

**Human and Animal Rights:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed Consent:** All participants gave signed consent to participate in the study and for their health records to be accessed in the future.

**Author contributions: NRF** Investigation, Writing - Original Draft; **LDW** Methodology, Formal analysis, Writing - Original Draft; **GB** Investigation, Resources, Writing - Review & Editing; **PT** Methodology, Data Curation; **MB** Writing - Review & Editing; **NCH** Conceptualization, Writing - Review & Editing; **EMD** Conceptualization, Writing - Review & Editing, Supervision, Project administration; **CC** Conceptualization, Writing - Review & Editing, Supervision, Project administration; **KAW** Conceptualization, Investigation, Writing - Review & Editing, Supervision, Project administration. All authors made substantial contributions to the manuscript and approved the final version.

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| **Supplementary Table A: Descriptive statistics for radial HR-pQCT parameters at baseline (2011-2012) and for changes in parameters from 2011-2012 to 2017**  |
|  |  |  |  |  |  |  |
| **Parameter [Median (lower quartile, upper quartile) values shown]** | **Males (n=115)** | **Females (n=99)** |
| **Baseline** | **Annual change (%)** | **P-value** | **Baseline** | **Annual change (%)** | **P-value** |
| Trabecular area (mm2) | 332 (291, 376) | **0.1 (0.0, 0.3)** | **<0.001** | 226 (202, 268) | **0.1 (0.0, 0.2)** | **<0.001** |
| Total volumetric bone density (mg/cm3) | 297 (266, 339) | ***-0.6 (-1.1, 0.0)*** | ***<0.001*** | 244 (215, 302) | ***-0.7 (-1.4, -0.2)*** | ***<0.001*** |
| Trabecular density (mg/cm3) | 186 (163, 210) | -0.1 (-0.6, 0.2) | 0.368 | 150 (127, 176) | -0.2 (-0.8, 0.2) | 0.053 |
| Trabecular number (mm-1) | 2.4 (2.3, 2.5) | 0.0 (-1.2, 0.8) | 0.920 | 2.2 (2.0, 2.4) | ***-0.5 (-1.3, 0.4)*** | ***0.004*** |
| Trabecular thickness (mm) | 0.066 (0.059, 0.071) | 0.0 (-0.9, 0.7) | 1.000 | 0.055 (0.051, 0.065) | 0.3 (-0.8, 1.0) | 0.550 |
| Trabecular separation (mm) | 0.36 (0.33, 0.38) | 0.0 (-0.8, 1.4) | 1.000 | 0.40 (0.36, 0.44) | **0.7 (-0.4, 1.4)** | **0.012** |
| Cortical area (mm2) | 67 (57, 75) | ***-1.0 (-1.8, -0.2)*** | ***<0.001*** | 43 (37, 49) | ***-1.0 (-1.6, 0.1)*** | ***<0.001*** |
| Cortical density (mg/cm3) | 827 (793, 870) | ***-0.4 (-0.7, -0.1)*** | ***<0.001*** | 811 (775, 848) | ***-0.6 (-0.8, -0.2)*** | ***<0.001*** |
| Cortical porosity (%) | 4.1 (3.1, 5.0) | **2.0 (-0.6, 4.4)** | **<0.001** | 3.7 (2.5, 4.5) | 1.5 (-1.3, 5.7) | 0.091 |
| Cortical thickness (mm) | 0.8 (0.7, 1.0) | ***-0.8 (-1.5, 0.1)*** | ***<0.001*** | 0.7 (0.6, 0.8) | ***-0.8 (-1.3, 0.1)*** | ***<0.001*** |
| Cortical pore diameter (mm) | 0.15 (0.15, 0.16) | 0.2 (-0.5, 1.4) | 0.129 | 0.15 (0.15, 0.17) | **0.3 (-0.5, 1.2)** | **0.039** |
|  |  |  |  |  |  |  |
| P-values correspond to tests that median annual percentage changes were zero and were calculated from sign tests |
| Median annual percentage changes that were significantly different from zero (p<0.05) are highlighted in bold (underlined for increases and italic for decreases) |

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| **Supplementary Table B: Odds ratios for previous fracture per standard deviation difference in both baseline values and changes in parameters** |
|  |  |  |  |  |  |  |  |  |
| **HR-pQCT radial parameter (SD)** | **Baseline values in 2011-2012** | **Annual percentage change from 2011-2012 to 2017** |
| **Sex-adjusted** | **Fully-adjusted\*** | **Sex-adjusted** | **Fully-adjusted\*** |
| **Odds ratio** **(95% CI)** | **P-value** | **Odds ratio** **(95% CI)** | **P-value** | **Odds ratio** **(95% CI)** | **P-value** | **Odds ratio** **(95% CI)** | **P-value** |
| Trabecular area | 1.34 (0.84,2.13) | 0.216 | ***1.86 (1.04,3.34)*** | ***0.038*** | 0.82 (0.57,1.17) | 0.265 | 0.81 (0.55,1.21) | 0.305 |
| Total volumetric bone density | 0.75 (0.51,1.11) | 0.152 | 0.70 (0.44,1.12) | 0.141 | 0.98 (0.69,1.39) | 0.902 | 0.97 (0.65,1.43) | 0.860 |
| Trabecular density | 0.85 (0.58,1.26) | 0.426 | 0.78 (0.49,1.23) | 0.284 | 0.69 (0.48,1.00) | 0.052 | 0.67 (0.44,1.01) | 0.059 |
| Trabecular number | 0.94 (0.65,1.36) | 0.746 | 0.93 (0.60,1.44) | 0.753 | 0.83 (0.58,1.18) | 0.293 | 0.77 (0.52,1.15) | 0.205 |
| Trabecular thickness | 0.82 (0.56,1.20) | 0.306 | 0.73 (0.47,1.13) | 0.161 | 1.02 (0.72,1.45) | 0.900 | 1.08 (0.73,1.58) | 0.707 |
| Trabecular separation | 1.11 (0.76,1.63) | 0.577 | 1.14 (0.73,1.77) | 0.576 | 1.22 (0.85,1.74) | 0.276 | 1.31 (0.87,1.96) | 0.195 |
| Cortical area | 0.78 (0.47,1.30) | 0.342 | 1.11 (0.61,2.03) | 0.739 | 1.07 (0.75,1.52) | 0.703 | 1.01 (0.67,1.50) | 0.971 |
| Cortical density | 0.78 (0.54,1.13) | 0.184 | 0.78 (0.50,1.21) | 0.260 | 1.04 (0.73,1.47) | 0.842 | 1.05 (0.71,1.57) | 0.800 |
| Cortical porosity | 0.94 (0.66,1.35) | 0.746 | 0.92 (0.60,1.40) | 0.685 | 0.93 (0.65,1.31) | 0.668 | 0.94 (0.63,1.42) | 0.780 |
| Cortical thickness | 0.71 (0.47,1.07) | 0.104 | 0.78 (0.48,1.26) | 0.308 | 1.02 (0.72,1.45) | 0.908 | 0.93 (0.62,1.39) | 0.733 |
| Cortical pore diameter | ***0.63 (0.44,0.92)*** | ***0.015*** | ***0.52 (0.34,0.80)*** | ***0.003*** | 1.20 (0.84,1.73) | 0.318 | 1.40 (0.92,2.11) | 0.116 |
|  |  |  |  |  |  |  |  |  |
| HR-pQCT: High resolution peripheral quantitative computed tomography; CI: Confidence interval |
| \*Adjusted for sex, age, height, BMI, dietary calcium, physical activity, smoking history (ever vs never), alcohol consumption and social class  |
| Odds ratio of less than one for annual percentage change in parameter shows that reduced declines are related to lower risk of previous fracture |
| Significant associations (p<0.05) are given in italics |

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| --- | --- |
| **Supplementary Table C: Pearson correlations between tibial HR-pQCT parameters associated with previous fracture** |  |
|  |  |
| **Tibial HR-pQCT parameters** | Trabecular area | Total volumetric bone density | Cortical area | Cortical density | Cortical thickness |
| Total volumetric bone density | -0.31\*\*\* |  |  |  |  |
| Cortical area | 0.08 | 0.76\*\*\* |  |  |  |
| Cortical density  | -0.12\* | 0.70\*\*\* | 0.73\*\*\* |  |  |
| Cortical thickness | -0.24\*\*\* | 0.83\*\*\* | 0.92\*\*\* | 0.73\*\*\* |  |
| Trabecular density† | -0.13\* | 0.23\*\*\* | 0.22\*\*\* | 0.20\*\*\* | 0.27\*\*\* |
| *\*\*\* p<0.01, \*\* p<0.05, \* p<0.1* |  |  |  |  |  |
| †Correlations presented for annual percentage changes in trabecular density; correlations presented for baseline values for all other parameters |

| **Supplementary Table D: Selected loci in relation to baseline values and annual percentage changes in tibial and radial HR-pQCT parameters** |  |
| --- | --- |
|  |  |  |  |  |  |  |  |  |
| **HR-pQCT parameter (SD)** | **rs1053051 (TMEM263): per extra T allele** | **rs7812088 (ABCF2): AG compared to GG** | **rs10226308 (TXNDC3): per extra G allele** | **rs3801387 (WNT16): per extra G allele** |
|
| **Estimate (95% CI)** | **P-value** | **Estimate (95% CI)** | **P-value** | **Estimate (95% CI)** | **P-value** | **Estimate (95% CI)** | **P-value** |
| **Tibial parameters (baseline values)** |  |  |  |  |  |  |  |
| Trabecular area | -0.07 (-0.24,0.10) | 0.426 | -0.28 (-0.59,0.03) | 0.072 | 0.07 (-0.17,0.30) | 0.584 | -0.02 (-0.23,0.18) | 0.810 |
| Total volumetric bone density | 0.13 (-0.03,0.30) | 0.112 | 0.18 (-0.13,0.48) | 0.251 | 0.02 (-0.21,0.25) | 0.856 | 0.03 (-0.17,0.23) | 0.767 |
| Trabecular density | 0.13 (-0.05,0.31) | 0.147 | 0.10 (-0.22,0.43) | 0.520 | 0.11 (-0.14,0.35) | 0.388 | 0.03 (-0.18,0.25) | 0.760 |
| Trabecular number | 0.00 (-0.17,0.18) | 0.958 | -0.10 (-0.42,0.22) | 0.533 | ***0.28 (0.04,0.53)*** | ***0.022*** | 0.03 (-0.18,0.24) | 0.758 |
| Trabecular thickness | 0.15 (-0.03,0.34) | 0.109 | 0.21 (-0.13,0.55) | 0.232 | -0.08 (-0.34,0.18) | 0.541 | 0.01 (-0.21,0.24) | 0.927 |
| Trabecular separation | -0.03 (-0.21,0.15) | 0.728 | 0.05 (-0.27,0.36) | 0.781 | ***-0.26 (-0.51,-0.02)*** | ***0.032*** | -0.05 (-0.26,0.16) | 0.666 |
| Cortical area | 0.06 (-0.07,0.18) | 0.385 | 0.10 (-0.13,0.33) | 0.384 | -0.03 (-0.21,0.15) | 0.734 | 0.01 (-0.14,0.16) | 0.879 |
| Cortical density | 0.04 (-0.12,0.20) | 0.636 | 0.00 (-0.28,0.29) | 0.974 | 0.06 (-0.15,0.28) | 0.560 | 0.03 (-0.16,0.21) | 0.784 |
| Cortical porosity | 0.07 (-0.11,0.25) | 0.449 | 0.16 (-0.17,0.48) | 0.353 | -0.04 (-0.29,0.22) | 0.766 | -0.18 (-0.40,0.04) | 0.104 |
| Cortical thickness | 0.09 (-0.06,0.24) | 0.244 | 0.21 (-0.07,0.49) | 0.136 | -0.06 (-0.27,0.15) | 0.571 | 0.01 (-0.17,0.20) | 0.874 |
| Cortical pore diameter | 0.10 (-0.09,0.28) | 0.290 | 0.28 (-0.05,0.61) | 0.095 | -0.02 (-0.27,0.24) | 0.892 | ***-0.23 (-0.44,-0.01)*** | ***0.042*** |
|  |  |  |  |  |  |  |  |  |
| **Tibial parameters (annual percentage changes)** |  |  |  |  |  |  |  |
| Trabecular area | -0.01 (-0.19,0.17) | 0.925 | 0.09 (-0.24,0.42) | 0.588 | -0.00 (-0.26,0.25) | 0.995 | -0.05 (-0.27,0.17) | 0.664 |
| Total volumetric bone density | 0.08 (-0.10,0.26) | 0.394 | -0.16 (-0.49,0.17) | 0.329 | 0.09 (-0.16,0.35) | 0.475 | -0.12 (-0.34,0.10) | 0.284 |
| Trabecular density | 0.08 (-0.11,0.26) | 0.397 | -0.10 (-0.43,0.23) | 0.543 | 0.22 (-0.04,0.47) | 0.092 | ***-0.28 (-0.50,-0.07)*** | ***0.011*** |
| Trabecular number | -0.17 (-0.35,0.02) | 0.076 | 0.13 (-0.21,0.47) | 0.447 | 0.04 (-0.22,0.30) | 0.785 | -0.14 (-0.36,0.09) | 0.233 |
| Trabecular thickness | ***0.19 (0.01,0.38)*** | ***0.044*** | -0.19 (-0.53,0.15) | 0.262 | 0.02 (-0.24,0.28) | 0.903 | 0.05 (-0.17,0.28) | 0.657 |
| Trabecular separation | 0.17 (-0.02,0.35) | 0.080 | -0.12 (-0.46,0.22) | 0.504 | -0.05 (-0.31,0.21) | 0.705 | 0.15 (-0.07,0.37) | 0.189 |
| Cortical area | -0.08 (-0.26,0.10) | 0.398 | -0.11 (-0.44,0.22) | 0.520 | -0.06 (-0.32,0.19) | 0.630 | 0.01 (-0.21,0.22) | 0.964 |
| Cortical density | 0.15 (-0.03,0.34) | 0.110 | 0.01 (-0.33,0.35) | 0.945 | 0.14 (-0.12,0.39) | 0.298 | -0.11 (-0.33,0.12) | 0.347 |
| Cortical porosity | ***-0.24 (-0.42,-0.06)*** | ***0.009*** | -0.02 (-0.35,0.31) | 0.895 | -0.09 (-0.34,0.16) | 0.496 | 0.05 (-0.17,0.26) | 0.653 |
| Cortical thickness | -0.13 (-0.31,0.06) | 0.175 | -0.10 (-0.43,0.24) | 0.566 | -0.02 (-0.27,0.23) | 0.877 | -0.00 (-0.22,0.22) | 0.992 |
| Cortical pore diameter | 0.09 (-0.10,0.27) | 0.352 | -0.26 (-0.60,0.08) | 0.130 | -0.11 (-0.37,0.15) | 0.404 | 0.12 (-0.10,0.34) | 0.277 |
|  |  |  |  |  |  |  |  |  |
| **Radial parameters (baseline values)** |  |  |  |  |  |  |  |
| Trabecular area | -0.05 (-0.21,0.11) | 0.520 | ***-0.30 (-0.60,-0.01)*** | ***0.046*** | 0.17 (-0.05,0.39) | 0.134 | -0.07 (-0.27,0.12) | 0.466 |
| Total volumetric bone density | ***0.25 (0.08,0.42)*** | ***0.005*** | 0.24 (-0.09,0.57) | 0.157 | -0.21 (-0.45,0.03) | 0.089 | 0.18 (-0.04,0.39) | 0.108 |
| Trabecular density | ***0.23 (0.06,0.40)*** | ***0.007*** | -0.06 (-0.38,0.26) | 0.725 | -0.07 (-0.31,0.17) | 0.554 | 0.15 (-0.06,0.36) | 0.164 |
| Trabecular number | 0.09 (-0.09,0.27) | 0.339 | -0.09 (-0.43,0.25) | 0.608 | 0.08 (-0.18,0.33) | 0.555 | 0.08 (-0.14,0.31) | 0.458 |
| Trabecular thickness | ***0.30 (0.12,0.48)*** | ***0.001*** | -0.01 (-0.35,0.33) | 0.948 | -0.15 (-0.41,0.10) | 0.235 | 0.15 (-0.08,0.37) | 0.197 |
| Trabecular separation | -0.14 (-0.31,0.04) | 0.129 | 0.09 (-0.25,0.42) | 0.609 | -0.03 (-0.28,0.21) | 0.785 | -0.09 (-0.31,0.13) | 0.403 |
| Cortical area | ***0.16 (0.03,0.29)*** | ***0.016*** | 0.16 (-0.08,0.41) | 0.196 | -0.13 (-0.31,0.05) | 0.160 | 0.04 (-0.12,0.21) | 0.613 |
| Cortical density | ***0.19 (0.01,0.38)*** | ***0.043*** | ***0.36 (0.01,0.71)*** | ***0.041*** | -0.16 (-0.42,0.10) | 0.213 | 0.12 (-0.11,0.35) | 0.320 |
| Cortical porosity | 0.06 (-0.14,0.25) | 0.571 | -0.25 (-0.61,0.11) | 0.169 | -0.09 (-0.36,0.18) | 0.505 | -0.08 (-0.32,0.16) | 0.510 |
| Cortical thickness | ***0.19 (0.02,0.36)*** | ***0.028*** | 0.26 (-0.06,0.57) | 0.106 | -0.21 (-0.44,0.02) | 0.079 | 0.08 (-0.12,0.29) | 0.429 |
| Cortical pore diameter | 0.07 (-0.13,0.27) | 0.516 | -0.09 (-0.46,0.29) | 0.647 | -0.15 (-0.43,0.12) | 0.280 | ***-0.26 (-0.50,-0.01)*** | ***0.039*** |
|  |  |  |  |  |  |  |  |  |
| **Radial parameters (annual percentage changes)** |  |  |  |  |  |  |  |
| Trabecular area | -0.08 (-0.28,0.11) | 0.394 | -0.12 (-0.49,0.25) | 0.513 | -0.07 (-0.34,0.20) | 0.615 | -0.18 (-0.42,0.06) | 0.149 |
| Total volumetric bone density | 0.07 (-0.13,0.27) | 0.517 | 0.10 (-0.28,0.49) | 0.596 | 0.04 (-0.24,0.32) | 0.784 | 0.17 (-0.08,0.42) | 0.187 |
| Trabecular density | -0.02 (-0.22,0.19) | 0.882 | 0.00 (-0.38,0.39) | 0.981 | -0.05 (-0.33,0.23) | 0.714 | -0.10 (-0.35,0.15) | 0.440 |
| Trabecular number | -0.08 (-0.28,0.12) | 0.415 | -0.16 (-0.54,0.21) | 0.389 | 0.09 (-0.18,0.37) | 0.504 | -0.20 (-0.44,0.05) | 0.113 |
| Trabecular thickness | 0.11 (-0.09,0.30) | 0.286 | 0.19 (-0.19,0.56) | 0.322 | -0.14 (-0.41,0.13) | 0.299 | 0.21 (-0.03,0.45) | 0.089 |
| Trabecular separation | 0.09 (-0.11,0.29) | 0.357 | 0.16 (-0.22,0.54) | 0.410 | -0.09 (-0.37,0.18) | 0.513 | 0.20 (-0.04,0.45) | 0.103 |
| Cortical area | 0.05 (-0.15,0.25) | 0.649 | -0.08 (-0.47,0.30) | 0.666 | 0.08 (-0.20,0.35) | 0.573 | 0.23 (-0.01,0.48) | 0.064 |
| Cortical density | 0.12 (-0.08,0.32) | 0.224 | 0.35 (-0.03,0.73) | 0.070 | 0.12 (-0.16,0.39) | 0.406 | 0.19 (-0.05,0.44) | 0.121 |
| Cortical porosity | -0.06 (-0.26,0.14) | 0.548 | -0.10 (-0.49,0.29) | 0.614 | 0.03 (-0.25,0.32) | 0.809 | -0.11 (-0.36,0.14) | 0.395 |
| Cortical thickness | 0.07 (-0.12,0.27) | 0.456 | -0.14 (-0.52,0.24) | 0.473 | 0.02 (-0.25,0.30) | 0.873 | 0.21 (-0.04,0.45) | 0.096 |
| Cortical pore diameter | 0.04 (-0.16,0.24) | 0.686 | 0.32 (-0.07,0.70) | 0.107 | -0.01 (-0.28,0.27) | 0.968 | 0.14 (-0.11,0.39) | 0.265 |

Estimates shown are standard deviation differences in HR-pQCT parameters according to each loci

Associations were adjusted for age and sex

Significant associations (p<0.05) are given in italics