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SHORT COMMUNICATION

Genetic factors influencing bone mineral content in a black South African population

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Abstract Bone mass differs according to ethnic classification, with individuals of African ancestry attaining the highest measurements across numerous skeletal sites. Elevated bone mass is even maintained in those individuals exposed to adverse environmental factors, suggesting a prominent genetic effect that may have clinical or therapeutic value. Using a candidate gene approach, we investigated associations of six candidate genes (*ESR1, TNFRSF11A, TNFRSF11B, TNFSF11, SOST* and *SPP1*) with bone mass at the hip and lumbar spine amongst pre-pubertal black South African children (mean age 10.6 years) who formed part of the longitudinal Birth to Twenty cohort. 151 black children were genotyped at 366 polymorphic loci, including 112 previously associated and 254 tagging single nucleotide polymorphisms (SNPs). Linear regression was used to highlight significant associations whilst adjusting for height, weight, sex and bone area. Twenty-seven markers (8 previously associated and 19 tag SNPs; $P < 0.05$) were found to be associated with either femoral neck (18) or lumbar spine (9) bone mineral content. These signals were derived from three genes, namely *ESR1* (17), *TNFRSF11B* (9) and *SPP1* (1). One marker (rs2485209) maintained its association with the femoral neck after correction for multiple testing ($P = 0.038$). When compared to results amongst Caucasian adults, we detected differences with respect to associated skeletal sites. Allele frequencies and linkage disequilibrium patterns were also significantly different between populations. Hence, our results support the existence of a strong genetic effect acting at the femoral neck in black South African children, whilst simultaneously highlighting possible causes that account for inter-ethnic bone mass diversity.

Keywords Bone mineral content · Genetics · African · *ESR1* · Birth to Twenty

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Introduction

Individuals of African ancestry attain a higher average bone mass (assessed as either bone mineral content [BMC] or bone mineral density [BMD]) compared to other ethnic groups, across numerous skeletal sites, specifically when corrected for covariates [1–3]. This has been documented both in admixed populations of African Americans and Tobagonian men [4], for example, as well as amongst non-admixed populations still resident on the African continent such as Somali women [5] and black South Africans [6]. This greater bone mass appears to be established from early childhood [7], implying an important genetic influence that is in line with strong heritability estimates for BMD (60–90 % in twin studies) [8]. Moreover, evidence suggests that elevated bone mass in individuals of African descent is maintained in spite of often numerous adverse environmental factors. For example, African-American women maintain greater bone mass than white women

despite a lower mean 25-hydroxyvitamin D concentration [9, 10]. Similarly, data from the National Health and Nutrition Examination Surveys (NHANES) demonstrated that non-Hispanic blacks had lower calcium intakes than their white and Hispanic counterparts [11], likely due to the higher frequency of lactose intolerance seen amongst black populations [9, 12]. Amongst a cohort of black South African children, participating in the Birth to Twenty (Bt20) longitudinal study, bone mass at the femoral neck (FN) was higher than in white children despite the former having a lower socioeconomic status [6], lower calcium and vitamin D intakes [13], unfavourable anthropometric measurements [2, 6, 14] and lower levels of physical activity [15]. Unravelling the genetic effects responsible for this phenomenon may yield important insights into bone biology, with possible clinical or therapeutic value.

To date, genetic investigations of bone health have rarely incorporated children [16]. Attention has usually been focused on adults, in an effort to identify susceptibility variants for osteoporosis [17–19], however, these studies tend to be complicated by environmental covariates that potentially mask associations between genotype and bone mass. Some have argued that such associations would be easier to detect during childhood, when genetic regulation of bone is at its peak and environmental influences have had limited time to exert their effects [16].

The aim of this study was to investigate possible genetic factors contributing to bone health in black South African children with a mean age of 10.6 years. With little previous genetic research for non-admixed Africans, we sought to interrogate six leading candidate genes known to influence BMD based on numerous independent studies amongst individuals of European descent. Specifically, we selected six of nine candidate genes that maintained significant association to either lumbar spine (LS) or FN BMD at a meta-analytical level, according to Richards and colleagues [20]. These included *ESR1* (oestrogen receptor α), *TNFRSF11B* (osteoprotegerin), *TNFRSF11A* (RANK), *TNFSF11* (RANKL), *SPP1* (osteopontin) and *SOST* (sclerostin). We hypothesised that whilst some single nucleotide polymorphic (SNP) markers in these genes might share significant association to bone mass in both Europeans and Africans, unique association signals may be detected in our non-admixed African sample. Such signals might offer potential clues to the elevated bone mass within this ethnic group, in addition to further highlighting the differences in genetic architecture when compared to Europeans.

Materials and methods

Subjects

Study participants included 151 black children from the Bone Health sub-cohort ($n = 682$) of the larger longitudinal Birth to Twenty cohort in Soweto, South Africa.[1] Briefly, the Birth to Twenty cohort was designed to track the growth and development of a sample of urban children across their first 20 years of life. Neonates, totalling 3273, were recruited from public sector hospitals in a 6-week period from April 23 to June 8, 1990, and were assessed annually thereafter. Numerous environmental and physiological variables were recorded during each year. The Bone Health sub-cohort was established 9 years into the study, and selected members were additionally required to undergo regular dual X-ray absorptiometry (DXA) scans to track bone mass [14]. Enrolment into the sub-cohort was based on the exclusion criteria of any pre-existing conditions that would impact on BMC, including rickets, tuberculosis, cancer, a history of endocrine disease, gastrointestinal disease associated with malabsorption, and regular medication use, specifically corticosteroids, anti-epileptic drugs, calcium and/or vitamin D supplementation. Since participants were recruited from the Soweto-Johannesburg metropolitan area, black participants were all south-eastern Bantu-speakers, belonging to the 'S' group according to Guthrie classification [21]. A previous study in which 18 ancestry informative markers were genotyped in 990 black Bt20 participants confirmed that no high-level population sub-structure was evident. Although numerous measurements were available, this study only used bone phenotypic data recorded at a mean age of 10.6 years in order to avoid complications posed by puberty, such as rapid growth and bone remodelling that hinders direct comparison between individuals [16].

Informed consent from a legal guardian was obtained prior to enrolment into the study. Ethical approval for this study was granted by the University of the Witwatersrand Human Research Ethics Committee (Medical); certificate number M10457.

Anthropometry

Measurements of height, to the last completed 1 mm, were taken using a wall-mounted stadiometer (Holtain, Crosswell, UK), whilst weight, to the nearest completed 0.1 kg, was recorded using a digital electronic instrument (Dismed, Halfway House, South Africa) based on standardized protocols [22]. Both instruments were regularly calibrated, and subjects wore minimal clothing when being weighed.

Measurement of bone

Measurements of bone area and mineral content at the hip (FN) and L1 to L4 vertebrae (LS) were performed using the

[1] Note that for the purposes of this study, 'black' is used to define individuals of African ancestry without known admixture, whilst 'white' denotes individuals of European descent.

Hologic QDR-4500A DXA machine (Bedford, MA, USA). Measurements of BMC were assessed and not BMD, as this has been found to be an inappropriate metric for children [23]. Using a spinal phantom, the coefficient of variation was calculated as 0.44 % for BMC (g) and 0.36 % for bone area (cm^2). A single technician conducted all readings to avoid an additional source of variation. Participants were wearing only light clothing at the time of measurement.

Sampling of blood and DNA extraction

DNA was extracted from a 5 ml sample of whole blood using the salting out procedure [24], and stored in Tris/EDTA (TE) buffer at 4 °C. Sample concentrations were measured using the Tecan Infinite® 200 PRO NanoQuant (Tecan Group Ltd., Männedorf, Switzerland) and normalized to 50 ng/µl for the purposes of this study.

SNP selection

Candidate genes were selected based on a recent meta-analysis conducted by the Genetic Factors for Osteoporosis (GEFOS) consortium [20]. Six genes strongly linked to either LS or FN BMD were selected for validation amongst black South Africans, namely *ESR1*, *TNFRSF11A*, *TNFRSF11B*, *TNFSF11*, *SOST* and *SPP1*. A total of 189 SNPs, spanning all six genes, had been found to generate strong association signals amongst Europeans ($P < 2.39 \times 10^{-6}$). Additionally, a panel of tag SNPs was selected for the purposes of identifying association signals that may be unique to this population, but only within genes *ESR1, TNFRSF11A, TNFRSF11B,* and *TNFSF11* due to kit size limitations. Tagger [25] was used to identify tag SNPs through a multi-marker tagging approach with a minor allele frequency of 1 % and an r^2 cutoff of 0.8, among publicly available African data (Yoruba) from the HapMap data set (Release 27, Phase II + III, February 2009, on NCBI B36 assembly, dbSNP b126).

All SNPs were assessed by the Illumina Assay Design Tool for compatibility with the GoldenGate genotyping assay. Low scoring SNPs (<0.6 on a scale of 0–1) were excluded. The final SNP panel consisted of 112 previously associated and 254 tag SNPs, which provided a coverage range of 90–96 % in genes *ESR1, TNFRSF11A, TNFRSF11B,* and *TNFSF11*. Information on these SNPs, including reference allele designation and allele frequency amongst different populations was retrieved using HapMart, an extension of BioMart [26].

Genotyping

Genotyping was performed using the GoldenGate genotyping assay with VeraCode microbeads on the Illumina BeadXpress™ platform (Illumina, USA) as described elsewhere [27].

Quality control

Raw data were examined using the genotyping module of Beadstudio (Framework version 3.1.3.0; module version 3.2.32). Illumina designed built-in assay controls were used as a measure of result quality and samples failing more than two such controls (out of five) were excluded. In terms of SNP performance, only SNPs with a Hardy–Weinberg equilibrium (HWE) P value >0.05 were analysed further. The genotype calls for the remaining SNPs and samples were individually assessed and ambiguous calls that could not be clarified were removed.

Data analysis

SAS Enterprise Guide version 4.2 (SAS Institute Inc., Cary, NC, USA) was used to assess the descriptive statistics of the cohort. Normality of the distribution for measured parameters was tested using a Chi-squared test for goodness-of-fit. Where a normal distribution was applicable, a two-sample t test was conducted in order to detect differences between participants, sub-divided by gender. In all other instances, a Wilcoxon–Mann–Whitney test was used to account for skew distributions. To compare allele frequencies between populations, a contingency table test was performed. Quanto (version 1.2.4) [28] was used to determine the power of the study.

gPLINK software (version 2.050) [29] was used to perform a linear regression analysis in order to test for associations between BMC and genotype, using an additive genetic model. Sex, height, weight and bone area were entered as covariates, and a label-swapping permutation procedure ($n = 1000$) was used to correct for multiple testing. A significance level threshold of 5 % ($\alpha = 0.05$) was adopted for all tests, and P values were rounded-off to three decimal places.

To further visualise the results for *ESR1*, LocusZoom (version 1.1) was used [30] (http://csg.sph.umich.edu/locuszoom/). These plots are based on data from the International HapMap Project (Phase II data), the 1000 Genomes Project (August 2009 release), and recent builds of both dbSNP (135) and the UCSC genome browser (GRCh37/hg19), where applicable.

Results

Descriptive statistics

Table 1 shows the descriptive statistics for the study participants ($n = 151$). Values for our sample were not significantly different from those reported elsewhere [6] for the larger bone health sub-cohort of the Bt20 study. When

Table 1 Descriptive statistics for black South African children

	Black boys ($n = 80$)	Black girls ($n = 71$)	P value
Age (years ± SD)	10.53 ± 0.30	10.55 ± 0.30	0.870
Height (cm ± SD)	136.9 ± 6.24	138.8 ± 5.99	0.072
Weight (kg ± SD)	31.74 ± 5.89	34.69 ± 8.17	**0.010**
FN BMC (g)	3.05 ± 0.36	2.77 ± 0.42	**<0.001**
FN bone area (cm^2)	4.08 ± 0.31	4.01 ± 0.28	0.168
LS BMC (g)	23.46 ± 3.79	25.72 ± 5.64	**0.010**
LS bone area (cm^2)	42.64 ± 4.17	42.95 ± 4.24	0.760

Bone measurements are unadjusted for covariates

Bold values indicate P values that are significant at 5 % level

FN femoral neck, *LS* lumbar spine, *SD* standard deviation, *cm* centimetres, *kg* kilograms, *g* grams, cm^2 square centimetres

considering differences between sex, black boys weighed significantly less than black girls. With respect to bone measurements, boys had higher unadjusted BMC at the FN, but lower BMC at the spine than black girls, prompting the inclusion of sex as a covariate.

Genotyping

A single SNP (rs9479134) failed to reach quality thresholds and was removed from further analysis. Twenty additional SNPs were excluded as their genotype frequencies were inconsistent with HWE ($P < 0.05$). Lastly, 16 SNPs had >10 % missingness (the proportion of individuals unsuccessfully genotyped for that SNP divided by total study participants) and were also excluded. The final SNP panel for analysis consisted of 329 SNPs, out of the 366 initially chosen.

Association results

Significant associations, prior to multiple testing correction, are listed in Table 2. All reported beta values indicate the effect on BMC [measured in grams (g)] with each additional minor allele, as determined by sample allele frequencies. Thus, a positive beta value indicates that the minor allele is associated with increased BMC, whereas a negative value indicates association with decreased BMC.

Twenty-seven SNPs (8 previously associated; 19 tag) achieved pointwise estimates for association with BMC that were significant at the 5 % level, prior to correction. Nine SNPs were linked to LS BMC and 18 to FN BMC, but no marker achieved significance for both skeletal sites. The majority of the associated SNPs were located in either *ESR1* (17) or *TNFRSF11B* (9), with one signal deriving from a SNP in *SPP1*. After adjusting P values for multiple testing, only one SNP marker (*ESR1,* rs2485209) maintained significance at the 5 % level. This marker had been previously associated to BMD at the LS amongst Caucasian adults [20]. Given the allele frequency of rs2485209 within this sample, we were 56 % powered to detect the effect of this SNP, based on a calculation using Quanto.

Allele frequencies of all significantly associated SNPs were contrasted across several populations (Table 2). The minor allele for rs2485209 is relatively common (>40 %) in all populations with the exception of the Yoruba population ($P < 0.001$), showing a highly significant difference between the two African populations. The potential functional effects of the SNPs were checked using the UCSC human genome browser (Feb. 2009, GRCh37/hg19 assembly); however, no notable effects were documented at the time of the study.

ESR1

Due to the number of positive associations, a closer inspection of *ESR1* was conducted. A LocusZoom plot showing association scores with FN BMC is displayed in Fig. 1, with r^2 measures of linkage disequilibrium (LD) calculated based on either Yoruba (YRI) or European (CEU) HapMap population data. rs2485209 is located towards the 5′ end of the gene, within the 150 kb region upstream of the first coding exon that contains numerous transcription start sites. Interestingly, this SNP shared little LD with other typed SNPs in close proximity, according to Yoruba population data. In contrast, numerous SNPs share a moderate level of LD with the marker amongst Europeans.

Discussion

Our modest exploratory study revealed a significant association between rs2485209 and adjusted FN BMC in black South African children. This marker resides within *ESR1*, upstream of the first coding exon, and was previously detected to influence BMD in adult Caucasians, although at a different skeletal site (LS). The allele associated with higher BMC had comparable frequencies between the current sample and CEU (Utah residents with Northern and Western European ancestry), CHB (Han Chinese from Beijing, China) and JPT (Japanese from Tokyo, Japan) individuals, but notably, it varied across African populations (0.26–0.44; see Table 2). Limited LD between rs2485209 and surrounding markers was observed in an African population (Yoruba, from Nigeria), but appeared more extensive amongst the European population. A total of 26 additional SNPs showed promising association with either FN or LS BMC, prior to correction, and these

Table 2 List of SNP markers generating significant association signals with bone mineral content in black SA children

Gene	SNP ID	Alleles A1[a]/A2	MAF (sample)	*P* value[b] (FN BMC)	*P* Value[b] (LS BMC)	Beta (effect size[c])	Published data (A1 allele frequencies)					
							YRI[d]	CEU[d]	CHB	JPT	LWK	MKK
ESR1	rs11155823	T/G	0.16	**0.032**	0.469	−0.100	0.06**	0**	0.10	0.09		
	rs2485209	C/A	0.44	**0.002**[e]	0.717	0.112	0.26**	0.41	0.42	0.43	0.36	0.25
	rs2504069	G/A	0.23	0.710	**0.028**	0.864	0.20	0.29	0.07	0.06		
	rs2982551	C/A	0.42	0.398	**0.037**	−0.677	0.31**	0.40	0.80	0.82	0.43	0.37
	rs3020377	A/G	0.15	**0.041**	0.401	0.085	0.23	0.70**	0.19	0.21		
	rs3020396	A/G	0.27	**0.013**	0.790	0.085	0.20	0.74**	0.38	0.36	0.29	0.41
	rs4458702	A/G	0.10	**0.037**	0.258	−0.129	0.05	0.20**	0.45	0.47	0.07	0.05
	rs532010	C/T	0.46	**0.033**	0.105	−0.065	0.44	0.36*	0.39	0.36	0.33	0.45
	rs6910500	A/G	0.07	**0.005**	0.939	0.174	0.06	0.02*	0	0		
	rs7744006	A/G	0.13	0.917	**0.042**	−0.978	0.22*	0**	0	0		
	rs9322332	C/A	0.45	**0.024**	0.337	−0.073	0.64**	0.60**	0.61	0.58		
	rs9340785	C/G	0.05	**0.032**	0.962	−0.161	0.12*	0**	0	0		
	rs9340820	G/T	0.16	**0.019**	0.955	0.095	0.17	0.02**	0.29	0.32	0.28	0.19
	rs9340939	A/C	0.13	0.700	**0.043**	−0.981	0.22*	0.01**	0.03	0	0.16	0.03
	rs9371234	A/C	0.17	**0.030**	0.451	−0.087	0.10	0**	0	0.06		
	rs9383598	T/C	0.14	**0.046**	0.791	−0.102	0.06**	0**	0.08	0.08	0.04	0.06
	rs9397453	A/G	0.22	**0.034**	0.406	−0.080	0.08**	0**	0.08	0.08	0.10	0.12
SPP1	rs2869722	G/A	0.15	**0.030**	0.446	0.101	0.12	0.33**	0.26	0.23		
TNFRSF11B	rs1032129	C/A	0.33	0.466	**0.050**	0.712	0.42	0.32	0.57	0.65		
	rs11573938	A/G	0.02	**0.022**	0.886	0.317	0.06*	0.14**	0.06	0	0.04	0.07
	rs1804854	C/T	0.33	0.373	**0.036**	−0.761	0.28	0**	0	0	0.33	0.21
	rs4520195	G/A	0.27	0.200	**0.014**	−0.977	0.23	0**	0	0	0.28	0.11
	rs7464496	T/C	0.06	**0.046**	0.946	0.141	0.06	0.55**	0.36	0.22		
	rs882763	T/C	0.33	0.356	**0.030**	−0.790	0.29	0**	0	0	0.33	0.21
	rs2035977	G/C	0.42	**0.015**	0.100	0.088	0.44	0.57**	0.44	0.38		
	rs2101752	G/T	0.23	0.056	**0.027**	0.925	0.21	0.56**	0.36	0.25		
	rs7839059	C/A	0.33	**0.036**	0.208	0.074	0.35	0.67**	0.80	0.76		

MAF minor allele frequency, *YRI* Yoruba from Ibadan, Nigeria; *CEU* Utah residents with Northern and Western European ancestry, *CHB* Han Chinese from Beijing, China, *JPT* Japanese from Tokyo, Japan, *LWK* Luhya from Webuye, Kenya, *MKK* Maasai from Kinyawa, Kenya

Bold values indicate *P* values that are significant at 5 % level

* $P < 0.05$, ** $P < 0.01$

[a] Minor (effect) allele

[b] Listed *P* values are those prior to correction for multiple testing

[c] Effect of each added minor allele on BMC (g) at skeletal sites with significant association. Negative values indicate a decrease in bone mass, whilst positive values indicate an increase

[d] HapMap allele frequency data for these populations were compared to the current study using a contingency table test

[e] *P* value after correction was 0.038

markers may prove interesting to reassess in a better powered study. Thus, in line with our hypothesis, we have replicated an association signal derived from European research, but have also discovered important differences that hint at the different genetic regulation of bone mass in Africans and/or at different ages.

ESR1 is one of the strongest candidate genes known to influence bone mass, having been replicated in four different study designs [8]. The receptor protein product mediates the largely beneficial effects of oestrogen on the maintenance of bone mass in both men and women, as has been previously documented [31, 32]. Structurally, the gene displays a complex design [33], possessing eight coding as well as eight non-coding exons; the latter confined to a 150 kb region at the 5′ end, which also contains two variable number tandem repeats. Each of these non-coding exons is preceded by an individual promoter sequence, creating multiple transcription start sites. To

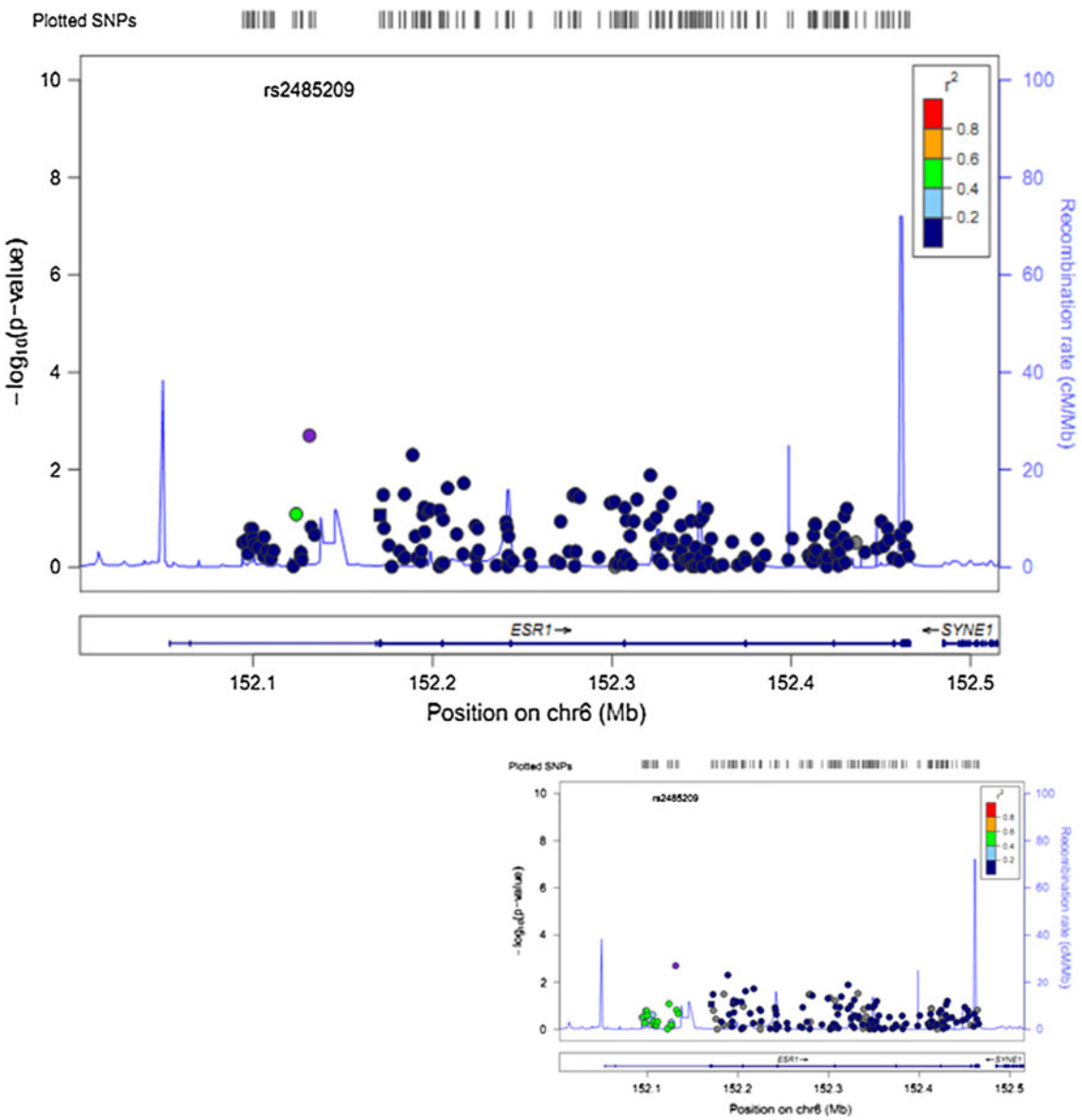

Fig. 1 LocusZoom plots for femoral neck association results within *ESR1*. The SNP marker with the leading association signal (rs2485209; $P = 0.038$ after correction for multiple testing) is coloured *purple*, with other SNP markers coloured according to the strength of r^2 linkage disequilibrium (LD) shared with the leading marker, based on Yoruba population data. rs2485209 maps to the 150 kb region upstream of the first coding exon, which contains several non-coding exons that have unique, individual promoters. Only one other marker is reported to share moderate (0.4–0.6) linkage disequilibrium with the leading SNP. The *inset* diagram shows the same results, but LD is calculated using data from European ancestry individuals. Here, rs2485209 is in moderate LD with numerous other markers. These fall between successive peaks of the blue line graph that measure, on the second *y*-axis, the recombination frequency during meiosis (colour figure online)

date, *ESR1* is known to encode 17 different transcripts (Ensembl release 65). Although the function of these has been difficult to investigate [34], variation near the 5′ end of *ESR1* has significant potential to impact on the expression of one or more transcripts, with downstream consequences for bone health.

Interestingly, rs2485209 is located within the 150 kb stretch of non-coding exons and their individual promoters. With links to different skeletal sites between our sample of black children (FN) and Caucasian adults (LS), there exists the possibility of two separate causal variants being tagged by this SNP. LD differences between Yoruba and European populations lend further support to this line of reasoning (Fig. 1). Although experimental confirmation is required, we speculate that this region of the gene may, in general, offer valuable insight into observed differences in inter-ethnic bone mass, and should be prioritised in future research. Discrepancies in associated skeletal sites might

also speak to genetic differences in bone regulation between childhood and adulthood, and/or between different skeletal sites, with some having argued that gene sets responsible for bone maintenance may differ by site and with age [35, 36].

Although failing to maintain significance, we briefly consider the 26 other markers that were associated to BMC prior to correction, as the false negative rate is suspected to be high given a small sample size. Amongst these markers are seven other previously associated SNPs, based on the GEFOS meta-analysis (Table 3). In addition to rs2485209, three of these seven tended towards an association with a different skeletal site than in Caucasian adults. Furthermore, five SNPs displayed statistically significant allele frequency differences between our sample and European individuals including rs2869722, rs7464496, rs2035877, rs2101752 and rs7939059 (see Table 2). Effect size comparisons were not made, as the unit of measurement differed between studies (BMC versus BMD) as well as the average age of participants (10.6 years versus >49 years). Of the 19 remaining markers, these appeared to strengthen the importance of *ESR1* and *TNFRSF11B* over other candidate genes, within this sample. Similarly, associations appeared more often at the FN; the skeletal site at which bone mass differences appear to be greatest between black and white South Africans [37, 38].

Several important limitations of this study need to be borne in mind. Most importantly, we lacked a suitable comparison for our study. To our knowledge, the only genome-wide publication on genetic contributions to bone phenotypes in 10-year-old children was that of Duren and colleagues [39]. They performed a search for loci linked to metacarpal thickness in 10-year-old children participating in the Fels Longitudinal Study. Although age-matched, the study investigated both a different phenotype and skeletal site, and the subsequent association signals did not map to the vicinity of *ESR1*. Thus, without sufficient research on the genetic mechanisms underpinning childhood bone health, we cannot fully clarify whether our results speak to the genetic factors that are important for Africans, or for children. Furthermore, the limited sample size meant we were substantively underpowered. BMC measurements were not all obtained within the same season, and thus may be subject to seasonal variances [40], although this was not suspected to be a major concern based on previous research [41]. Of the 37 SNPs failing quality control 27 were tag SNPs, causing a loss of coverage in genes *ESR1, TNFRSF11B, TNFRSF11A* and *TNFSF11*. The remaining ten markers were previously associated SNPs that would need to be retested to ascertain their validity to this population. We only tested for associations under an additive model, possibly missing out on dominant and recessive effects. Lastly, there is a current lack of consensus on the best method to correct BMC for covariates, with each corrective method producing different results [6, 42].

Based on our modest yet promising results, future genetic research into the bone mass of individuals of African ancestry should be encouraged. Results from a larger sample size may afford insight into a number of smaller effect sizes across a broader range of genes, as well as possible sex-specific effects [43, 44], which we were unable to investigate here. Moreover, evidence may accumulate to support the theory of a genetic 'set-point' for bone mass [45, 46], which is currently a highly feasible explanation for the bone mass observed in black Bt20 members, despite so many adverse environmental effects. Replication studies amongst separate African population groups, however, should also be prioritised to help validate findings and investigate possible local, population-specific, environmental factors [47].

In conclusion, we have provided the first glimpse into the genetic factors regulating the elevated bone mass of African individuals. These results support the importance of both *ESR1* and *TNFRSF11B* as candidate genes that

Table 3 Comparison between present study results and those from the GEFOS meta-analysis

Gene	SNP ID	South Africa			GEFOS meta-analysis [20]		
		Allele	*P* value	Skeletal site	Allele	*P* value[a]	Skeletal site
ESR1	rs2485209	C	0.002	FN	C	3.20E−07	LS
	rs2504069	G	0.028	LS	G	1.10E−08	LS
	rs2982551	C	0.037	LS	C	2.10E−09	LS
SPP1	rs2869722	G	0.030	FN	G	9.60E−07	LS
TNFRSF11B	rs7464496	T	0.046	FN	C	1.70E−09	LS
	rs2035977	G	0.015	FN	C	6.40E−08	LS
	rs7839059	C	0.036	FN	A	4.50E−08	FN
	rs2101752	G	0.027	LS	T	5.00E−08	LS

FN femoral neck, *LS* lumbar spine

[a] *P* value determined under a fixed effects model

influence bone mass. However, SNP allele frequency differences, LD dissimilarities and discordance of associated skeletal sites provide important signs of the influence genetic variation may have in creating bone mass diversity, specifically between Europeans and Africans.

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Conflict of interest None.

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