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# Identification of a novel locus on chromosome 2q13 which predisposes to clinical vertebral fractures independently of bone density

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1           **Identification of a novel locus on chromosome 2q13 which predisposes**  
2           **to clinical vertebral fractures independently of bone density**

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99

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101 **ABSTRACT**

102 **Objectives:** To identify genetic determinants of susceptibility to clinical vertebral fractures, an  
103 important complication of osteoporosis. **Methods:** Here we conduct a genome-wide  
104 association study in 1,553 postmenopausal women with clinical vertebral fractures and 4,340  
105 controls, with a 2-stage replication involving 1,028 cases and 3,762 controls. Potentially causal  
106 variants were identified using eQTL data from transiliac bone biopsies and bioinformatic  
107 studies. **Results:** A locus tagged by rs10190845 was identified on chromosome 2q13 which  
108 was significantly associated with clinical vertebral fracture ( $p=1.04 \times 10^{-9}$ ) with a large effect  
109 size (odds ratio 1.74, 95% CI 1.06 – 2.6). Bioinformatic analysis of this locus identified several  
110 potentially functional SNPs which are associated with expression of the positional candidate  
111 genes *TTL* (Tubulin Tyrosine Ligase) and *SLC20A1* (Solute Carrier Family 20 Member 1).  
112 Three other suggestive loci were identified on chromosomes 1p31, 11q12 and 15q11. All these  
113 loci were novel and had not previously been associated with BMD or clinical fractures.  
114 **Conclusion:** We have identified a novel genetic variant that is associated with clinical vertebral  
115 fractures by mechanisms that are independent of BMD. Further studies are now in progress to  
116 validate this association and evaluate the underlying mechanism.

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119 **KEYWORDS:** Osteoporosis, Gene polymorphism, Bone Mineral Density, *TTL*, *SLC20A1*

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135 **1. INTRODUCTION**

136 Osteoporosis is a common disease with a strong genetic component. It is characterised by low  
137 bone mineral density (BMD), deterioration in the microstructural architecture of bone and an  
138 increased risk of fragility fractures. Vertebral fractures are an early and important complication  
139 of osteoporosis.[1] They are characterised by loss of height and deformity of the affected  
140 vertebrae and associated with increased risk of other fractures.[2] It has been estimated that  
141 between 8-30% of patients with radiological evidence of vertebral fractures (so called  
142 morphometric fractures) come to medical attention for reasons that are incompletely  
143 understood.[3,4] In contrast, other patients with vertebral fractures come to medical attention  
144 because of symptoms such as back pain, kyphosis, and height loss, and are defined as having  
145 clinical vertebral fractures.[5-7] Clinical vertebral fractures are associated with a markedly  
146 increased risk of future fractures and increased mortality.[8] Major advances have been made  
147 in identifying genetic variants that regulate BMD and some variants have also been identified  
148 that predispose to non-vertebral fractures.[9-20] However, the genetic determinants of  
149 vertebral fractures are poorly understood. A previous genome-wide association study (GWAS)  
150 published by Oei and colleagues involving a discovery cohort of 8,717 cases and 21,793  
151 controls failed to identify any significant genetic predictors of radiographic vertebral fracture  
152 at a genome-wide significant level.[21] However, in this study, the vertebral fractures were  
153 defined simply on the basis of morphometric analysis of spinal radiographs. It is well  
154 recognised however that the morphometric techniques employed in this study may have  
155 identified vertebral deformities that were not fractures.[22] The aim of the present study was  
156 to re-evaluate the predictors of clinical vertebral fractures by genome wide association study  
157 to try and gain new insights into this important and poorly understood clinical problem.

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159 **2. PATIENTS AND METHODS**

160 The study involved a discovery phase with 1,553 clinical vertebral fracture cases and 4,340  
161 controls, a first replication phase of 694 cases and 2,105 controls, and a second replication  
162 phase of 334 cases and 1,657 controls, as summarised in Supplementary Table 1. The genome  
163 wide association study was performed using standard methodology as detailed in the  
164 Supplementary Text 1.

165  
166 **3. RESULTS**

167 **3.1. Characteristics of the study populations**

168 The mean ( $\pm$ standard deviation) age of the patients with clinical vertebral fractures was  
169 71.3 $\pm$ 9.3 years with a bone mineral density T-score at the lumbar spine of -2.72 $\pm$ 1.4; and at the  
170 femoral neck of -2.57 $\pm$ 1.1. The controls were not matched with the cases by age and did not  
171 undergo phenotyping for vertebral fracture on the basis that clinical vertebral fractures are  
172 uncommon in the general population (estimated incidence of 9.8/1000 person-years in 75-84  
173 year olds)[23]. While it is possible that clinical vertebral fractures may have occurred in some  
174 controls in later life this is unlikely to have substantially affected the results of the analysis,  
175 other than to have potentially slightly reduced its power.[24] This approach has been used  
176 previously for genome-wide studies in various common diseases including diabetes, Paget's  
177 disease, and rheumatoid arthritis.[25,26]

178 We identified 334 clinical vertebral fracture female cases from the UK Biobank cohort with a  
179 mean age ( $\pm$ standard deviation) of 58.8 $\pm$ 7.7 years, and they were age-matched with 1,657  
180 female controls from the same cohort.

### 181 **3.2. Genome-wide association analysis of the discovery sample**

182 Since different genotyping platforms were used in the analysis of the different cohorts that  
183 constitute the discovery sample, association analysis was conducted following imputation of  
184 all genotypes into the CEU panel of HapMap II reference (see Patients and Methods section).  
185 Following imputation, we analysed 2,366,456 SNPs and identified 31 with suggestive evidence  
186 of association with vertebral fracture ( $p \leq 10^{-4}$ ). Details are summarised in Supplementary Table  
187 2, the Manhattan and quantile-quantile plots are shown in Supplementary Figures 2 and 3. Each  
188 study was corrected by genomic control; genomic inflation factors ranged between  $\lambda=1.001$  to  
189  $\lambda=1.046$  for genotyped SNPs and  $\lambda=1.006$  to  $\lambda=1.036$  after imputation.

### 190 **3.3. Replication and combined analysis**

191 We analysed the 31 suggestively associated SNPs identified in the discovery cohort  
192 (Supplementary Table 4) and seven additional SNPs that had been significantly associated with  
193 clinical fractures in a previous GWAS (Supplementary Table 5) in the replication sample.[10]  
194 Four SNPs showed nominal association ( $p < 0.05$ ) with clinical vertebral fractures at replication  
195 (Table 1). The combined discovery and replication analysis corrected for age identified one  
196 SNP (rs10190845) on chromosome 2q13 with genome-wide significant evidence of association  
197 with clinical vertebral fractures ( $p = 1.27 \times 10^{-8}$ ). The predisposing allele had a frequency of 0.034  
198 in cases compared with 0.022 in controls and the odds ratio for susceptibility to fracture was  
199 1.75 [95% CI: 1.44-2.12] (Figure 1). The results were similar without age correction ( $p = 4.9 \times 10^{-8}$ ;  
200 odds ratio 1.66 [95% CI: 1.38-1.99]). Conditional analysis on rs10190845 did not reveal any  
201 secondary association signals at the locus (Supplementary Figure 4). Three other SNPs on

202 chromosomes 1p31, 11q12 and 15q11 were suggestively associated with vertebral fracture in  
203 the combined analysis (Table 1 and Supplementary Figures 5 and 6). None of these regions  
204 have previously been found to be associated with BMD or fracture in previous GWAS.[10,13]  
205 The top SNP (rs10190845) maps to a region which contains eleven potential candidate genes  
206 (Figure 2). This region has previously been implicated as a genetic regulator of bone density  
207 by Estrada and colleagues[10] who reported that rs17040773 within *ANAPC1* (Anaphase  
208 Promoting Complex Subunit 1) was associated with femoral neck BMD ( $p=1.5 \times 10^{-9}$ ), but not  
209 with clinical fractures ( $p=0.79$ ). rs17040773 is not in linkage disequilibrium with rs10190845  
210 in our population ( $r^2=0.006$ ), and, in keeping with this, when we performed conditional analysis  
211 on rs17040773, we confirmed that rs10190845 remained significantly associated with clinical  
212 vertebral fractures ( $p=2.09 \times 10^{-8}$ ; odds ratio 1.73 [95% CI: 1.43-2.09]). In order to test whether  
213 the variants associated with clinical vertebral fractures played a role in BMD, we tested the  
214 rs10190845 variant for association with volumetric vertebral bone mineral density in females  
215 on the dataset from Nielson and colleagues.[27] We did not find any association for the variant  
216 and BMD ( $p=0.23$ ). This suggests that rs10190845 constitutes an independent signal which  
217 predisposes to clinical vertebral fracture by mechanisms that are independent of an effect on  
218 BMD.

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234 **Table 1. Variants showing suggestive or significant association with vertebral fracture**

Chr	SNP	Position	A	Discovery (n = 5,893)			Replication (n = 2,799)			Combined* (n = 8,692)				UK Biobank replication (n = 1,991)			Total** (n = 10,683)			
				AF	p	OR (95% CI)	AF	p	OR (95% CI)	p	OR (95% CI)	I2	Q p	AF	p	OR (95% CI)	p	OR (95% CI)	I <sup>2</sup>	Q p
2	rs10190845	112192944	A	0.03	2.4x10 <sup>-5</sup>	1.70 (1.33- 2.17)	0.05	1.60x10 <sup>-4</sup>	1.84 (1.34- 2.53)	1.27x10 <sup>-8</sup>	1.75 (1.45- 2.12)	5.9	0.39	0.05	0.027	1.66 (1.06- 2.60)	1.04x10 <sup>-9</sup>	1.75 (1.45- 2.12)	0.0	0.48
11	rs7121756	57980425	A	0.29	5.2x10 <sup>-5</sup>	1.22 (1.11- 1.35)	0.28	0.011	1.23 (1.05- 1.45)	1.27x10 <sup>-6</sup>	1.23 (1.13- 1.33)	0.0	0.67	0.29	0.35	1.09 (0.91- 1.32)	4.39x10 <sup>-7</sup>	1.22 (1.13- 1.32)	49.0	0.03
15	rs2290492	92464744	A	0.23	3.4x10 <sup>-5</sup>	1.24 (1.12- 1.37)	0.21	0.021	1.23 (1.03- 1.46)	1.61x10 <sup>-6</sup>	1.24 (1.13- 1.35)	53.7	0.02	0.22	0.44	1.08 (0.88- 1.33)	2.51x10 <sup>-7</sup>	1.23 (1.13- 1.33)	75.6	1.1x10 <sup>-5</sup>
1	rs1360181	68248452	C	0.16	8.4x10 <sup>-5</sup>	1.25 (1.12- 1.41)	0.17	0.008	1.30 (1.07- 1.56)	1.87x10 <sup>-6</sup>	1.26 (1.14- 1.41)	7.7	0.57	0.17	0.38	0.90 (0.72- 1.14)	1.09x10 <sup>-5</sup>	1.22 (1.12- 1.33)	32.2	0.57

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236 The allele (A) and allele frequency (AF) for each of the variants is shown along with the p value for association, odds ratio (OR) and 95%  
 237 confidence interval (95% CI). Q p values correspond to Cochran's Q p-values. The values shown are adjusted for age but similar results were  
 238 obtained for unadjusted association tests. Position refers to Human Genome Assembly GRCh38.p11.

239 \*Combined results showed the meta-analysis for discovery and replication stage.

240 \*\*Total results showed the meta-analysis including the second replication in the UK Biobank cohort.

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247 A second replication for the significant hit on chromosome 2 and suggestive SNPs on  
248 chromosomes 1, 11 and 15 was performed in 334 clinical vertebral fracture cases and 1,657  
249 controls from UK Biobank. The top hit (rs10190845) on chromosome 2 was found nominally  
250 associated with clinical vertebral fractures ( $p=0.027$ ,  $OR=1.66[1.060-2.600]$ ,  $MAF=0.049$ ). No  
251 association was found for the suggestive SNPs in this cohort (Table 1).

252 Meta-analysis of the discovery and the two replication stages showed a combined p-value for  
253 rs10190845= $1.04 \times 10^{-9}$  ( $OR=1.74[1.06-2.6]$ ) with no evidence of heterogeneity between  
254 cohorts ( $I^2=0.0$ ,  $p=0.48$ ) (Table 1).

255 The SNPs rs7121756 on chromosome 11 and rs2290492 on chromosome 15 showed significant  
256 heterogeneity among cohorts (Cochrane's  $Q<0.05$ ), and a random effect analysis was  
257 performed. rs7121756 remained suggestively associated with clinical vertebral fractures  
258 ( $p=1.01 \times 10^{-6}$ ), whilst rs2290492 showed a marginal association ( $p=0.004$ ).

### 259 **3.4.Functional evaluation of chromosome 2q13 locus**

260 This analysis focused on a linkage disequilibrium block of approximately 700kb surrounding  
261 the top hit rs10190845. We identified a total of 936 SNPs within the region which were  
262 analysed in the GWAS ( $n=376$ ) or which were in linkage disequilibrium ( $r^2$  value of  $> 0.7$ )  
263 with rs10190845, or which showed suggestive association to clinical vertebral fractures  
264 ( $p<5 \times 10^{-3}$ ). We imputed the genotypes for the SNPs within the region of interest using the 1000  
265 Genomes phase 3 panel as reference and tested the SNPs for association with clinical vertebral  
266 fractures. We removed 878 of the SNPs since they showed no association with clinical vertebral  
267 fractures in our dataset ( $p>0.05$ ). The remaining 58 candidate SNPs were tested for association  
268 with the level of expression of genes within the candidate locus using a bone-derived gene  
269 expression dataset (eQTLs)[28] (Tables 2, 3 and Supplementary Figure 7). This resulted in the  
270 identification of nine SNPs which were eQTLs for genes within the region. In order to gain  
271 insight into the functional basis of the association at 2q13 we used SuRFR[29] which integrates  
272 functional annotation and prior biological knowledge to identify potentially causal genetic  
273 variants, to assess these 9 SNPs along with the top hit rs10190845 (Table 2 and Supplementary  
274 Figure 7).

275 **Table 2. Functionality of SNPs in 2q13 region, ranked by SuFR**

SuFR Rank	SNP ID	R <sup>2</sup> with rs10190845	A (AF)	GWAS p-value (Discovery cohort only)	OR (95%CI)	Location	GERP Value	DNase HS sit	DNase Foot	Ernst Score	Position Score	MAF Score	Enhancer score	TFBS score	Total score	eQTL	eQTL gene(s)	eQTL p
1	rs35586251	0.17	A (0.02)	2.09x10 <sup>-4</sup>	1.69 (1.28-2.24)	Exon <i>FBLN7</i>	4.47	0	0	7	5	0.02	0	0	9.89	Yes	<i>TTL</i>	6.6 x 10 <sup>-6</sup>
2	rs77172864	0.79	G (0.03)	4.96x10 <sup>-5</sup>	1.68 (1.31-2.17)	Intergenic	0.18	0	0	1	3	0.02	0	0	8.56	Yes	<i>SLC20A1</i>	0.0001
3	rs10190845	1	A (0.03)	2.4x10 <sup>-5</sup>	1.70 (1.33-2.17)	Intergenic	0	0	0	2	3	0.96	0	0	8.06	No	-	-
4	rs77996972	0.22	T (0.02)	2.11x10 <sup>-4</sup>	1.69 (1.28-2.23)	Intron <i>FBLN7</i>	1.77	313	0	7	1	0.02	0	0	7.61	Yes	<i>TTL</i> <i>SLC20A1</i>	3.8 x 10 <sup>-6</sup> 5.5 x 10 <sup>-5</sup>
5	rs75814334	0.22	T (0.02)	2.11x10 <sup>-4</sup>	1.69 (1.28-2.23)	Intron <i>FBLN7</i>	0.43	239	0	8	1	0.02	0	0	7.56	Yes	<i>TTL</i> <i>SLC20A1</i>	2.1 x 10 <sup>-6</sup> 6.6 x 10 <sup>-5</sup>
6	rs74792868	0.22	A (0.02)	2.1x10 <sup>-4</sup>	1.69 (1.28-2.24)	Intron <i>FBLN7</i>	0	0	0	9	1	0.02	0	0	7.5	Yes	<i>TTL</i> <i>SLC20A1</i>	2.0 x 10 <sup>-5</sup> 2.8 x 10 <sup>-5</sup>
6	rs72943913	0.29	G (0.03)	5.48x10 <sup>-5</sup>	1.67 (1.30-2.14)	Intron <i>ZC3H8</i>	0.15	0	0	3	1	0.02	0	0	6.46	Yes	<i>SLC20A1</i>	0.0001
7	rs112275607	0.22	A (0.02)	2.13x10 <sup>-4</sup>	1.69 (1.28-2.24)	Intron <i>FBLN7</i>	0	0	0	8	1	0.02	0	0	6.83	Yes	<i>TTL</i> <i>SLC20A1</i>	2.8 x 10 <sup>-6</sup> 6.2 x 10 <sup>-5</sup>
8	rs113085288	0.06	T (0.02)	1.79x10 <sup>-4</sup>	1.70 (1.29-2.24)	Intron <i>FBLN7</i>	0	0	0	7	1	0.02	0	0	6.08	Yes	<i>SLC20A1</i>	4.1 x 10 <sup>-6</sup>
9	rs113428223	0.29	T (0.03)	4.55x10 <sup>-5</sup>	1.70 (1.31-2.20)	Intron <i>ZC3H6</i>	0	0	0	2	1	0.02	0	0	5.61	Yes	<i>SCL20A1</i>	0.0001

276 A (AF): allele (allele frequency); GERP: Genomic evolutionary rate profiling; DNase HS: DNase hypersensitivity; DNase foot: DNase footprint;

277 Ernst score: classes of chromatin states (recurrent combinations of chromatin marks); MAF: minor allele frequency; TFBS: transcription factor

278 binding site. Gene names: *FBLN7*: Fibulin 7; *ZC3H8*: Zinc Finger CCCH-Type Containing 8; *ZC3H6*: Zinc Finger CCCH-Type Containing 6.

279

280

281 **Table 3. Correlation between genotypes for potentially functional SNP and bone-specific expression of genes in the candidate region**  
 282

RANK	SNP	GENE	PROBE	A1	A2	FRQ	BETA	SE	P
1	rs35586251	<i>TTL</i>	224896_s_at	A	G	0.017	0.65	0.13	6.62x10 <sup>-6</sup>
2	rs77172864	<i>SLC20A1</i>	230494_at	G	A	0.013	-0.46	0.11	0.00011
4	rs77996972	<i>TTL</i>	224896_s_at	T	C	0.012	0.67	0.13	3.80x10 <sup>-6</sup>
		<i>SLC20A1</i>	230494_at	T	C	0.012	-0.49	0.11	5.50x10 <sup>-5</sup>
5	rs75814334	<i>TTL</i>	224896_s_at	T	C	0.013	0.67	0.13	2.10x10 <sup>-6</sup>
		<i>SLC20A1</i>	230494_at	T	C	0.013	-0.48	0.11	6.60x10 <sup>-5</sup>
6	rs74792868	<i>TTL</i>	224896_s_at	A	G	0.012	0.66	0.14	2.00x10 <sup>-5</sup>
		<i>SLC20A1</i>	230494_at	A	G	0.012	-0.53	0.12	2.80x10 <sup>-5</sup>
6	rs72943913	<i>SLC20A1</i>	230494_at	G	A	0.013	-0.46	0.11	0.00011
7	rs112275607	<i>TTL</i>	224896_s_at	A	G	0.013	0.67	0.13	2.80x10 <sup>-6</sup>
		<i>SLC20A1</i>	230494_at	A	G	0.013	-0.48	0.11	6.02x10 <sup>-5</sup>
8	rs113085288	<i>SLC20A1</i>	230494_at	T	A	0.008	-0.72	0.14	4.06x10 <sup>-6</sup>
9	rs113428223	<i>SLC20A1</i>	230494_at	T	C	0.013	-0.46	0.11	0.0001

283  
 284 The data shown are only for the associations which were significant after Bonferroni correction (p value for significance  $\leq 0.0002$ ). A1: allele 1,  
 285 A2: Allele 2, FRQ: frequency of allele 1, BETA: effect size on regression analysis referred to A1 allele, SE: standard error of beta estimate, probe  
 286 IDs obtained from the Affymetrix HG U133 2.0 plus array. Gene names: *TTL*: Tubulin Tyrosine Ligase; *SLC20A1*: Solute Carrier Family 20  
 287 Member 1 (also known as *PIT1*).  
 288

289 The top ranking variant identified by SuRFR, rs35586251, located within exon 3 of *FBLN7* is  
290 a non-synonymous substitution (p.Val119Met). However, analysis using various *in silico*  
291 software tools yielded inconsistent results with regard to functionality of this SNP at the protein  
292 level (Supplementary Table 6). The other 9 SNPs are associated with expression of *TTL*,  
293 *SCL20A* or both genes. The variant that ranked top by SuRFR, rs35586251, was associated  
294 with increased expression of *TTL* ( $p=6.6 \times 10^{-6}$ ). Four other variants were also associated with  
295 both increased expression of *TTL* and reduced expression of *SLC20A1* (p-values ranging from  
296  $2.1 \times 10^{-6}$  to  $10^{-5}$ ). The second ranking variant, rs77172864, in strong LD with the GWAS top  
297 hit ( $r^2=0.79$ ), was associated with reduced expression of *SLC20A1* ( $p=10^{-4}$ ) (Tables 2 and 3).  
298 The variants listed on Table 2 were tested in the UK Biobank cohort for further association  
299 with clinical vertebral fractures (Supplementary Table 7). Although none of them was  
300 significantly associated with the trait, a trend of significance was found for SNPs rs72943913,  
301 rs77172864, and rs113428223 ( $p=0.06$ , OR=1.66), and all of them identified as eQTLs for  
302 *SLC20A1* gene in bone. These variants showed a lower frequency (MAF=0.03) than the top hit  
303 (MAF=0.05), which could require a greater sample size to detect associations with the trait.

### 304 **3.5. Association between clinical vertebral fractures and other osteoporosis related** 305 **phenotypes**

306 In order to determine if there is overlap between the SNPs identified as associated with lumbar  
307 spine BMD in previous GWAS with those associated with clinical vertebral fracture in this  
308 study, we evaluated 50 SNPs that have been associated with lumbar spine BMD at a genome-  
309 wide significant level in previous studies in our dataset.[10,11,13,30,31] Four variants were  
310 nominally associated with clinical vertebral fracture after Bonferroni correction (Table 4). We  
311 also analysed 15 variants previously associated with clinical fracture,[13] of which three were  
312 associated with clinical vertebral fractures in this study. We also analysed the SNPs identified  
313 by Nielson and colleagues[27] as genome-wide significant predictors of volumetric vertebral  
314 bone mineral density for association with clinical vertebral fractures in our dataset. Of the six  
315 genome-wide significant SNPs identified by Nielson et al, we found that one was significantly  
316 associated with clinical vertebral fractures after Bonferroni correction (rs12742784,  
317  $p=6.24 \times 10^{-5}$ ). The BMD-increasing variants in Table 4 conferred a reduced risk of clinical  
318 vertebral fractures in our study, whilst the variants associated with appearance of clinical  
319 fractures in previous studies were also associated with a higher risk of developing a clinical  
320 vertebral fracture in our data.

321

322 **Table 4. Association between known genetic determinants of spine BMD and clinical vertebral fractures in the combined GWAS dataset.**

Previous studies									Present study	
Study	SNP	Locus	Candidate gene	Phenotype	Method	Allele	Beta <sup>1</sup>	p	Beta <sup>2</sup>	p
Estrada	rs1346004	2q24.3	<i>GALNT3</i>	LS-BMD	DXA	A	-0.06	3.87x10 <sup>-30</sup>	+0.16	0.0002
Estrada	rs4727338	7q21.3	<i>SLC25A13</i>	LS-BMD	DXA	C	+0.07	2.13x10 <sup>-35</sup>	-0.15	0.0004
Estrada	rs6426749	1p36.12	<i>ZBTB40</i>	LS-BMD	DXA	C	+0.1	1.86x10 <sup>-44</sup>	-0.22	0.0003
Styrkarsdottir	rs7524102	1p36	<i>WNT4</i>	LS-BMD	DXA	A	-0.11	9.2x10 <sup>-9</sup>	+0.23	0.0002
Estrada	rs4727338	7q21.3	<i>SLC25A13</i>	Clinical fracture	Clinical records and X-rays	G	+0.08	5.9x10 <sup>-11</sup>	+0.14	0.0004
Estrada	rs6426749	1p36.12	<i>ZBTB40</i>	Clinical fracture	Clinical records and X-rays	G	+0.07	3.6x10 <sup>-6*</sup>	+0.22	0.0003
Estrada	rs6959212	7p14.1	<i>STARD3NL</i>	Clinical fracture	Clinical records and X-rays	T	+0.05	7.2x10 <sup>-5*</sup>	+0.15	0.001
Nielson	rs12742784	1p36.12	<i>ZBTB40</i>	Vertebral BMD	qCT imaging	T	+0.09	1.05x10 <sup>-10</sup>	-0.20	6.24x10 <sup>-5</sup>

323

324 The variants shown are those that were significant after Bonferroni correction for testing 56 BMD variants (p threshold for association 0.0009)  
 325 and 16 fracture variants (p threshold for association 0.003). \*SNP significantly associated with clinical fracture after Bonferroni correction (p  
 326 threshold at Estrada et al 5x10<sup>-4</sup>).

327 Beta<sup>1</sup> showed the effect for the previous studies (LS-BMD, clinical fracture and vertebral BMD).

328 Beta<sup>2</sup> showed the effect for the present study on clinical vertebral fracture

329 Gene names: *GALNT3*: Polypeptide N-Acetylgalactosaminyltransferase 3); *SLC25A13*: Solute Carrier Family 25 Member 13; *ZBTB40*: Zinc  
 330 Finger And BTB Domain Containing 40; *WNT4*: Wnt Family Member 4; *STARD3NL*: StAR Related Lipid Transfer Domain Containing 3 N-  
 331 Terminal Like).

332 Method column shows the technique used to evaluate the BMD or assess the fracture (DXA: dual energy X-ray absorptiometry, CT: quantitative  
 333 computerised tomography)

#### 334 4. DISCUSSION

335 Many advances have been made in defining the genetic determinants of bone mineral density  
336 and fractures through large scale genome-wide association studies, genome sequencing studies  
337 and linkage studies in rare bone diseases.[32] For example, linkage studies have shown that  
338 loss-of-function and gain-of-function variants in *LRP5* cause early onset osteoporosis[33] and  
339 high bone mass[34] respectively, whereas loss of function mutations affecting *SOST* and *LRP4*  
340 have been identified as causes of high bone mass and osteosclerosis.[35,36] Genome-wide  
341 association studies and genome sequencing studies have also been successful in identifying  
342 multiple loci that regulate bone mineral density[9-11,30,37] and a smaller number that  
343 predispose to clinical fractures.[10,30]

344 Although vertebral fractures are one of the most common and important complications of  
345 osteoporosis, relatively little is known about the genetic determinants of this type of  
346 fracture.[38] In a previous study of 8,717 cases and 21,793 controls, Oei and colleagues failed  
347 to identify any locus with significant evidence of association with morphometric vertebral  
348 fractures.[21] In the present study however, we were successful in identifying one genome-  
349 wide significant variant that predisposed to clinical vertebral fractures, which was replicated in  
350 several populations. We also detected loci that might play a role in clinical vertebral fractures  
351 (showing suggestive association at the genome-wide level), but further studies need to be  
352 performed in further cohorts to confirm or refute these associations. A likely reason for the  
353 difference between our findings and those of Oei et al, is varying case definition. Here, we  
354 studied patients with clinical vertebral fractures as opposed to morphometric vertebral  
355 deformities, many of which may not be true fractures.[22] The genome-wide significant SNP  
356 identified in the present study, rs10190845, shows one of the largest effect size so far detected  
357 in the field of osteoporosis genetics (OR=1.75[1.45-2.12]). Most of the signals associated with  
358 BMD or fracture to date showed a very low effect (ORs between 0.90 and 1.10),[12,13] with a  
359 few exceptions.[20]

360 rs10190845 maps to chromosome 2q13, a region previously associated with low femoral neck  
361 bone density.[10] However, when conditioning on rs17040773, the previously reported top  
362 SNP at the locus,[10] the association with rs10190845 remained significant, indicating that  
363 rs10190845 represents a novel signal.

364 In order to determine if there was an overlap between the results of this study and those  
365 previously reported, we analysed 71 SNPs that have previously been associated with either  
366 spine BMD or clinical fractures and identified seven variants that were significantly associated  
367 with clinical vertebral fracture in this study, after Bonferroni correction (threshold for

368 significance 0.0009 for BMD and 0.003 for clinical fractures). However, the association for  
369 these variants did not reach genome-wide significance, therefore, they were not selected in the  
370 GWAS analysis. The SNPs associated with low BMD as well as increased risk of clinical  
371 fractures in previous studies were associated with an increased risk of clinical vertebral  
372 fractures in this study and those associated with an increased risk of clinical fractures in  
373 previous studies were associated with an increased risk of clinical vertebral fractures in this  
374 study.

375 Furthermore, when we analysed six SNPs that were significantly associated with vertebral bone  
376 mineral density on quantitative computerised tomography (qCT) analysis[27] one locus on  
377 chromosome 1p36, close to *ZBTB40*, was identified and significantly associated with clinical  
378 vertebral fracture in this study. These results support the importance of *ZBTB40* as a predictor  
379 of clinical fractures and suggest that the mechanism of association is most probably mediated  
380 by changes in BMD. The observations in this study, when taken together with the findings of  
381 Nielson and Estrada[10,27] indicate that there is a partial overlap between loci that regulate  
382 lumbar spine BMD, and clinical vertebral fractures. However, there are some genetic  
383 determinants of clinical vertebral fracture which are unique and which operate independently  
384 of BMD.

385 In order to identify the mechanisms by which 2q13 predisposes to vertebral fracture we  
386 conducted bioinformatics analyses to determine if rs10190845 or other SNPs nearby were  
387 likely to be functional variants. These studies identified several potentially functional SNPs in  
388 the same LD block as rs10190845, which might account for the association we observed. The  
389 top ranking SNP from SuRFR analysis was rs35586251, which was strongly associated with  
390 expression of the *TTL* gene within the candidate locus (Supplementary Figure 8). However, the  
391 second ranking SNP, rs77172864 (Supplementary Figure 9), in strong LD with the GWAS top  
392 hit, was significantly associated with the expression of *SLC20A1*. Several other SNPs were also  
393 significantly associated with expression of *TTL* and/or *SLC20A1*, raising the possibility that  
394 alterations in expression of one or both genes might account for the predisposition to clinical  
395 vertebral fractures. Association analysis performed using UK Biobank cohort for these SNPs  
396 showed a trend of association for markers regulating *SLC20A1* gene, which also showed some  
397 degree of linkage disequilibrium, with the GWAS top hit. The lack of significant association  
398 might be due to their low allele frequency (MAF=0.03), which means that a larger sample size  
399 may be required to detect a strong association. The Tubulin Tyrosine Ligase encoded by *TTL*  
400 is involved in regulation of the cytoskeleton. Previous studies have shown that *TTL* is involved  
401 in neuronal development[39] and injury signalling,[40] raising the possibility that variants that



402 regulate *TTL* might be involved in regulating pain perception, which could account for the fact  
403 that predisposing variants have not previously been associated with BMD. Other mechanisms  
404 might also be possible and further studies need to be performed in order to address the role of  
405 *TTL* in clinical vertebral fracture. The other main candidate gene, *SLC20A1*, encodes Pit1,  
406 which facilitates the entry of inorganic phosphate into the cytoplasm.[41] Previous studies have  
407 shown that *SLC20A1* is involved in mineralisation.[42-45] Altered expression of this gene  
408 could convey risk for vertebral fractures via an effect on bone mineralisation. Although  
409 *SLC20A1* presents as the candidate gene for association with clinical vertebral fractures in this  
410 study, it has not been identified previously as a predictor of BMD or fractures. This opens for  
411 alternative mechanisms, or that *TTL* rather than *SLC20A1* is the candidate gene within the 2q13  
412 locus.

413 Limitations of the study include the fact that the total sample size was relatively small and the  
414 power to detect alleles of modest effect size was limited. It is possible that we may have missed  
415 associations between rare variants and clinical vertebral fractures since the imputation we  
416 performed was against HapMap reference panel rather than larger panels that increase  
417 imputation power particularly against low frequency variants. Although case definition was  
418 clinically based, there was no significant heterogeneity in the associations we observed across  
419 centres.

420 Strengths of the present study are that it has provided important new information on the genetic  
421 determinants of clinical vertebral fracture and that results, despite the sample size, have been  
422 validated in two independent replication stages.

#### 423 **4.1. Conclusion**

424 Genome wide association analysis identified a significant association between a marker on  
425 chromosome 2 and clinical vertebral fractures in postmenopausal women, a finding validated  
426 in several independent populations.

427 It is of interest that the top hit and other suggestive hits identified acted independently of BMD,  
428 bringing to attention other bone microarchitectural modalities that determine fracture  
429 susceptibility. This suggests that the variants identified might be acting as markers for  
430 perception of pain or other factors that are associated with the clinical presentation of vertebral  
431 fractures. We also found that some of the variants previously identified as regulators of spine  
432 BMD were associated with clinical vertebral fractures, but with effects that were weaker than  
433 the top hit and other suggestive hits. Taken together, the data suggest that the genetic basis of  
434 clinical vertebral fracture is complex involving variants that act independently of BMD as well

435 as those that are associated with spine BMD. Further research is now warranted to fully  
436 investigate the mechanisms involved.

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503

504

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645 **Fig 1. Cohort specific association between rs10190845 and clinical vertebral fracture**

646 The point estimates (squares) and 95% confidence intervals (horizontal lines) for individual  
647 studies are shown with the summary indicated by the diamond using a fixed effect model.  
648 Summaries are shown for meta-analysis with discovery cohorts only (Summary\_discovery),  
649 with the first replication cohorts only (Summary\_replication), and for the whole 3-stage meta-  
650 analysis (Summary\_meta-analysis). “BRITISH-WTCCC” shows the results for the combined  
651 cohorts CAIFOS, AOGC, DOES, and EPIC, and the control cohort WTCCC2. “Scottish  
652 replication” corresponds to EDOS-ORCADES cohorts, “Italian\_replication\_1” study  
653 corresponds to Florence-InCHIANTI cohorts and “Italian\_replication\_2” study comprises the  
654 Turin and Siena cohorts. Cohort sizes are reflected by square dimensions.

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657 **Fig 2. Regional association plots of susceptibility locus for clinical vertebral fracture**

658 The figure shows the results after imputation using 1000G v3 as reference panel. The SNPs are  
659 colour coded according to the extent of LD with the SNP showing the highest association signal  
660 from the combined analysis (represented as a purple diamond). The estimated recombination  
661 rates (cM/Mb) from HapMap CEU release 22 are shown as light blue lines, and the blue arrows  
662 represent known genes in the region. The red line shows the threshold for genome-wide  
663 significance ( $p = 5 \times 10^{-8}$ )