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

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**ABSTRACT**

**Objectives:** To identify genetic determinants of susceptibility to clinical vertebral fractures, an important complication of osteoporosis. **Methods:** Here we conduct agenome-wide association study in 1,553 postmenopausal women with clinical vertebral fractures and 4,340 controls, with a 2-stage replication involving 1,028 cases and 3,762 controls. Potentially causal variants were identified using eQTL data from transiliac bone biopsies and bioinformatic studies. **Results:** A locus tagged by rs10190845 was identified on chromosome 2q13 which was significantly associated with clinical vertebral fracture (p=1.04x10-9) with a large effect size (odds ratio 1.74, 95% CI 1.06 – 2.6). Bioinformatic analysis of this locus iodentified several potentially functional SNPs which are associated with expression of the positional candidate genes *TTL* and *SLC20A1*. Three other suggestive loci were identified on chromosomes 1p31, 11q12 and 15q11. All these loci were novel and had not previously been associated with BMD or clinical fractures. **Conclusion:** We have identified a novel genetic variant that is associated with clinical vertebral fractures by mechanisms that are independent of BMD. Further studies are now in progress to validate this association and evaluate the underlying mechanism.

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

1. **INTRODUCTION**

Osteoporosis is a common disease with a strong genetic component. It is characterised by low bone mineral density (BMD), deterioration in the microstructural architecture of bone and an increased risk of fragility fractures. Vertebral fractures are an important complication of osteoporosis.[1] They are characterised by loss of height and deformity of the affected vertebrae and are associated with an increased risk of other fractures.[2] It has been estimated that only 8-30% of patients with radiological evidence of vertebral fractures on radiographs (so called morphometric fractures) come to medical attention.[3,4] For reasons that are incompletely understood, many other patients with vertebral fractures present clinically with symptoms such as back pain, kyphosis, and height loss, and these patients are said to have clinical vertebral fractures.[5-7] Clinical vertebral fractures are associated with a markedly increase risk of future fractures and increased mortality, indicating their importance as a marker of poor clinical outcome in osteoporosis.[8] Major advances have been made in identifying genetic variants that regulate BMD and some variants have also been identified that predispose to non-vertebral fractures.[9-20] However, the genetic determinants of vertebral fractures are poorly understood. A previous genome-wide association study published by Oei and colleagues involving a discovery cohort of 8,717 subjects with morphometric vertebral deformities and 21,793 controls failed to identify any significant genetic predictors of vertebral fracture at a genome-wide significant level.[21] A limitation of this study was the diversity of methods and criteria used to define the presence of vertebral deformities in different cohorts[21] and the lack of a consensus on what constitutes a true vertebral fracture on imaging[22]. In view of these issues, the aim of this study was to perform a genome-wide association study to identify genetic variants that predisposed to clinical vertebral fractures, a more clearly defined phenotype.

1. **PATIENTS AND METHODS**

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

1. **RESULTS**
   1. **Characteristics of the study populations**

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

We identified 335 vertebral fracture female cases from the UK Biobank cohort with a mean age (± standard deviation) of 58.82 ± 7.72 years, and they were age-matched with 1,657 female controls from the same cohort. One 73-year old woman with vertebral fracture had to be excluded since it was not possible to match this individual with an age-matched control. Following this exclusion, there was no significant difference in age (mean ± standard deviation) between cases (58.78 ± 7.69) and controls (58.76 ± 7.68) (p=0.96).

* 1. **Genome-wide association analysis**

Since different genotyping platforms were used in the different cohorts, association analysis was conducted following imputation of all genotypes into the CEU panel of HapMap II reference (see Patients and Methods section). Following imputation, we analysed 2,366,456 SNPs and identified 31 with suggestive evidence of association with vertebral fracture (p < 10-4). Details are summarised in Supplementary Table 2, and the Manhattan and quantile-quantile plots from the discovery sample are shown in Supplementary Figures 2 and 3. Each study was corrected by genomic control, and genomic inflation factors ranged between = 1.001 to  = 1.046 for genotyped SNPs and  = 1.006 to  = 1.036 after imputation.

* 1. **Replication analysis**

We analysed the 31 suggestively associated SNPs identified in the discovery cohort (Supplementary Table 4) and seven additional SNPs that had been significantly associated with clinical fractures in a previous GWAS (Supplementary Table 5).[10] The combined discovery and replication analysis corrected for age identified one SNP (rs10190845) on chromosome 2q13 with genome-wide significant evidence of association with clinical vertebral fractures (p = 1.27x 10-8). The predisposing allele had a frequency of 0.034 in cases compared with 0.022 in controls and the odds ratio for susceptibility to fracture was 1.75 [95% CI: 1.44-2.12] (Figure 1). The results were similar without age correction (p=4.9 x 10-8; odds ratio 1.66 [95% CI: 1.38 – 1.99]). Conditional analysis on rs10190845 showed that the association with the trait was driven by a single signal, and no secondary signals were detected (Supplementary Figure 4). Three other SNPs on chromosomes 1p31, 11q12 and 15q11 were suggestively associated with vertebral fracture (Table 1 and Supplementary Figures 5 and 6). None of these regions have previously been found to be associated with BMD or fracture in other GWAS.[10,13]

The top hit maps to a region which contains eleven potential candidate genes (Figure 2). This region has previously been implicated as a genetic regulator of bone density by Estrada and colleagues[10] who reported that rs17040773 within *ANAPC1* was associated with femoral neck BMD (p = 1.5 x 10-9), but not with clinical fractures (p = 0.79). The rs17040773 was not in linkage disequilibrium with rs10190845 (r2=0.006), and, therefore, when we performed conditional analysis on rs17040773, we confirmed that rs10190845 remained significantly associated with clinical vertebral fractures (p= 2.09 x 10-8; odds ratio 1.73 [95% CI: 1.43-2.09]). In order to test whether the variants associated with clinical vertebral fractures played a role in BMD, we tested the rs10190845 variant for association with volumetric vertebral bone mineral density in females on the dataset from Nielson and colleagues.[31] We did not find any association for the variant and BMD (p=0.23). This suggests that rs10190845 constitutes an independent signal which predisposes to vertebral fracture by mechanisms that are independent of an effect on BMD.

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

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | **Discovery**  **(n = 5,893)** | | | **Replication**  **(n= 2,799)** | | | **Combined\***  **(n= 8,692)** | | | | **UK Biobank replication**  **(n= 1,991)** | | | **Total\*\***  **(n= 10,683)** | | | |
| **Chr** | **SNP** | **Position** | **A** | **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)** | **I2** | **Q p** |
| 2 | rs10190845 | 112666992 | A | 0.03 | 2.4x10-5 | 1.70  (1.33-2.17) | 0.05 | 1.60x10-4 | 1.84  (1.34-2.53) | 1.27x10-8 | 1.75  (1.45-2.12) | 5.9 | 0.39 | 0.05 | 0.027 | 1.66  (1.06-2.60) | 1.04x10-9 | 1.75  (1.45-2.12) | 5.9 | 0.39 |
| 11 | rs7121756 | 57504473 | A | 0.29 | 5.2x10-5 | 1.22  (1.11-1.35) | 0.28 | 0.011 | 1.23  (1.05-1.45) | 1.27x10-6 | 1.23  (1.13-1.33) | 0.0 | 0.67 | 0.29 | 0.35 | 1.09  (0.91-1.32) | 4.39x10-7 | 1.22  (1.13-1.32) | 49.0 | 0.03 |
| 15 | rs2290492 | 90808978 | A | 0.23 | 3.4x10-5 | 1.24  (1.12-1.37) | 0.21 | 0.021 | 1.23  (1.03-1.46) | 1.61x10-6 | 1.24  (1.13-1.35) | 53.7 | 0.02 | 0.22 | 0.44 | 1.08  (0.88-1.33) | 2.51x10-7 | 1.23  (1.13-1.33) | 75.6 | 1.1x10-5 |
| 1 | rs1360181 | 68486723 | C | 0.16 | 8.4x10-5 | 1.25  (1.12-1.41) | 0.17 | 0.008 | 1.30  (1.07-1.56) | 1.87x10-6 | 1.26  (1.14-1.41) | 7.7 | 0.57 | 0.17 | 0.38 | 0.90  (0.72-1.14) | 1.09x10-5 | 1.22  (1.12-1.33) | 32.2 | 0.57 |

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% 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 obtained for unadjusted association tests.

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

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

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

Meta-analysis of the discovery and the two replication stages showed a final p-value for rs10190845 = 1.04x10-9 (OR=1.74 [1.06 – 2.6]) with no evidence of heterogeneity between cohorts (I2=0.0, p= 0.48).

The SNPs rs7121756 on chromosome 11 and rs2290492 on chromosome 15 show significant heterogeneity among cohorts (Cochrane’s Q < 0.05), and a random effect analysis was performed. Rs7121756 remained suggestively associated with clinical vertebral fractures (p = 1.01 x 10-6), whilst rs2290492 showed a marginal association (p = 0.004).

* 1. **Functional evaluation of chromosome 2q13 locus**

In order to gain insight into the functional basis of the association at 2q13 we used SuRFR[27] which integrates functional annotation and prior biological knowledge to identify potentially causal genetic variants. This analysis focused on a linkage disequilibrium block of approximately 700kb surrounding the top hit rs10190845. We identified a total of 936 SNPs within the region which were analysed in the GWAS (n=376) or which were in linkage disequilibrium (r2 value of > 0.7) with rs10190845, or which showed suggestive association to vertebral fractures (p < 5 x 10-3). We imputed the genotypes for the SNPs within the region of interest using the 1000 Genomes phase 3 panel as reference and tested the SNPs for association with clinical vertebral fractures. We removed 878 of the SNPs since they showed no association with clinical vertebral fractures in our dataset (p > 0.05). The remaining 58 candidate SNPs were tested for association with the level of expression of genes within the candidate locus using a bone-derived gene expression dataset (eQTLs)[28] (Tables 2 and 3 and Supplementary Figure 7). This resulted in the identification of nine SNPs which were eQTLs for genes within the region. These SNPs were analysed by SuRFR along with the top hit rs10190845 (Table 2 and Supplementary Figure 7).

**Table 2. Functionality of SNPs in 2q13 region, predicted by SuRFR**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Rank** | **SNP ID** | **R2 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) | 2.09x10-4 | 1.69  (1.28-2.24) | *Exon FBLN7* | 4.47 | 0 | 0 | 7 | 5 | 0.02 | 0 | 0 | 9.89 | Yes | *TTL* | 6.6 x 10-6 |
| 2 | rs77172864 | 0.79 | G (0.03) | 4.96x10-5 | 1.68  (1.31-2.17) | Intergenic | 0.18 | 0 | 0 | 1 | 3 | 0.02 | 0 | 0 | 8.56 | Yes | *SCL20A1* | 0.0001 |
| 3 | rs10190845 | 1 | A (0.03) | 2.4x10-5 | 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) | 2.11x10-4 | 1.69  (1.28-2.23) | Intron *FBLN7* | 1.77 | 313 | 0 | 7 | 1 | 0.02 | 0 | 0 | 7.61 | Yes | *TTL SLC20A1* | 3.8 x 10-6 5.5 x 10-5 |
| 5 | rs75814334 | 0.22 | T (0.02) | 2.11x10-4 | 1.69  (1.28-2.23) | Intron *FBLN7* | 0.43 | 239 | 0 | 8 | 1 | 0.02 | 0 | 0 | 7.56 | Yes | *TTL SLC20A1* | 2.1 x 10-6 6.6 x 10-5 |
| 6 | rs74792868 | 0.22 | A (0.02) | 2.1x10-4 | 1.69  (1.28-2.24) | Intron *FBLN7* | 0 | 0 | 0 | 9 | 1 | 0.02 | 0 | 0 | 7.5 | Yes | *TTL SLC20A1* | 2.0 x 10-5 2.8 x 10-5 |
| 6 | rs72943913 | 0.29 | G (0.03) | 5.48x10-5 | 1.67  (1.30-2.14) | Intron *ZC3H8* | 0.15 | 0 | 0 | 3 | 1 | 0.02 | 0 | 0 | 6.46 | Yes | *SLC20A1* | 0.0001 |
| 7 | rs112275607 | 0.22 | A (0.02) | 2.13x10-4 | 1.69  (1.28-2.24) | Intron *FBLN7* | 0 | 0 | 0 | 8 | 1 | 0.02 | 0 | 0 | 6.83 | Yes | *TTL SLC20A1* | 2.8 x 10-6 6.2 x 10-5 |
| 8 | rs113085288 | 0.06 | T (0.02) | 1.79x10-4 | 1.70  (1.29-2.24) | Intron *FBLN7* | 0 | 0 | 0 | 7 | 1 | 0.02 | 0 | 0 | 6.08 | Yes | *SLC20A1* | 4.1 x10-6 |
| 9 | rs113428223 | 0.29 | T (0.03) | 4.55x10-5 | 1.70  (1.31-2.20) | Intron *ZC3H6* | 0 | 0 | 0 | 2 | 1 | 0.02 | 0 | 0 | 5.61 | Yes | *SCL20A1* | 0.0001 |

A (AF): allele (allele frequency); GERP: Genomic evolutionary rate profiling; DNAase HS: DNase hypersensitivity; DNase foot: DNase footprint; Ernst score: classes of chromatin states (recurrent combinations of chromatin marks); MAF: minor allele frequency; TFBS: transcription factor binding site.

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

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

The data shown are only for the associations which were significant after Bonferroni correction (p value for significance <0.0002). A1: allele 1, 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 IDs obtained from the Affymetrix HG U133 2.0 plus array.

The top ranking variant identified by SuRFR, rs35586251, located within exon 3 of *FBLN7* is a non-synonymous substitution (p.Val119Met). However, analysis using various *in silico* software tools yielded inconsistent results with regard to functionality of this SNP at the protein level (Supplementary Table 6). The other SNPs ranked as potentially functional by SuRFR were associated with expression of *TTL*, *SCL20A* or both genes. The top ranking functional variant rs35586251 was associated with increased expression of *TTL* (p=6.6 x 10-6). Four other variants were also associated with increased expression of *TTL* and reduced expression of *SLC20A1* (p-values ranging from 2.1 x 10-6 to 10-5). The second ranking variant, rs77172864, in strong LD with the GWAS top hit (r2=0.79), was associated with reduced expression of *SLC20A1* (p = 10-4) (Tables 2 and 3).

The variants listed on Table 2 were tested on the UK Biobank cohort for further association with vertebral fractures (Supplementary Table 7). Although none of them was significantly associated with the trait, a trend of significance was found for SNPs rs72943913, rs77172864, and rs113428223 (p=0.06, OR=1.66), all of them identified as eQTLs for *SLC20A1* gene. These variants showed a lower frequency than the top hit (MAF=0.03).

* 1. **Association between clinical vertebral fractures and other osteoporosis related phenotypes**

In order to determine if there was overlap between the SNPs identified as associated with lumbar spine BMD in previous GWAS with those associated with clinical vertebral fracture in this study, we evaluated 50 SNPs that have been associated with lumbar spine BMD at a genome-wide significant level in previous studies in our dataset.[10,11,13,29,30] This resulted in the identification of four variants that were nominally associated with clinical vertebral fracture after Bonferroni correction (Table 4). Of the 15 variants previously associated with clinical fracture,[13] three were associated with clinical vertebral fractures in this study. We also analysed the SNPs identified by Nielson and colleagues[31] as genome-wide significant predictors of volumetric vertebral bone mineral density for association with clinical vertebral fractures in our dataset. Of the six genome-wide significant SNPs identified in Nielson et al, we found that one was significantly associated with clinical vertebral fractures after Bonferroni correction (rs12742784, p=6.24 x 10-5).

**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 | Allele | Beta1 | p | Beta2 | p |
| Estrada | rs1346004 | 2q24.3 | GALANT3 | LS-BMD | A | ‐0.06 | 3.87x10‐30 | +0.16 | 0.0002 |
| Estrada | rs4727338 | 7q21.3 | SLC25A13 | LS-BMD | C | +0.07 | 2.13x10‐35 | -0.15 | 0.0004 |
| Estrada | rs6426749 | 1p36.12 | ZBTB40 | LS-BMD | C | +0.1 | 1.86x10‐44 | -0.22 | 0.0003 |
| Styrkarsdottir | rs7524102 | 1p36 | WNT4 | LS-BMD | A | -0.11 | 9.2x10-9 | +0.23 | 0.0002 |
| Estrada | rs4727338 | 7q21.3 | SLC25A13 | Clinical fracture | G | +0.08 | 5.9x10-11 | +0.14 | 0.0004 |
| Estrada | rs6426749 | 1p36.12 | ZBTB40 | Clinical fracture | G | +0.07 | 3.6x10-6\* | +0.22 | 0.0003 |
| Estrada | rs6959212 | 7p14.1 | STARD3NL | Clinical fracture | T | +0.05 | 7.2x10-5\* | +0.15 | 0.001 |
| Nielson | rs12742784 | 1p36.12 | ZBTB40 | Vertebral BMD | T | +0.09 | 1.05x10-10 | -0.20 | 6.24x10-5 |

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

Beta1 showed the effect for the previous studies (LS-BMD, clinical fracture and vertebral BMD).

Beta2 showed the effect for the present study on clinical vertebral fracture

1. **DISCUSSION**

Many advances have been made in defining the genetic determinants of bone mineral density and fractures through large scale genome-wide association studies, genome sequencing studies and linkage studies in rare bone diseases.[32] For example, linkage studies have shown that loss-of-function and gain-of-function variants in *LRP5* cause early onset osteoporosis[33] and high bone mass[34] respectively, whereas loss of function mutations affecting *SOST* and *LRP4* have been identified as causes of high bone mass and osteosclerosis.[35,36] Genome-wide association studies and genome sequencing studies have also been successful in identifying multiple loci that regulate bone mineral density[9-11,29] and a smaller number that predispose to clinical fractures.[10,29]

Although vertebral fractures are one of the most common and important complications of osteoporosis, relatively little is known about the genetic determinants of this type of fracture.[37] In a previous study of 8,717 cases and 21,793 controls, Oei and colleagues failed to identify any locus with significant evidence of association with morphometric vertebral deformities.[21] In the present study however, we were successful in identifying one genome-wide significant variant that predisposed to clinical vertebral fractures, which was replicated in several populations. We also detected some suggestive loci that might play a role in vertebral fractures, but further studies need to be performed in further cohorts to confirm or refute these associations. Most likely the reason for the difference between the findings in this study and that study of Oei et al, is that the case definition was different. Here we studied patients with clinical vertebral fractures as opposed to morphometric vertebral deformities.[22] The genome-wide significant SNP identified in the present study, rs10190845, shows one of the largest effect size so far detected in the field of osteoporosis genetics (OR= 1.75 [1.45-2.12]). Apart of some exceptions,[20] most of the signals associated with BMD or fracture to date showed a very low effect (ORs between 0.90 and 1.10).[12,13] It maps to chromosome 2q13, a region previously associated with low femoral neck bone density.[10] However, when conditioning on rs17040773, the previously reported top SNP at the locus,[10] the association with rs10190845 remained significant, indicating that it is a novel signal.

In order to determine if there was an overlap between the results of this study and those previously reported, we analysed 51 SNPs that have previously been associated with either spine BMD or clinical fractures and identified seven variants that were significantly associated with clinical vertebral fracture in this study. The SNPs that were associated with low BMD in previous studies were associated with an increased risk of clinical vertebral fractures in this study and those associated with an increased risk of clinical fractures in previous studies were associated with an increased risk of clinical vertebral fractures in this study.

Furthermore, when we analysed six SNPs that were significantly associated with vertebral bone mineral density on quantitative computerised tomography (qCT) analysis[31] we identified one locus on chromosome 1p36, close to *ZBTB40*, that was significantly associated with clinical vertebral fracture in this study. These results support the importance of *ZBTB40* as a predictor of clinical fractures and suggest that the mechanism of association is most probably mediated by changes in BMD. The observations in this study, when taken together with the findings of Nielson and Estrada[10,31] indicate that there is a partial overlap between loci that regulate lumbar spine BMD, and clinical vertebral fractures, but that there are some genetic determinants of clinical vertebral fracture which are unique and which operate independently of BMD.

In order to identify the mechanisms by which 2q13 predisposes to vertebral fracture we conducted functional and bio-informatic studies to determine if rs10190845 or other SNPs nearby were likely to be functional variants. These studies identified several potentially functional SNPs in the same LD block as rs10190845, which might account for the association we observed. The top ranking SNP from SuRFR analysis was rs35586251, which was strongly associated with expression of the *TTL* gene within the candidate locus (Supplementary Figure 8). However, the second ranking SNP, rs77172864 (Supplementary Figure 9), in strong LD with the GWAS top hit, was significantly associated with the expression of *SLC20A1*. Several other SNPs were also significantly associated with expression of *TTL* and/or *SLC20A1,* raising the possibility that alterations in expression of one or both genes might account for the predisposition to vertebral fractures. Association analysis performed using UK Biobank cohort for these SNPs showed a trend of association for markers regulating *SLC20A1* gene, which also showed some degree of linkage disequilibrium, with the GWAS top hit. The lack of significant association might be due to their low allele frequency (MAF=0.03), which means that a larger sample size may be required to detect a strong association. The *TTL* gene encodes a tubulin tyrosine ligase involved in regulation of the cytoskeleton. Previous studies have shown that *TTL* is involved in neuronal development[38] and injury signalling,[39] raising the possibility that variants that regulate *TTL* might be involved in regulating pain perception, which could account for the fact that predisposing variants have not previously been associated with BMD. Other mechanisms might also be possible and further studies need to be performed in order to address the role of *TTL* in vertebral fracture. The other main candidate gene, *SLC20A1*, encodes Pit1, which facilitates the entry of inorganic phosphate into the cytoplasm.[40] Previous studies have shown that *SLC20A1* is involved in mineralisation,[41-44] which raises the possibility that differences in expression of this gene could be involved in regulating susceptibility for vertebral fractures by an effect on bone mineralisation. Although there is a trend towards *SLC20A1* as the candidate gene for association with clinical vertebral fractures, it has not been identified previously as a predictor of BMD or fractures. This suggests that an alternative mechanism may be operative, or that *TTL* rather than *SLC20A1* is the candidate gene within the 2q13 locus.

Limitations of the study include the fact that the total sample size was relatively small and the power to detect alleles of modest effect size was limited. It’s possible that we may have missed associations between rare variants and clinical vertebral fractures since the imputation we performed was against HapMap reference panel. Although case definition was clinically based there was no significant heterogeneity in the associations we observed across centres.

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

* 1. **Conclusion**

Genome wide association analysis identified a significant association between a marker on chromosome 2 and clinical vertebral fractures in postmenopausal women, which has been validated in several independent populations.

It is of interest that the top hit and other suggestive hits identified acted independently of BMD. This suggests that the variants identified might be acting as markers for perception of pain or other factors that are associated with the clinical presentation of vertebral fractures. We also found that some of the variants previously identified as regulators of spine BMD were associated with clinical vertebral fractures in this study but with effects that were weaker than the top hit and other suggestive hits. Taken together, the data suggest that the genetic basis of clinical vertebral fracture is complex involving variants that act independently of BMD as well as those that are associated with spine BMD. Further research is now warranted to fully investigate the mechanisms involved.

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

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



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

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

