



TRANSLATIONAL SCIENCE

Genome-wide association study identifies genetic variants which predict the response of bone mineral density to teriparatide therapy

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ABSTRACT

Objectives Teriparatide (TPTD) is an effective treatment for osteoporosis but the individual response to therapy is variable for reasons that are unclear. This study aimed to determine whether the response to TPTD might be influenced by genetic factors.

Methods We searched for predictors of the response of bone mineral density (BMD) to TPTD using a two-stage genome-wide association study in 437 patients with osteoporosis from three referral centres. Demographic and clinical data including the response of BMD to treatment at the lumbar spine and hip were extracted from the medical records of each participant.

Results Allelic variation at rs6430612 on chromosome 2, close to the *CXCR4* gene was associated with the response of spine BMD to TPTD at a genome wide significant level ($p=9.2 \times 10^{-9}$, $\beta=-0.35$ (-0.47 to -0.23)). The increase in BMD was almost twice as great in AA homozygotes at rs6430612 as compared with GG homozygotes with intermediate values in heterozygotes. The same variant was also associated with response of femoral neck and total hip BMD ($p=0.007$). An additional locus on chromosome 19 tagged by rs73056959 was associated with the response of femoral neck BMD to TPTD ($p=3.5 \times 10^{-9}$, $\beta=-1.61$ (-2.14 to -1.07)).

Conclusions Genetic factors influence the response to TPTD at the lumbar spine and hip with a magnitude of effect that is clinically relevant. Further studies are required to identify the causal genetic variants and underlying mechanisms as well as to explore how genetic testing for these variants might be implemented in clinical practice.

INTRODUCTION

Osteoporosis is a common disease characterised by low bone mineral density (BMD) and changes in the microstructure of bone, which lead to an increased risk of fragility fractures.¹ Treatment costs in the UK alone were estimated as £2.1 billion annually in 2020.² While oral bisphosphonates are the first line of treatment for many patients,³ there is evidence from clinical trials⁴⁻⁶ and observational studies⁷ that teriparatide (TPTD) is more effective than oral bisphosphonates in patients with severe osteoporosis of the spine and vertebral fractures. Although TPTD is an effective treatment, previous studies have shown that the response to therapy is variable

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The response of bone mineral density (BMD) to the bone anabolic treatment teriparatide (TPTD) in patients with osteoporosis varies but the reasons are unclear.

WHAT THIS STUDY ADDS

⇒ This study shows for the first time that the response of BMD to TPTD at both spine and hip is influenced by genetic factors.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Genotyping for TPTD response variants may be of clinical value in personalising osteoporosis treatment to decide whether TPTD would be the optimal therapy.

for reasons that are unclear.^{7,8} Since genetic factors are known to be important in regulating BMD and susceptibility to osteoporosis,⁹ we investigated the hypothesis that genetic factors might also influence the response to TPTD therapy. This was achieved by performing a two-stage genome wide association study in patients undergoing TPTD therapy for the treatment of osteoporosis as part of everyday clinical practice. As TPTD is used both as a primary treatment in patients for severe osteoporosis and as a second-line treatment in patients who respond inadequately to antiresorptive therapy, we included both groups of patients in the genome wide association analysis (GWAS). We subsequently performed an interaction analysis to determine to what extent previous antiresorptive therapy had influenced the genotype-specific response to TPTD therapy for the SNP that reached genome wide significance.

PATIENTS AND METHODS**Patients**

The study group comprised individuals who were undergoing treatment with TPTD for osteoporosis as part of their usual clinical care. Participants from three secondary care referral centres were included in the study from Edinburgh (UK), Aarhus (Denmark) and Ljubljana (Slovenia). Participants were recruited between June 2005 and July 2016 in the Edinburgh centre, from July 2003 to October



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2013 in the Danish centre and from July 2008 to July 2014 in the Slovenian centre. Adherence to the injection treatment was confirmed at follow-up clinical visits at either face-to-face or telephone consultations at 3–4 months into the treatment course; at the half-way point at 9 or 12 months and at the end of treatment at 18 or 24 months. In keeping with a previous study which looked at clinical predictors of response to TPTD therapy,⁸ adherence to treatment was excellent and was estimated to be at least 90% or better across the whole study cohort.

BMD was measured at the lumbar spine (average of vertebrae L1–L4), total hip and femoral neck prior to starting TPTD therapy and at the end of treatment according to normal clinical practice. In patients where it was not possible to obtain measurements in all four lumbar vertebrae due to technical reasons such as vertebral fractures or osteoarthritis, we took the average of the evaluable vertebrae. The BMD measurements were made by dual X-ray absorptiometry (DEXA) using Hologic QDR4500 densitometers. Clinical and demographic data were obtained from the participants' medical records at each centre. Serum 25(OH)D was measured at the local hospital laboratories using the methodology employed at the time they commenced TPTD. Dietary calcium intake was estimated by food frequency questionnaire. Self-reported physical activity was recorded at the time of baseline DEXA by questionnaire at the Edinburgh centre in which participants were asked to record whether they were ambulant (on their feet for ≥ 4 hours a day) or sedentary (on their feet for <4 hours a day). Additionally, participants were asked to record whether they undertook high-impact sports (such as running, snow-sports or ball games) or low impact sports (such as walking, swimming and yoga).

Treatment received

A total of 314 patients (72%) had had been treated with TPTD over a 24-month period, but the remainder had undergone 18 months treatment based on the approvals in place at the time mandated by the European Medicines Agency.

Genome-wide association study

Genotyping was performed on DNA samples extracted from venous blood in all 437 participants using the Axiom chip from Affymetrix using standard methodology at the Wellcome Trust Clinical Research Facility at the University of Edinburgh. Allele calls were generated by GenomeStudio GenCall V.6.3.0 and all data were used to assign genotypes. Technical details of quality control measures, methods of imputation, expression quantitative trait analysis and meta-analysis of results from the different centres are all provided in online supplemental information. We randomly assigned 295 of the included participants (67.5%) to the discovery sample and the remaining 142 (32.5%) to the replication sample, with a similar proportion of individuals from each centre in both cohorts.¹⁰

Statistical methods

Standardised residuals for percentage of change in lumbar spine BMD and femoral neck BMD following treatment with TPTD treatment corrected for age, duration of treatment, centre, gender and two principal components were used for the Genome Wide Association Study (GWAS) in PLINK. In keeping with normal practice for GWAS studies,¹¹ we used a two-stage approach assigning two-thirds of individuals at random to the discovery cohort and the remaining one-third to the replication cohort. Further details on the GWAS analysis are described in online supplemental information. We did not include bisphosphonate

therapy into the GWAS model since the primary aim of the study was to identify predictors of response to TPTD whether or not they had been previously treated with bisphosphonates. Instead, we elected to evaluate the possible influence of bisphosphonate therapy on responses to TPTD¹² by looking for evidence of an interaction between genotype, previous bisphosphonate therapy and changes in BMD at the lumbar spine and hip by a two-way analysis of variance using SPSS version 25.

Reporting guidelines

The Strengthening the Reporting of Genetic Association Studies (STREGA)¹³ guidelines were followed in reporting the results of this study.

RESULTS

Characteristics of the studied populations

The baseline characteristics of the study populations are shown in [table 1](#). Most participants (94%) were female with an average age of 69 years. The lowest BMD at all sites (lumbar spine, femoral neck and total hip) were observed in the Edinburgh cohort, with intermediate values in the Danish cohort and highest values in the Slovenia cohort. It is probable that these differences in BMD were related to the fact that the number of individuals who were previously treated with bisphosphonates prior TPTD, ranged from 8.9% of subjects in the Edinburgh cohort to 96% of those in the Slovenia cohort.

The overall responses of BMD to TPTD treatment at the lumbar spine, femoral neck and total hip are shown in [table 1](#). There was no significant difference in age, proportion of males and females, or the percentage change in BMD following TPTD treatment at the lumbar spine or femoral neck in the discovery and replication cohorts, nor was there a difference in the proportion who received 18 months or 24 months therapy. There was a small difference between the BMD change at total hip in the Danish cohort between discovery and replication, $p=0.042$ (online supplemental table 1).

Genetic variants associated with response of BMD to TPTD

The results of the combined analysis from discovery and replication cohorts for change in lumbar spine BMD are shown in [figure 1](#), which illustrates the Manhattan plot and quantile-quantile plots. Additional variants on chromosome 15 and 19 showed a suggestive association with change in spine BMD ([table 2](#)). Imputation analysis did not identify any further significant associations with LS-BMD change. Suggestive associations that were driven by singleton SNPs were not considered for further analysis.

The results of the combined analysis from discovery and replication cohorts for change in femoral neck BMD are shown in [figure 2](#), which illustrates both the Manhattan plot and quantile-quantile plots. We identified one locus tagged by the SNP rs73056959 that was significantly associated with changes in femoral neck BMD. We also identified a variant on chromosome 2, distinct from the locus associated with response of lumbar spine BMD, where there was a suggestive association with change in femoral neck BMD ([table 2](#)).

Full details of the loci which were significantly or suggestively associated with changes in BMD at the lumbar spine and femoral neck are shown in [table 2](#), which provides information on allele frequencies, p values, beta-coefficients and 95% CIs separately in the discovery and replication cohorts as well for the full cohort.

Regional plots of the regions with genome wide significant evidence of association with response of spine BMD and femoral

Table 1 Baseline clinical characteristics and overall response to teriparatide of the study population

	Edinburgh	Denmark	Slovenia	Total
No	214	85	138	437
Age	69.6±8.7	68.4±8.5	68.7±10.8	69.1±9.4
Female	201 (94.0%)	64 (75.3%)	136 (98.5%)	401 (91.8%)
Spine T-score	-4.40±0.52	-3.03±1.28	-2.50±1.18	-3.52±1.29
Femoral neck T-score	-2.88±0.74	-2.63±0.98	-2.30±1.10	-2.66±0.95
Total hip T-score	-2.67±0.89	-2.31±1.06	-1.80±0.92	-2.32±1.01
History of vertebral fractures	109 (50.9%)	85 (100.0%)	125 (90.6%)	319 (73.0%)
History of non-vertebral fractures	99 (46.3%)	31 (36.5%)	72 (52.2%)	202 (46.2%)
Previous bisphosphonate treatment	19 (8.9%)	47 (55.3%)	133 (96.4%)	214 (49.0%)
TPTD 18 months	86 (40%)	36 (42.3%)	1 (0.7%)	123 (28.2%)
TPTD 24 months	128 (60%)	49 (57.7%)	137 (99.3%)	314 (71.8%)
Change in lumbar spine BMD (g/cm ²)	0.09±0.05	0.07±0.06	0.05±0.06	0.07±0.06
Change in lumbar spine BMD (%)	15.60±8.38	10.06±8.45	6.71±8.60	11.72±9.35
Change in femoral neck BMD (g/cm ²)	0.01±0.08	0.007±0.04	0.008±0.07	0.01±0.07
Change in femoral neck BMD (%)	2.23±7.81	1.93±7.9	1.08±8.69	1.78±8.15
Change in total hip BMD (g/cm ²)	0.01±0.09	0.01±0.03	-0.002±0.04	0.007±0.07
Change in total hip BMD (%)	1.57±6.92	2.36±5.28	0.01±7.15	1.20±6.76

Values are numbers and percentages or mean±SD. The changes in BMD at all sites refer to the difference between the values at beginning and end of study in absolute terms expressed as g/cm² or percentage difference. Note that measurements at the hip sites were only available in 403 participants due to image artefacts or metalwork as the result of previous fractures.

BMD, bone mineral density; TPTD, teriparatide.

neck BMD are shown in online supplemental figures 1 and 2, respectively. The chromosome 2 locus that was associated with response of spine BMD contains several genes, but the top hit was nearest *CXCR4*, which is a gene with known effects on bone metabolism. The lactase (*LCT*) gene is also in this locus and variants in this gene have previously been associated with BMD, but the association remained significant after a conditional analysis considering *LCT* variants. Accordingly, after removal of the *LCT* signal (lowest $p=6.7\times 10^{-4}$), the rs6430612 was strongly associated with the response to TPTD ($p=9.4\times 10^{-7}$). This was well below the threshold for significance ($p=2.3\times 10^{-4}$) after accounting for the number of single nucleotide polymorphisms within the area of interest by Bonferroni's correction.

The Chromosome 19 locus associated with response of femoral neck BMD contains many genes. The top hit rs73056959 was in an intergenic region between *PEG3/ZIM2* and *USP29/ZIM3* genes and was not in an area with features of a regulatory region. The genes nearby are involved in cell proliferation and DNA

binding, as well as deubiquitination and stabilisation of proteins, but none had a clear role in bone metabolism.

The association between response of lumbar spine and hip BMD to TPTD treatment and carriage of allelic variants at rs6430612 is shown in figure 3A–C. Individuals homozygous for the A allele at rs6430612 had, on average, a 16% increase in spine BMD compared with a 7.3% for homozygotes for the G allele, with intermediate responses in heterozygotes. Response of femoral neck and total hip BMD to TPTD was also significantly associated with rs6430612 allelic variants (figure 3B,C).

The response of lumbar spine, femoral neck and total hip BMD to TPTD treatment in relation to carriage of the rs73056959 variant which was a genome wide predictor of change in femoral neck BMD is shown in figure 3D–F. Variants at rs73056959 were not significantly associated with change in lumbar spine BMD (figure 3D, $p=0.20$), but were significantly associated with change in both femoral neck BMD (figure 3E, $p=4\times 10^{-5}$) and total hip BMD (figure 3F, $p=3.3\times 10^{-4}$).

In view of the differences in the proportion of individuals previously treated with bisphosphonates in different centres, we performed an interaction analysis using two-way ANOVA to explore the relation between genotype, previous therapy and the change in BMD. This showed that rs6430612 genotype ($p<0.001$) and previous bisphosphonate treatment ($p<0.001$) were both significant predictors of the response of lumbar spine BMD to TPTD therapy but there was no significant interaction between genotype and previous treatment in determining response ($p=0.215$). At the femoral neck site, rs6430612 genotype was not a significant predictor of change in BMD ($p=0.245$) nor was previous bisphosphonate treatment ($p=0.06$) but here there was a significant genotype–treatment interaction ($p=0.008$). At the total hip, rs6430612 genotype was a significant predictor ($p=0.038$) along with previous treatment ($p=0.001$) but with no significant genotype–treatment interaction ($p=0.170$).

A similar analysis for rs73056959 showed no significant association between genotype and change in lumbar spine BMD

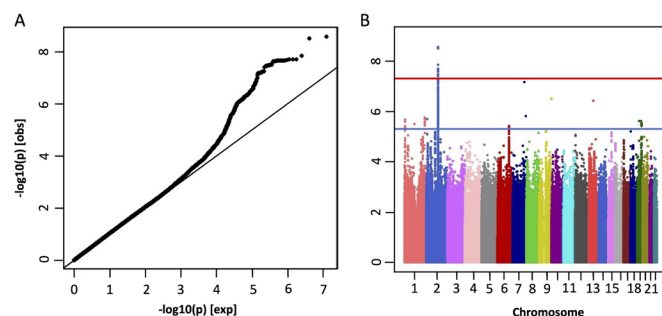


Figure 1 Genome wide association study for response of spine BMD to teriparatide. A shows the Q-Q-plot for imputed SNPs associated with the percentage change in lumbar spine in response to teriparatide treatment. B shows the Manhattan plot of the signals associated with the response. The red line shows the threshold for genome wide significance ($p=5\times 10^{-8}$), and the blue line shows the suggestive threshold for genome wide significance $p=5\times 10^{-6}$. BMD, bone mineral density.

Table 2 Genotyped variants showing significant or suggestive association with the response of BMD to teriparatide

Chr	Trait	SNP	A	Discovery (n=295)				Replication (n=142)			Combined (n=437)			I ²	I ² P value
				AF	P value	β (95% CI)	AF	P value	β (95% CI)	P value	β (95% CI)				
2	LS	rs6430612	G	0.38	4.3×10 ⁻⁶	−0.34 (−0.48 to −0.20)	0.39	4.9×10 ⁻⁴	−0.38 (−0.59 to −0.17)	9.2×10 ⁻⁹	−0.35 (−0.47 to −0.23)	0.0	0.76		
15	LS	rs12439872	G	0.20	3.7×10 ⁻⁴	−0.32 (−0.50 to −0.15)	0.26	0.001	−0.42 (−0.67 to −0.16)	3.1×10 ⁻⁶	−0.35 (−0.50 to −0.20)	0.0	0.57		
19	LS	rs875704	A	0.15	1.4×10 ⁻⁴	−0.39 (−0.59 to −0.19)	0.12	0.002	−0.57 (−0.94 to −0.22)	1.6×10 ⁻⁶	−0.44 (−0.62 to −0.26)	0.0	0.39		
2	FN	rs10932371	A	0.31	1.1×10 ⁻⁴	0.38 (0.19 to 0.57)	0.27	0.003	0.36 (0.12 to 0.60)	1.1×10 ⁻⁶	0.37 (0.22 to 0.52)	0.0	0.93		
19	FN	rs73056959	A	0.05	1.4×10 ⁻⁶	−2.25 (−3.16 to −1.34)	0.05	1.4×10 ⁻⁴	−1.28 (−1.94 to −0.62)	3.5×10 ⁻⁹	−1.61 (−2.14 to −1.07)	65	0.09		

The combined results shown were corrected by the genomic inflation factor (λ) as described in the methods section of online supplemental material giving details of the GWAS methodology. Trait-LS signifies change in lumbar spine BMD and FN signifies change in femoral neck BMD; A, signifies allele (G for guanosine and A for adenine), AF signifies allele frequency. The p values for association, beta statistics and their 95% CIs are shown. The I² value indicates heterogeneity between the discovery and replication cohorts and the I² p value indicates if the p value for significance of the heterogeneity statistic.

AF, allele frequency; BMD, bone mineral density; Chr, chromosome; GWAS, genome wide association analysis; SNP, single nucleotide polymorphism.

($p=0.079$), a significant association with previous treatment ($p=0.001$) but no significant genotype–treatment interaction ($p=0.796$). At the femoral neck site, there was a significant association with rs73056959 genotype ($p<0.001$), no significant association with previous treatment ($p=0.184$) but a significant genotype–treatment interaction ($p=0.018$). At the total hip site, there was a significant association with rs73056959 genotype ($p<0.001$), no association with previous treatment ($p=0.432$) and no genotype–treatment interaction ($p=0.139$). Taken together, these data indicate that at the lumbar spine and total hip, there is a significant association between rs6430612 genotype and previous treatment on the response of spine BMD but no genotype–treatment interaction. For rs73056959, change in total hip BMD was associated with genotype but not with previous treatment and there was no interaction. For femoral neck BMD genotype–treatment interactions were observed with both rs6430612 and rs73056959.

To explore the possibility that other lifestyle and demographic variables such as smoking, alcohol use, dietary calcium intake, self-reported exercise, body mass index, sex, age and baseline serum 25(OH)D levels might have differed between genotype response groups, we studied these factors in relation to rs6430612 and rs73056959 genotypes which were predictors of percent change in lumbar spine BMD and hip BMD, respectively (online supplemental tables 2 and 3). The results did not show significant differences in these variables according to genotype with the exception of rs6430612 where GG homozygotes who

responded least well to TPTD had a higher dietary calcium intake than the other groups. We speculate that this difference was a chance finding which was unrelated to a poor TPTD response.

DISCUSSION

Teriparatide is an effective treatment for osteoporosis.¹⁴ It is particularly valuable in those with severe spinal osteoporosis complicated by vertebral fractures and glucocorticoid-induced osteoporosis where randomised trials have shown to be more effective than oral bisphosphonates at preventing vertebral fractures.^{6 15}

In some countries, access to TPTD therapy is limited to individuals who have had an inadequate response to standard therapies because costs are considerably higher than bisphosphonates. This was the case in Slovenia at the time participants were recruited where use of TPTD was largely restricted to patients who had not responded adequately to standard therapy. Recent clinical guidelines have recommended that TPTD should be considered as first-line therapy in postmenopausal women with vertebral fractures because it is more effective than standard care with bisphosphonates in preventing new vertebral fractures.^{16 17} Although TPTD is more effective than oral bisphosphonates in this situation, there is greater burden for the patient in that the standard course of therapy involves daily self-administered subcutaneous injections for a 2-year period. Reflecting this fact, a previous audit based in the Edinburgh centre reported that 15.8% of patients who were offered TPTD therapy declined because they were unwilling to self-inject.¹⁸

The individual treatment response to TPTD is known to be variable.⁸ In a previous study of 312 TPTD-treated patients, we reported that the average increase in spine BMD was 13.7%, with an SD of 9.7%, reflecting the fact that some patients experience a very robust increase in spine BMD with TPTD, whereas for a sizeable proportion, the increase is no greater than with an oral bisphosphonate.⁷ In view of this, it would be of clinical value to be able to inform patients about how well they are likely to respond when treated with TPTD, so that they can make a more informed decision on treatment choice.¹⁹

To try and facilitate this, previous research has been conducted aiming at predicting the response to TPTD. In one study, an inverse correlation between body mass index and response of spine BMD to TPTD treatment was observed⁸ but this was not confirmed in another study.⁷ Changes in serum levels of the biomarker PINP measured after 3 months of TPTD therapy have been associated with BMD response at 2 years of treatment²⁰ but this does not help clinicians to identify patients who would benefit from the therapy before starting it.

Here, we have used a pharmacogenomic approach to identify possible genetic determinants of response to TPTD in real-world

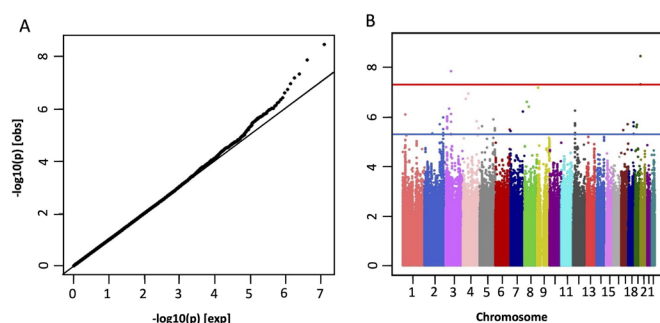


Figure 2 Genome wide association study for response of femoral neck BMD to teriparatide. (A) Q-Q-plot for imputed and genotyped SNPs associated with the percentage change in femoral neck BMD following teriparatide treatment. (B) Manhattan plot showing the signals associated with the response. The variant on chromosome 3 was not considered further according to the protocol since it was derived from a single SNP. The red line shows the threshold for genome wide significance ($p=5\times 10^{-8}$), and the blue line shows the suggestive threshold for genome wide significance $p=5\times 10^{-6}$. BMD, bone mineral density; SNP, single nucleotide polymorphism.

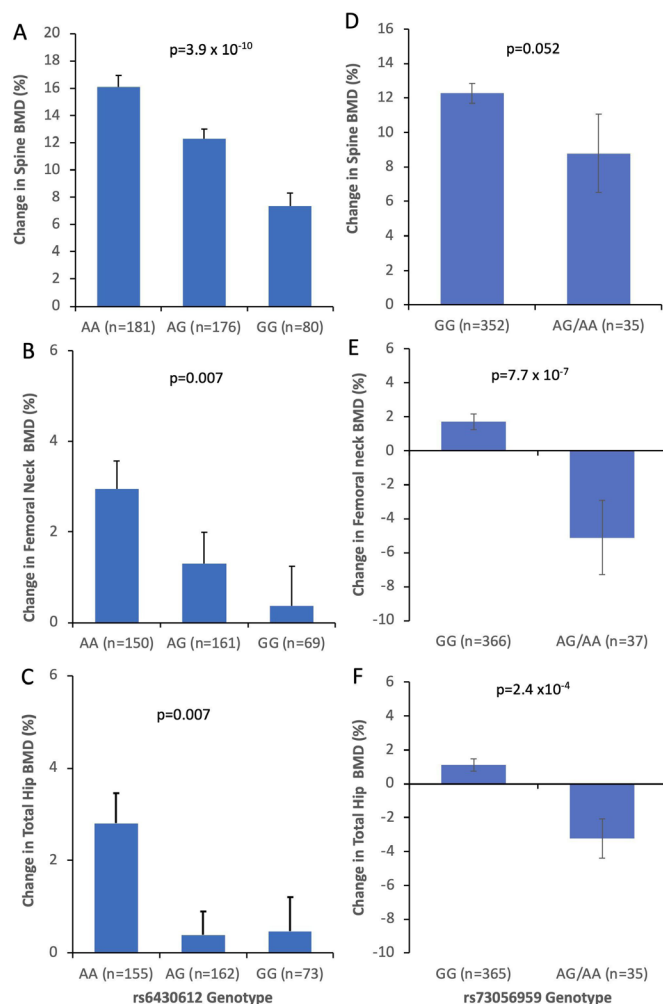


Figure 3 Relation between allelic variants at the genome wide significant hits and percentage changes in BMD following teriparatide treatment at the lumbar spine, femoral neck and total hip. Changes in BMD in relation to allelic variants at rs6430612 are shown in A–C. Changes in BMD in relation to alleles at rs73056959 locus are shown in D–F. Data from one subject who was a AA homozygote at the rs73056959 locus was combined with AG heterozygotes for the purpose of statistical analysis. Note that the numbers for each genotype type differ between panels due to the fact that hip measurements were not available in all subjects due to technical factors. Values in the graphs are means \pm SEM. BMD, bone mineral density.

clinical practice. We included patients who had received TPTD as primary therapy for severe osteoporosis and those who had responded inadequately to antiresorptive therapy with bisphosphonates. We found a genome wide significant locus for response of lumbar spine BMD to TPTD treatment, tagged by the rs6430612 SNP on chromosome 2 and a genome wide significant locus for response of femoral neck BMD to treatment, tagged by the rs73056959 SNP on chromosome 19.

Because many patients had previously been treated with bisphosphonates, we performed an interaction analysis to determine to what extent previous bisphosphonate therapy had influenced the response to TPTD therapy for the SNP that reached genome wide significance. As expected,^{12 21} we found that the increase in spine BMD was influenced by previous bisphosphonate treatment but we found that there was no genotype–bisphosphonate interaction for rs6430612 alleles, which were associated with response of spine BMD to TPTD at a genome

wide significant level. A similar pattern was found in relation to rs73056959 alleles and response of total hip BMD where there was no significant genotype–bisphosphonate interaction. At the femoral neck, there was a significant genotype–bisphosphonate interaction ($p = 0.018$) although the statistical strength of this association was much weaker than that for the allelic association with change in BMD ($p < 0.001$). Although our data confirm that previous bisphosphonate treatment influences response to TPTD, our results indicate that the allelic association observed at both rs6430612 for change in spine BMD and rs73056959 for change in hip BMD applies both to bisphosphonate naïve and bisphosphonate-treated patients.

The allelic variants at the chromosome 2 locus were of particularly large effect size with a 50% greater increase in spine BMD in homozygotes for the A allele as compared with homozygotes for the G allele. Interestingly, variants at rs6430612 were also predictive of response of femoral neck and total hip BMD even though this SNP was not identified as a predictor of change in femoral neck BMD at the GWAS stage.

Allelic variants at the chromosome 19 also showed a large effect on the change of femoral neck BMD in that carriers of the G allele showed an increase in BMD at femoral neck and total hip whereas carriers of the A allele exhibited bone loss at both sites. Bone loss at the upper femur is recognised to occur in a proportion of patients treated with TPTD^{12 22 23} and our observations suggest that genotyping at rs73056959 may provide a biomarker for this phenomenon.

While the findings reported here are of potential clinical interest, the study had several limitations. The sample size was small for a GWAS-based approach but despite this we identified two loci for treatment response which exceeded the threshold for genome wide significance. In this regard, a previous systematic review by Maranville and Cox²⁴ reported that the effect size for pharmacogenetic phenotypes was twice as large as for other phenotypes. They attributed this to the fact that these are phenotypes that represent interaction effects between biological measurements and drug treatment.²⁴ A second weakness is that we did not formally assess the effects of adherence with treatment on responses but we estimated that adherence was at least 90% for the participants included in the study. We cannot completely exclude the possibility that small differences in adherence may have occurred, but this would be expected to reduce the significance of the associations observed rather than cause false positive associations. A third weakness was the fact that we included participants who had received treatment with TPTD for both 18 months and 24 months. This variation in duration of treatment was, however, determined at random, due to a change in the product licence for TPTD mandated by the European Medicines Agency in July 2008. To compensate for the difference in treatment duration, we corrected the duration of treatment in each individual as part of the GWAS analysis. A fourth weakness was the fact that the study was performed in centres from three different European Countries, but the GWAS was corrected for centre and for two principal components to adjust for population-based differences in genetic background of the study populations to compensate for this.

A fifth limitation is that we did not have comprehensive information on lifestyle variables in all individuals that might potentially have affected treatment response. We had information on all individuals for age, sex, body mass index and current smoking status and these did not differ by genotype group for the SNP associated with TPTD response. We had information on alcohol intake, dietary calcium intake and 25(OH)D levels in most individuals and these also did not differ by genotype group except

that dietary calcium intake was higher in GG homozygotes at rs6430612 who responded least well to TPTD, but this lifestyle factor did not differ for genotypes at rs73056959. We feel it is implausible that the higher dietary calcium in the GG genotype group at rs6430612 could have been responsible for the poorer response of spine BMD and it is likely that the statistical difference observed could have occurred by play of chance. Data were available for physical activity and participation in sports for about 50% of subjects from the Edinburgh cohort. Like the other variables, these did not differ significantly according to genotype group. Taken together, these data indicate that is very unlikely that environmental confounding factors played a significant role in the associations we observed.

While the observations made in this study have clinical relevance both in patients who have previously been treated with bisphosphonates and those who have not, the mechanisms underlying the associations reported will require further studies. Such studies, while relevant to offering insights into the possible mechanisms responsible for the associations we observed, would be beyond the scope of the present paper. The top hit SNP for response of lumbar spine BMD was close to the *CXCR4* gene and rs6430612 was an eQTL for this gene ($p=0.01$). Allele A of rs6430612 associated with good response to TPTD increased the expression of *CXCR4* in blood.²⁵ The *CXCR4* gene encodes a receptor for stromal cell derived factor 1 (SDF-1), which is a chemokine that is widely expressed.²⁶ Conditional deletion of *CXCR4* in osteoblast precursors reduces bone mass in mice,²⁷ and mice lacking *CXCR4* in haematopoietic stem cells exhibit increased bone resorption and enlarged osteoclasts.^{28,29} These observations make *CXCR4* a potentially interesting candidate as a mediator of response to TPTD, but further mechanistic studies in vitro and in vivo will be required to investigate this. Other genes within this locus include *DARS*, *ZRANB3* and *MCM6* but none of these genes is known to have a role in bone metabolism.

The variant on chromosome 19 that was associated with response to TPTD at the femoral neck was in an intergenic region between *PEG3/ZIM2* and *USP29/ZIM3* genes. We found no evidence that this SNP was in a regulatory region on bioinformatic analysis. While none of the genes in this region are known to regulate bone metabolism, some members of the USP (ubiquitin-specific protease) family have been proposed to regulate PTH-induced bone formation.³⁰

The most important outcome of osteoporosis treatment is fracture risk reduction. The study had a relatively small population and inadequate duration of follow-up to investigate genotype effects on fracture risk reduction, but it has recently been demonstrated that increases in BMD with osteoporosis treatments is a very strong predictor of fracture risk reduction.³¹

In summary we have, for the first time, identified genetic variants which are significantly associated with response of spine and femoral neck BMD to the bone anabolic drug TPTD. It will now be of interest to conduct further studies to explore the role which genotyping for these variants might play in selecting patients for TPTD treatment in routine clinical practice.

The most likely scenario to implement these findings clinically would be to offer genotyping as a decision aid to patients being considered for TPTD treatment. They could be asked to rate their likelihood of accepting TPTD blinded to genotype and repeat the question after they know their genotype. An analogous approach has previously been used to examine the influence on knowledge of relative risk reduction versus absolute risk reduction in fracture occurrence with different osteoporosis treatments on patients' likelihood of accepting treatment.¹⁹

The ability to give patients an indication of how well they are likely to respond to TPTD is clinically relevant since the treatment burden of daily TPTD injections is higher than with oral bisphosphonates, annual bisphosphonate infusions and monthly romosozumab injections.³² However, if patients felt that they were likely to be a good responder to TPTD this may help physicians and patients to make a more informed decision which is at the core of patient-centred medicine.

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