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=9.2×10−8 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=3.5×10−6, 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.

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 determine whether TPTD would be the optimal therapy.

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 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 2020 in Aarhus and June 2006 to May 2016 in Ljubljana.

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Osteoporosis

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, 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 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 quantitation 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.

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,13 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 TPTD 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) 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 both 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

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 was 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=4×10−7) and total hip BMD (figure 3F, p=3.3×10−7).

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 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.
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. 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.

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. 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.

The individual treatment response to TPTD is known to be variable. 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. 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.

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 observed but this was not confirmed in another study. 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 treatment 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

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

<table>
<thead>
<tr>
<th>Chr</th>
<th>Trait</th>
<th>SNP</th>
<th>AF</th>
<th>Discovery (n=295)</th>
<th>Replication (n=142)</th>
<th>Combined (n=437)</th>
<th>Genotype</th>
<th>P value</th>
<th>β (95% CI)</th>
<th>Genotype</th>
<th>P value</th>
<th>β (95% CI)</th>
<th>Genotype</th>
<th>P value</th>
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<tbody>
<tr>
<td>2</td>
<td>LS</td>
<td>rs6430612</td>
<td>G</td>
<td>0.38</td>
<td>4.3×10^{-4}</td>
<td>-0.34 (−0.48 to -0.20)</td>
<td>0.39</td>
<td>4.9×10^{-4}</td>
<td>-0.38 (−0.59 to -0.17)</td>
<td>9.2×10^{-9}</td>
<td>-0.35 (−0.47 to -0.23)</td>
<td>0.0</td>
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<tr>
<td>15</td>
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<td>G</td>
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<td>3.7×10^{-4}</td>
<td>-0.32 (−0.50 to -0.15)</td>
<td>0.26</td>
<td>0.001</td>
<td>-0.42 (−0.67 to -0.16)</td>
<td>3.1×10^{-5}</td>
<td>-0.35 (−0.50 to -0.20)</td>
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<tr>
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<td>A</td>
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<td>1.4×10^{-4}</td>
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<td>0.12</td>
<td>0.002</td>
<td>-0.57 (−0.94 to -0.22)</td>
<td>1.6×10^{-6}</td>
<td>-0.44 (−0.62 to -0.26)</td>
<td>0.0</td>
<td>0.39</td>
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<tr>
<td>2</td>
<td>FN</td>
<td>rs10932371</td>
<td>A</td>
<td>0.31</td>
<td>1.1×10^{-4}</td>
<td>0.38 (0.19 to 0.57)</td>
<td>0.27</td>
<td>0.003</td>
<td>0.36 (0.12 to 0.60)</td>
<td>1.1×10^{-6}</td>
<td>0.37 (0.22 to 0.52)</td>
<td>0.0</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>FN</td>
<td>rs73056959</td>
<td>A</td>
<td>0.05</td>
<td>1.4×10^{-4}</td>
<td>-2.25 (−3.16 to -1.34)</td>
<td>0.05</td>
<td>1.4×10^{-4}</td>
<td>-1.28 (−1.94 to -0.62)</td>
<td>3.5×10^{-7}</td>
<td>-1.61 (−2.14 to -1.07)</td>
<td>65</td>
<td>0.09</td>
<td></td>
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</table>

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 rs73056959 and A for adrenalin); AF signifies allele frequency. The p values for association, beta statistics and their 95% CIs are shown. The I^2 value indicates heterogeneity between the discovery and replication cohorts and the F^2 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.

**Figure 2** Genome wide association study for response of femoral neck BMD to teriparatide. (A) QQ-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=5×10^{-8}), and the blue line shows the suggestive threshold for genome wide significance p=5×10^{-6}. BMD, bone mineral density; SNP, single nucleotide polymorphism.
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 total hip BMD to TPTD 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 on chromosome 19 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 ± SEM. BMD, bone mineral density.

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 on chromosome 19 are shown in D–F. Data from one subject who was an 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 ± SEM. BMD, bone mineral density.
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. The CXCR4 gene encodes a receptor for stromal cell derived factor 1 (SDF-1), which is a chemokine that is widely expressed. Conditional deletion of CXCR4 in osteoblast precursors reduces bone mass in mice, and mice lacking CXCR4 in haematopoietic stem cells exhibit increased bone resorption and enlarged osteoclasts. 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.

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.

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.

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. 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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Contributors NA and SHR conceived the study and obtained funding; NA wrote the first draft of the paper and performed the statistical analysis; SHR, AA, BL-P and OMEA contributed to the genetic analysis and interpretation of the results; NA, SHR, AA, KB, PR, BO, TK, JM and BLL contributed to data curation; SHR, PR, BO, TK, JM and BLL contributed to recruitment of participants. All authors critically reviewed the manuscript for intellectual content. All authors approved the final version of the manuscript. SHR is guarantor and accepts full responsibility for the work and/or the conduct of the study, had access to the data and controlled the decision to publish.

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Competing interests BLL has served on advisory boards and received lecture honoraria from Amgen, UCB, Gideon-Richter, Astellas and Astra-Zeneca. She holds research grants from Novo Nordisk and Amgen. SHR holds research grants from Amgen, Eli Lilly and UCB.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the design, or conduct, or reporting, or dissemination plans of this research. Coversations (including but not limited to local

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

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REFERENCES


Supplementary Material

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

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Methods

Sample size

Power calculation was performed in SPSS v27 and the sample size of 437 individuals in this study was sufficient to reach a statistical power of at least 90% for a common allele (frequency = 0.4) with medium sized effect (beta = 0.4).

Genotyping quality control

Standard quality control was performed in each cohort separately using PLINK v1.07 software (http://pngu.mgh.harvard.edu/purcell/plink/) [1] as described previously [2]. Low quality samples (based on call rate, excess of heterozygosity, gender mismatch, European ancestry and cryptic familial relationships) and low-quality SNP results (based on call rate, deviation from Hardy-Weinberg equilibrium, frequency of the minor allele and missingness degree) were excluded from the analysis (Suppl Figure S3). Only individuals with clinical information for BMD at baseline and at the end of treatment were selected for further analysis. Subsequently, the data from all participants were combined and subjects were allocated at random to the discovery cohort (66% of individuals) or replication cohort (33% of individuals). Genotyping cluster plots were manually inspected to only select significant and suggestive SNPs with high quality genotyping data.

Imputation

Imputation was performed separately in the discovery and replication cohorts, using the Michigan Imputation Server (https://imputationserver.sph.umich.edu) [3]. This is an open source for free genotype imputation based on Minimac3 v2.0.1 that uses 1000Genomes phase 3 v5 as reference panel. Quality control was performed and SNPs with imputation \( r^2 < 0.3 \) and MAF<0.05 were excluded in each dataset.

Genome wide Association analysis

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 genome wide association analysis in PLINK. Following implementation of quality control measures outlined above, 594,480 SNPs from 295 patients were available for the discovery stage with regard to changes in lumbar spine BMD by GWAS and 594,474 SNPs from 270 patients were available for the discovery stage for changes in BMD at the femoral neck site by GWAS. The discovery and replication GWAS were independently tested for association by PLINK software [1] using linear regression. After imputation and quality control, selected SNPs from the
discovery and replication datasets were independently tested for association using SNPTEST v2.4.1 software [4] and the frequentist additive test.

**Meta-analysis**

Results from the discovery and replication analysis were combined by meta-analysis using METAL software version released in 2011 [5] and corrected by the genomic inflation factor $\lambda$. Autosomal SNPs with MAF>0.05 were meta-analysed using the fixed-effect, inverse-variance meta-analysis of the software. SNPs with excess of heterogeneity ($I^2 \leq 0.05$), standard error <0.01 and minor allele frequency <0.01 were removed for the analysis.

Regional associations were obtained from the significant loci by LocusZoom v0.4.8 software [6]. The nearest gene located within the recombination area of the SNP, as the region within two recombinant peaks based on the linkage disequilibrium principle, was reported as the candidate gene.

**Allele response to TPTD**

The SNPs with p value < 5x10^{-8} (Bonferroni’s correction for multiple testing) at meta-analysis were selected to generate an allele score for changes in BMD in response to TPTD treatment. Singletons, defined as SNPs with no neighbouring markers in strong linkage disequilibrium were excluded. Statistical analysis was performed by ANOVA with Welch’s correction and Mann-Whitney non-parametric t-test in SPSS v26. GraphPad Prism v9.2 was used to plot the allele score.

**Conditional analysis**

Since the top hit SNP rs6430612 for spine BMD was in the same region of the genome as the Lactase gene (LCT) which is known to affect bone density, conditional analysis was performed for the SNPs rs3213890 and rs746857 which are situated within the Lactase gene (LCT) [1]. These SNPs were selected for the conditional analysis because they are in complete LD ($r^2=1$) with rs1042712, an established functional LCT variant [7] which was not present in the current GWAS. Linkage disequilibrium was checked using LDlink website (https://ldlink.nci.nih.gov) [8]. Cryptic signals within the most significant locus were browsed by conditional analysis on the top SNP using PLINK.

**Expression quantitative trait locus analysis**

Intragenic SNPs were tested for expression quantitative trait locus (eQTL) analysis using publicly available RNA data from peripheral blood, using the eQTLGen (https://www.eqtlgen.org) [9], GTEx (https://gtexportal.org/) [10], and FIVEx (https://fivex.sph.umich.edu) resources [11].
**Supplementary Table S1. Response of BMD to TPTD treatment in discovery and replication cohorts.**

<table>
<thead>
<tr>
<th>Discovery</th>
<th>Edinburgh</th>
<th>Denmark</th>
<th>Slovenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>145</td>
<td>57</td>
<td>93</td>
</tr>
<tr>
<td>Age</td>
<td>69 ± 9</td>
<td>68 ± 9</td>
<td>69 ± 11</td>
</tr>
<tr>
<td>Female sex</td>
<td>137 (94.5%)</td>
<td>44 (77.2%)</td>
<td>91 (97.8%)</td>
</tr>
<tr>
<td>TPTD 18 months</td>
<td>55 (37.9%)</td>
<td>23 (40.3%)</td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>TPTD 24 months</td>
<td>90 (62.1%)</td>
<td>34 (59.6%)</td>
<td>92 (97.9%)</td>
</tr>
<tr>
<td>Change in spine BMD (%)</td>
<td>15.75 ± 7.89</td>
<td>10.09 ± 8.03</td>
<td>6.96 ± 8.88</td>
</tr>
<tr>
<td>Change in femoral neck BMD (%)</td>
<td>2.30 ± 8.60</td>
<td>1.17 ± 8.26</td>
<td>1.15 ± 8.66</td>
</tr>
<tr>
<td>Change in total hip BMD (%)</td>
<td>1.34 ± 7.55</td>
<td>1.52 ± 4.87</td>
<td>0.25 ± 7.78</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Replication</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>69</td>
<td>28</td>
<td>45</td>
</tr>
<tr>
<td>Age</td>
<td>70 ± 8</td>
<td>69 ± 7</td>
<td>68 ± 11</td>
</tr>
<tr>
<td>Female sex</td>
<td>64 (92.7%)</td>
<td>20 (71.4%)</td>
<td>45 (100%)</td>
</tr>
<tr>
<td>TPTD 18 months</td>
<td>31 (44.9%)</td>
<td>13 (46.4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>TPTD 24 months</td>
<td>38 (55.1%)</td>
<td>15 (53.6%)</td>
<td>45 (100%)</td>
</tr>
<tr>
<td>Change in spine BMD (%)</td>
<td>15.29 ± 9.44</td>
<td>10.01 ± 9.53</td>
<td>6.21 ± 8.17</td>
</tr>
<tr>
<td>Change in femoral neck BMD (%)</td>
<td>2.07 ± 5.79</td>
<td>3.52 ± 6.97</td>
<td>0.92 ± 8.75</td>
</tr>
<tr>
<td>Change in total hip BMD (%)</td>
<td>2.06 ± 5.31</td>
<td>4.02 ± 5.64 *</td>
<td>-0.55 ± 5.56</td>
</tr>
</tbody>
</table>

Values are numbers and percentages or means ± SD. TPTD = teriparatide, BMD = bone mineral density. The only significant difference between discovery and replication cohorts was with respect to change in total hip BMD in the Danish cohort where the p-value for the difference was \( p=0.042 \).
Supplementary Table S2.
Lifestyle and demographic variables in relation to rs6430612 genotypes

<table>
<thead>
<tr>
<th>Variable</th>
<th>AA genotype (n=181)</th>
<th>AG genotype (n=176)</th>
<th>GG genotype (n=80)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>69 ± 8</td>
<td>69 ± 10</td>
<td>69 ± 10</td>
<td>0.709</td>
</tr>
<tr>
<td>Female</td>
<td>169 (91.7%)</td>
<td>159 (90.3%)</td>
<td>75 (93.7%)</td>
<td>0.658</td>
</tr>
<tr>
<td>Male</td>
<td>15 (8.3%)</td>
<td>17 (9.7%)</td>
<td>5 (6.3%)</td>
<td></td>
</tr>
<tr>
<td>BMI (Kg/M²)</td>
<td>24.3 ± 4.8</td>
<td>24.6 ± 4.5</td>
<td>25.1 ± 3.9</td>
<td>0.368</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>67.1 ± 33.2</td>
<td>69.9 ± 28.5</td>
<td>64.2 ± 25.6</td>
<td>0.362</td>
</tr>
<tr>
<td>Dietary calcium (mg/day)</td>
<td>883 ± 275</td>
<td>924 ± 272</td>
<td>1053 ± 251</td>
<td>1.0x10^-5</td>
</tr>
<tr>
<td>Current smoker</td>
<td>34 (19.3%)</td>
<td>35 (19.8%)</td>
<td>19 (23.7%)</td>
<td>0.710</td>
</tr>
<tr>
<td>Alcohol intake (u/week)</td>
<td>0 (0 – 7)</td>
<td>1.5 (0 – 7)</td>
<td>4 (0 – 10)</td>
<td>0.672</td>
</tr>
<tr>
<td>Daily physical activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>29/110 (26.4%)</td>
<td>31/86 (36%)</td>
<td>7/18 (38.9%)</td>
<td></td>
</tr>
<tr>
<td>Ambulant</td>
<td>81/110 (26.4%)</td>
<td>55/86 (64%)</td>
<td>11/18 (61.9%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Participation in sports</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>54/110 (49.0%)</td>
<td>46/86 (52.32%)</td>
<td>12/18 (66.6%)</td>
<td></td>
</tr>
<tr>
<td>Low impact</td>
<td>55/110 (50%)</td>
<td>40/86 (46.5%)</td>
<td>5/18 (5.5%)</td>
<td>0.38</td>
</tr>
<tr>
<td>High impact</td>
<td>1/110 (0.9%)</td>
<td>4/86 (4.6%)</td>
<td>1/18 (5.5%)</td>
<td></td>
</tr>
</tbody>
</table>

The p-values are derived from one way ANOVA across groups with Welch’s correction for continuous variables or X² test for categorical variables. Continuous variables are shown as N, mean ± standard deviation, except alcohol intake, which was shown as N, median (interquartile range). Categorical variables are shown as N (%). Data were available for age, sex, BMI and current smoking on all individuals. Data were available on 311 (71%) subjects for 25(OH)D; 430 (98%) for dietary calcium intake; 299 (68.4%) for alcohol intake; 214 (49%) for physical activity and 218 (50%) for participation in sports.


**Supplementary Table S3.**

Lifestyle and demographic variables in relation to rs73056959 genotypes

<table>
<thead>
<tr>
<th>Variable</th>
<th>GG genotype (n=366)</th>
<th>AG/AA genotype (n=37)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>69 ± 9</td>
<td>71 ± 9</td>
<td>0.170</td>
</tr>
<tr>
<td>Female</td>
<td>334 (91.3%)</td>
<td>32 (86.5%)</td>
<td>0.365</td>
</tr>
<tr>
<td>Male</td>
<td>32 (8.7%)</td>
<td>4 (11.1%)</td>
<td></td>
</tr>
<tr>
<td>BMI (Kg/M²)</td>
<td>24.8 ± 4.99</td>
<td>26.5 ± 6.8</td>
<td>0.154</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>66.2 ± 27.7</td>
<td>73.4 ± 24.7</td>
<td>0.176</td>
</tr>
<tr>
<td>Dietary calcium (mg/day)</td>
<td>952 ± 273</td>
<td>912 ± 250</td>
<td>0.247</td>
</tr>
<tr>
<td>Current smoker</td>
<td>76 (21.2%)</td>
<td>6 (16.2%)</td>
<td>0.670</td>
</tr>
<tr>
<td>Alcohol intake (u/week)</td>
<td>0 (0–7)</td>
<td>0 (0–9.5)</td>
<td>0.434</td>
</tr>
<tr>
<td>Daily physical activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>67/162 (41.4%)</td>
<td>8/11 (73%)</td>
<td>0.042</td>
</tr>
<tr>
<td>Ambulant</td>
<td>95/162 (58.6%)</td>
<td>3/11 (27%)</td>
<td></td>
</tr>
<tr>
<td>Participation in sports</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>80/162 (49.4%)</td>
<td>9/11 (81.8%)</td>
<td>0.058</td>
</tr>
<tr>
<td>Low impact</td>
<td>79/162 (48.8%)</td>
<td>2/11 (18.2%)</td>
<td></td>
</tr>
<tr>
<td>High impact</td>
<td>3/162 (1.9%)</td>
<td>0/11 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

The p-values are derived from one way ANOVA with Welch’s correction across groups for continuous variables or \( \chi^2 \) test for categorical variables. Continuous variables are shown as N, mean ± standard deviation, except alcohol intake, which was shown as median (interquartile range). Categorical variables are shown as N (%). Data were available for age, sex, BMI, dietary calcium intake and current smoking on all individuals. Data were available on 311 (77.1%) subjects for 25(OH)D; for 299 (74.1%) on alcohol intake; for 184 (45.6%) on physical activity and 170 (41.1%) for participation in sports.
Supplementary Figure S1. Regional association plot of the chromosome 2 locus for response of lumbar spine BMD to TPTD.

The red horizontal line shows the genome-wide significant threshold ($5 \times 10^{-8}$).

$p = 4.09 \times 10^{-9}$
Supplementary Figure S2. Regional association plot of the chromosome 19 locus for response of femoral neck BMD to TPTD.

The red horizontal line shows the genome-wide significant threshold \(5 \times 10^{-8}\).
Supplementary Figure S3.

Flowchart on the selection of the samples for the meta-analysis of lumbar spine and femoral neck BMD in each cohort

- Edinburgh: N = 281
  - Sample call rate
  - Missing genotype data
  - Excess of heterozygosity
  - European ancestry
  - Cryptic familial relationship
  - Missing clinical information or scanning artifacts

- Denmark: N = 123
  - Number not specified

- Slovenia: N = 198
  - Sample call rate
  - Missing genotype data
  - Excess of heterozygosity
  - European ancestry
  - Cryptic familial relationship
  - Missing clinical information or scanning artifacts

Total N = 602

- LSBMD = 214
  - LSBMD = 85
    - LSBMD = 138
      - LSBMD = 146
  - LSBMD = 173
  - LSBMD = 84
  - LSBMD = 146
  - LSBMD = 437
  - LSBMD = 403

Total N LSBMD = 437
Total N FNBMD = 403
References

7. Wellcome Trust Case Control C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661-78.