Hyperthyroidism, and bone mineral density : dissecting the causal association with Mendelian randomization analysis

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Abstract

Introduction

Untreated hyperthyroidism is associated with accelerated bone turnover, low bone mineral density (BMD) and increased susceptibility to fragility fractures. Although treatment appears to improve or even reverse some of these adverse skeletal effects, there is limited guidance on routine BMD assessment in hyperthyroid patients following treatment. By using Mendelian randomization (MR) analysis, we aimed to assess the causal association of hyperthyroid thyroid states with BMD and fractures using the UK Biobank.

Methods

This MR analysis included data from 473,818 participants (women: 54% of the total sample, the median age of 58.0 years (IQR=50-63 years), median body mass index (BMI) of 26.70 (IQR+24.11-29.82 kg/m^2) as part of the UK biobank study. The study outcomes were heel BMD assessed by quantitative ultrasound of the heel and self-reported fractures. Beta-weighted genetic risk score analysis was performed using 19 Single Nucleotide Polymorphisms (SNPs) for Graves' disease, 9 SNPs for hyperthyroidism and 11 SNPs for autoimmune thyroiditis. Since the unadjusted risk score, MR is equivalent to the inverse-variance weighted method; the genetic risk score analysis was adjusted for age, gender, and BMI. Sensitivity analyses were conducted using the Mendelian randomization-Egger (MR-Egger) and the inverse-variance weighted estimate methods. Replication analysis was performed using the GEnetic Factors for Osteoporosis (GEFOS) consortium data.

Results

MR analysis using beta-weighted genetic risk score showed no association of genetic risk for Graves' disease (Beta = -0.01, P-value=0.10), autoimmune thyroiditis (Beta = -0.006 P-value=0.25) and hyperthyroidism (Beta = -0.009, P-value=0.18) with heel ultrasound BMD.

MR Egger and inverse-variance MR methods in UK Biobank and GEFOS consortium confirmed these findings. The genetic risk for these hyperthyroid conditions was not associated with an increased risk of fractures.

Conclusion

Our study shows that excess genetic risk for Graves' autoimmune thyroiditis and hyperthyroidism does not increase the risk for low BMD and is not associated fractures in the Caucasian population. Our findings do not support routine screening for osteoporosis following definitive treatment of hyperthyroid states.

Introduction

Hyperthyroidism, characterized by excess thyroid hormone synthesis and secretion from the thyroid gland, affects approximately 1 in 100 individuals [1-3]. Graves' disease and multinodular goitre are the most common causes of hyperthyroidism in young/middle-aged and older individuals respectively [2, 3]. Untreated overt hyperthyroidism has well-documented skeletal consequences including low bone mineral density (BMD), secondary osteoporosis, and increased fragility fracture risk, which result from high and more frequent initiation of bone turnover (i.e., simultaneous increases in bone formation and resorption) [4-11]. Impaired bone health has also been shown among patients with subclinical hyperthyroidism (TSH concentrations below the normal reference range and free T3 and T4 levels within the normal reference range) [12-14] and euthyroid individuals with relatively low TSH and relatively high free T4 levels, albeit within the reference range [15, 16]. The treatment options for hyperthyroidism commonly include antithyroid drugs, radioactive iodine therapy, and near-total or total thyroidectomy [1, 17]. Although some studies have shown increases in bone formation, improvements in BMD and reduction in fracture risk [5, 17-20], others suggest persistent, low BMD even after treatment, especially in populations at risk of osteoporosis such as postmenopausal women [17, 21, 22].

Current guidelines for the diagnosis and management of osteoporosis (National Institute for Clinical Excellence NICE) clinical guideline CG146, ESCEO and IOF [23], Scientific Advisory council of osteoporosis Canada [24], American Association of Clinical Endocrinologists/American College of Endocrinology [25] recommend estimation of the absolute risk of the fracture using the FRAX score. The clinical risk factors included in the FRAX score do not consider patients treated for hyperthyroidism, and hence, these patients are likely to obtain a low FRAX score. As a result, patients who have received or are currently receiving treatment for hyperthyroidism are unlikely to be offered routine screening (i.e., a

dual-energy X-ray absorptiometry [DXA] scan) for monitoring changes in BMD and osteoporosis status. As such, it is critical to understand whether populations with imbalances in thyroid hormones are susceptible to ongoing bone loss, once they have definitive treatment for their hyperthyroidism. While available population-based studies have shown at least partial BMD recovery [5, 17-22], these are often limited by low statistical power, cross-sectional design or short-term follow-up and are confounded by factors such as age, sex, body mass index (BMI), presence of other endocrine disorders, lifestyle factors (e.g., physical activity, smoking and alcohol consumption) or concurrent use of medications, which can affect BMD. These confounding factors can be overcome to some extent by Mendelian randomization analysis [26], which can uncover causal relationships between selected thyroid disorders and BMD, whilst avoiding reverse causality (i.e., the disease cannot affect genotype) by using Mendel's Laws of Inheritance [27]. This law postulates that alleles segregate randomly from parents to offspring [28] and thus, offspring genotypes are randomly distributed in the population and are unlikely to be associated with confounders. An assessment of the association of excess genetic risk for hyperthyroid states such as Graves' with BMD and fractures, will help in understanding if these states have a direct causal role in lowering of the BMD and increasing risk of fractures. If such an association exists, it will support careful monitoring of BMD and periodic basement of fracture susceptibility in these patients. However, if there is no such association, it would support the current NICE guidelines.

The aim of the present investigation was to assess the effects of genetic susceptibility to hyperthyroidism on BMD and history of fragility fractures using the UK Biobank data and data from the GEnetic Factors for OSteoporosis (GEFOS) Consortium [29].

Methods

Study population

UK Biobank is a prospective cohort of 502,635 participants (5.5% response rate) aged 40-69 years. All participants were recruited between 2006 and 2010 and attended one of the 22 assessment centres across UK, where they provided information on sociodemographic, lifestyle and health parameters and underwent physical and medical assessments [30]. Blood samples were also collected for genotyping and biochemical analyses. UK Biobank participants were linked to their hospital inpatient, cancer-registry and death registry data. Both genotype data and phenotype data (BMD) were available for 473,818 participants.

The UK Biobank protocol complied with the Declaration of Helsinki and was approved by the North West Multi-Centre Research Ethics Committee. Participants provided their informed consent on the touchscreen before taking part. The UK Biobank protocol is available online (http://www.ukbiobank.ac.uk/wp-content/uploads/2011/11/UK-Biobank-Protocol.pdf). Additional details of the UK Biobank have been previously published.[30]

Quantitative heel ultrasound and history of fractures

Quantitative ultrasound of the heel was performed using the Sahara Clinical Sonometer (Hologic, Bedford, Massachusetts) according to a standardized protocol. Trained staff checked if participants were able to undergo both left and right heel ultrasound measurement. Those with open wounds, breaks or sores around the heel, or metal parts (such as pins) in the heel did not undertake measurement of that heel. Each centre used the same machine model, and quality control was performed daily with a phantom according to the manufacturer's instructions. The mean values for BMDs (g/cm^2) and BMD T-scores for the left and right heel were computed. The BMD data were available for both the left or right heal; we included the lower value of BMD in the analysis. History of fractures was assessed by self-reported data on fracture occurrence (yes or no) over the past 5 years (n=50,729).

Power calculation

The power calculation was carried out with publicly available power calculator for Mendelian randomization study available at https://shiny.cnsgenomics.com/mRnd/. The power calculation was performed for a definitive outcome, all-cause fractures in the UKbiobank study. A sample size of 473818 with around 10% prevalence of all-cause fractures (at the level of significance 0.05 and with 1% variance explained for the association between allele score and exposure variable) gives more than 50% power to detect an odds ratio of 1.1 and more than 90% power to detect an odds ratio of 1.2.

Replication Cohort

We used publicly available data from GWAS for BMD from the GEFOS consortium, which identified novel loci for BMD at the femoral neck, lumbar spine, and forearm; sites of common osteoporotic fractures. Forearm BMD data were not used in the present study because of the relatively low number of participants (n = 8143). We did not include the femoral neck BMD as the discordance rate between the femoral neck, and lumbar spine BMD was as high as 33%, and in older patients with osteoporosis assessment of lumbar spine, BMD is preferred [29]. The mendelian randomization (MR) analysis conducted on lumbar spine BMD comprised 28,498 participants from eight cohorts of European ancestry. The mean age of the participants in the available cohorts ranged from 17.7 to 80.2 years, and 34% of the participants in the meta-analysis were men.

SNP Instrument selection

We used the NGHRI-EBI catalogue of GWAS studies to identify uncorrelated SNPs associated with Graves' disease, hyperthyroidism and autoimmune thyroiditis with P-value $<10^{-5}$. As of September 2018, the Catalogue contains 5687 GWAS comprising 71,673 variant-trait

associations from 3567 publications [31]. We identified 19 independent loci for Graves' disease [32-35], 9 independent loci for hyperthyroidism [36] and 11 independent loci for autoimmune thyroid disease [32] (Supplementary Table 1). We used genetic instruments for Graves' disease, patients with hyperthyroidism due to other causes, and patients with autoimmune thyroiditis (including Hashimoto's thyroiditis which in some cases is characterized by transient hyperthyroidism or hashitoxicosis), to cover most causes of hyperthyroidism.

Risk-score based MR analysis To assess the effect of genetic predisposition to Graves' disease, hyperthyroidism and hypothyroidism on BMD we constructed a beta-weighted (wGRS) genetic risk score using 19 independent SNPs for Graves' disease, 9 independent SNPs for hyperthyroidism and 11 independent SNPs for autoimmune thyroiditis. The wGRS was calculated by multiplying each known β-coefficient (from NHGRI catalogue) for each phenotype by the number of corresponding risk alleles using Plink. The weighted genetic risk score wGRS was adjusted for age, gender, BMI and top five principal components. Sensitivity analyses was performed in the self-reported Caucasian population in the UKbiobank database. In order to correct for multiple testing in the wGRS analysis, Bonferroni correction was applied a P-value of (0.05/12) 0.004 was considered statistically significant.

Replication and sensitivity analyses for Mendelian randomization

To explore potential pleiotropic effects, we carried out three sensitivity meta-analyses: simple and weighted median and Mendelian-randomisation-Egger regression methods using the R Program MendelianRandomization [27, 37]. Simple and weighted median MR analysis provide estimations that are robust to the inclusion of up to 50% invalid instruments in a Mendelian randomization analysis[38].

Results

The MR analysis was performed on 473,818 participants from the UK biobank with 54% females, with a median age of 58.00 (IQR=50-63), median BMI of 26.70 (IQR=24.11-29.82) and median BMD of 0.449 g/cm^2(IQR=044-0.53). In the entire study cohort, 50729 individuals reported at least one incidence of fracture in the last five years. **Supplementary Tables 2 3 and 4** show the association of the known NGHRI SNPs for Grave's disease, hyperthyroidism and autoimmune thyroid disease with BMD.

Genetic risk score-based MR analysis for BMD and fractures

We performed a genetic risk score based MR analysis for BMD and fractures in UKbiobank population. To account for multiple testing, a Bonferroni corrected P-value of 0.004 was considered statistically significant.

MR analysis using beta-weighted genetic risk score adjusted for age, gender and BMI and top 5 principle component showed no association of genetic risk for Graves' disease (Beta = -0.01 P-value=0.10), autoimmune thyroiditis (Beta = -0.006 P-value=0.25) and hyperthyroidism (Beta = -0.009 P-value=0.18) with BMD. Also, the MR analysis using beta-weighted genetic risk score showed no association of genetic risk for Graves' disease (OR= 1.001, P-value=0.92), autoimmune thyroiditis (OR=0.99, P-value=0.25) and hyperthyroidism (OR=1.03 ,P-value=0.14) with self reported fractures in the last five years.

Sensitivity analysis in Caucasian population

We performed a sensitivity analysis with a genetic risk-score based MR in self-reported Caucasian population (n=409633) with GWAS data. MR analysis in the caucasian population using beta-weighted genetic risk score adjusted for age, gender and BMI and top 5 principle component showed no association (P<0.004) of genetic risk for Graves' disease (Beta = -0.01 P-value= 0.02), autoimmune thyroiditis (Beta = -0.01 P-value= 0.34) and hyperthyroidism

(Beta = -0.007 P-value= 0.34) with BMD. Also, the MR analysis using beta-weighted genetic risk score showed no association of genetic risk for Graves' disease (OR=1.01, P-value=0.6), autoimmune thyroiditis (OR=1.02, P-value=0.87) and hyperthyroidism (OR=0.98, P-value=0.31) with self-reported fractures in the last five years.

Replication analysis in GEFOS consortium using MendelianRandomization

Figure 1a 1b and 1c show the results of causal effects of hyperthyroid states on BMD estimated using each SNP separately in the UKBiobank data. We also show the analysis in both UKBiobank and GEFOS consortium using MR Egger, weighted median and IVW methods. In the UKBiobank data, the MR estimates using MR Egger method showed no association of genetic risk for Graves' disease (beta=-0.001, P-value=0.967), hyperthyroidism (beta=0.011, P-value=0.847) and autoimmune thyroid disease (beta=0.012, P-value=0.502) with BMD (**Table 1).** In the GEFOS consortium data, the MR estimates using MR Egger method showed no association of genetic risk for Graves' disease (beta=-0.003, P-value=0.928), hyperthyroidism (beta=0.118, P-value=0.161) and autoimmune thyroiditis (beta=-0.025, P-value=0.359) with BMD (**Table 1).** A meta-analysis of the MR-Egger Estimates from UK-biobank, and GEFOS consortium using fixed-effect models showed no association of genetic risk for Graves' disease, hyperthyroidism and autoimmune thyroid disease with bone mineral density (**Supplementary Table 5**).

Discussion

In this MR study, we demonstrated that excess genetic risk for Graves' disease, hyperthyroidism and autoimmune thyroiditis is not associated with low BMD or history of fractures in the UK Biobank.

Several epidemiological studies in adults have shown an association between a hyperthyroid status and low BMD [6, 14-16]. For example, a meta-analysis, which assessed alterations in

BMD and fractures risk in patients with hyperthyroidism, demonstrated significant BMD reductions and increased fracture risk in untreated patients [6]. Subclinical hyperthyroidism, individuals with TSH at the lower end and free T4 at the upper end of the normal euthyroid range, have also been associated with unfavourable bone outcomes including more significant bone loss and fracture risk [14-16]. The results of these observational studies do not establish a causal association as they are constrained by confounders such as duration of hyperthyroidism, variations in treatment and response to treatment, and other comorbidities. Evidence from observational and interventional studies on the effects of treatment for hyperthyroidism on bone outcomes has yielded mixed results. Although some studies have shown full recovery of BMD [5, 17-20], some others suggest the only partial restoration of bone mass and lower BMD [17, 21, 22].

Although a causal association between thyroid states and BMD is more likely to be established with MR studies, these remain limited. In a recent study, van Vliet et al. looked at 20 genetic variants that were previously identified for circulating TSH levels and found no evidence that a genetically determined circulating TSH concentration was associated with femoral neck or lumbar spine BMD [39]. They also found no association of variants in the TSHR gene and BMD. In contrast, two earlier genetic studies which explored the association between TSH and BMD found that the Asp727Glu polymorphism in the TSHR gene (rs1991517) was associated with higher BMD [40, 41]. This finding was, however, no longer significant after adjustments for BMI [40, 41], and available studies have failed to identify any further common TSHR genetic variants in association with BMD.

To the best of our knowledge, this is the first MR study looking at the effect of genetic predisposition to treated Graves' hyperthyroidism and autoimmune thyroiditis on BMD. The MR estimates obtained using the MR-Egger, and weighted median analysis were consistent, and do not support a causal association between treated hyperthyroid conditions and BMD.

Further, we did not show an association between hyperthyroid states and fractures, with these findings extending our understanding on the importance of thyroid function in the skeleton in middle-aged and older adults. The lack of association between hyperthyroid states and skeletal fractures may partially reflect the lack of association of thyroid function with BMD. An alternative explanation may be that treated hyperthyroidism is not associated with other skeletal parameters including bone geometry, microstructure and material, or even extra-skeletal characteristics including physical performance or falls [42, 43].

Our study is strengthened by the employment of the MR approach, which lessens systematic biases such as confounders and reverse causality, commonly affecting the results of conventional observational studies [27]. Another strength of our study is that we used data from large-scale genetic databases, which enabled us to explore the associations of hyperthyroidism, BMD and fracture risk in a precise way. Our study also has several limitations. UK biobank does not have information about the treatment received for hyperthyroid states; hence we are unable to adjust for this in the analysis. We performed the replication analysis in the publicly available GEFOS consortium using the Mendelian Randomization program [27, 37] implemented in software R. Since this genotype data has been imputed to HapMap data and not the 1000 genomes data, not all GWAS SNPs associated with underlying thyroid disorders could be used for the replication analysis. This could have led to under- or overestimation of the association of the genetic susceptibility to underlying thyroid disorder and BMD. In this study, we used commonly used measures (inverse variance weighted method, along with the classical effect weighted genetic score analysis) for reporting the results of the MR analysis [44]. It is, however, likely that the MR-Egger and other MR methods are susceptible to bias from weak instruments and are affected by low statistical power [44]. Also, the genetic instrument for the MR analysis was performed in the UK Biobank cohort consisted of participants of predominantly European ancestry, which may reduce generalizability to non-

European populations. Further, participants in the UK Biobank are not representative of the UK population, and there is evidence of a 'healthy volunteer' selection bias, which may have contributed to the null associations observed in the present investigation. Also, our genetic instruments do not capture hyperthyroidism due to solitary toxic and multinodular goitre hence MR analysis cannot assess the effect of these hyperthyroid conditions on BMD. Finally, in the UK Biobank, quantitative ultrasound was used to assess BMD of the heel in nearly the total sample, whereas DXA measurements in the hip/spine were performed only in a subset of participants. Nevertheless, associations between calcaneal quantitative ultrasound, well-established risk factors for osteoporosis and increased fracture risk have been previously shown [45, 46].

Conclusion

In summary, our study does not support a causal association between increased genetic risk for hyperthyroidism and the risk for low BMD. Our results support the current guidelines, which do not recommend routine long term screening for osteoporosis following treatment for hyperthyroidism. However, further MR studies in diverse populations are needed to confirm these findings.

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Data availability statement: The data that support the findings of this study are openly available in the UKBIObank data

Figure 1a 1b and 1c legend: Figure 1a 1b and 1c shows the results of causal effects of hyperthyroid states on BMD estimated using each SNP separately in the UKBiobank data and the MR-Egger and Inverse-variance weighted MR estimates.

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