Universiteit
Leiden
The Netherlands

Differentiated thyroid carcinoma : treatment and clinical consequences of therapy

Hoftijzer, H.C.

Citation
Hoftijzer, H. C. (2011, May 12). *Differentiated thyroid carcinoma : treatment and clinical consequences of therapy*. Retrieved from https://hdl.handle.net/1887/17641

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/17641

Note: To cite this publication please use the final published version (if applicable).

7

The type 2 deiodinase Thr92Ala polymorphism is associated with increased bone turnover and decreased femoral neck bone mineral density

Karen A. Heemstra, Hendrieke C. Hoftijzer, Wendy M. van der Deure, Robin P. Peeters, Eric Fliers, Bente C. Appelhof, Wilmar M. Wiersinga, Eleonora P. Corssmit, Theo J. Visser, Johannes W. Smit,

Clinical Endocrinology (Oxford), 2009 Aug; 71(2):279-283

Abstract

The role of type 2 deiodinase (D2) in the human skeleton remains unclear. The D2 polymorphism Thr92Ala has been associated with lower enzymatic activity, which could result in lower local triiodothyronine (T3) availability in bone. We therefore hypothesized that the D2-Thr92Ala polymorphism may influence bone mineral density (BMD) and bone turnover. We studied 154 patients (29 men, 125 women: 79 estrogen-replete, 46 estrogen-deficient) with cured differentiated thyroid carcinoma. BMD and bone turnover markers [bone-specific alkaline phosphatase (BAP), cross-linking terminal C-telopeptide of type I collagen (CTX), procollagen type 1 aminoterminal propeptide (P1NP), and cross-linked N-telopeptide of type I collagen (NTX)] were measured. Effects of the D2-Thr92Ala polymorphism on BMD and bone turnover markers were assessed by a linear regression model, with age, gender, estrogen state, body mass index (BMI), serum calcium, 25-hydroxyvitamin D, parathyroid hormone (PTH), thyroid-stimulating hormone (TSH), and free thyroxine (T4) as covariables. Sixty patients were wild type (Thr/Thr), 66 were heterozygous (Thr/Ala), and 28 were homozygous (Ala/Ala) for the D2 polymorphism. There were no significant differences in any covariables between the three genotypes. Subjects carrying the D2-Thr92Ala polymorphism had consistently lower femoral neck and total hip densities than wild-type subjects ($p = 0.028$), and this was accompanied by significantly higher serum P1NP and CTX and urinary NTX/creatinine levels. We conclude that in patients with cured differentiated thyroid carcinoma, the D2-Thr92Ala polymorphism is associated with a decreased femoral neck BMD and higher bone turnover independent of serum thyroid hormone levels, which points to a potential functional role for D2 in bone.

Introduction

The involvement of thyroid hormone in bone metabolism has been well documented clinically, ranging from decreased skeletal development in childhood hypothyroidism (1–3), to accelerated growth in childhood hyperthyroidism (4), to an increased risk for osteoporosis in overt and subclinical hyperthyroidism (5–8). Although clinical observations suggest a clear involvement of thyroid hormone in bone metabolism, the molecular mechanisms by which thyroid hormone acts on bone so far have been only partially uncovered. Triiodothyronine (T3) promotes osteoblastic proliferation, differentiation, and apoptosis and, by induction of interleukin 6 (IL-6), prostaglandins, and RANKL, probably also promotes osteoclast formation and activation. This suggests that osteoblasts are the primary target cells for T3 in the regulation of bone remodeling (1,2,9–12). A functional role of thyroid stimulating hormone (TSH) on skeletal development and metabolism has been proposed on the basis of data obtained in animal studies (13–15) and in humans (16,17). This was disputed, however, by data obtained in thyroid hormone receptor (TR)–deficient mice, which indicated that bone remodeling was predominantly mediated by T3 via TRalpha (18,19). It also has been reported recently that in humans there is a significant association between bone mineral density (BMD) and serum thyroid hormone concentrations rather than TSH (20).

Most actions of thyroid hormone are mediated by the active form of thyroid hormone, T3. Circulating and local T3 concentrations are regulated mainly by the iodothyronine deiodinases D1, D2, and D3 (21). D2 is essential for the local production of T3 through deiodination of triiodothyroxine (T4). Although earlier studies on the role and functional expression of iodothyronine deiodinase enzymes in the skeleton have been equivocal (12,14,22–25), a recent study reported normal growth in mice with deficiencies in D1 and D2, indicating that D2 may not be critical in skeletal development (26). This notion was supported in a recent study that demonstrated that D2 activity is restricted to mature osteoblasts, suggesting a possible role for D2 in mature osteoblast function (27). Devising a study to address the potential role of deiodinases, including D2, on skeletal metabolism is difficult in humans, but study of the effects of functional D2 polymorphisms on BMD and bone turnover in humans may shed light on this role.

Several polymorphisms in D2 have been described (28–30). The single-nucleotide polymorphism (SNP) in D2-Thr92Ala has been associated with body mass index (BMI) and insulin resistance in subjects with obesity and type 2 diabetes mellitus (28,29), although this was not confirmed in the Framingham Offspring Study (31). In a study by Canani and colleagues (28), the maximal velocity of D2 was decreased by 3- to 10-fold in thyroid and skeletal muscle of carriers of the D2-Thr92Ala polymorphism.

This effect was observed in the absence of differences in D2 mRNA level or in the biochemical protein properties of the 92Ala allele. It was therefore suggested that either a functionally relevant SNP occurs in linkage disequilibrium in the Thr92Ala polymorphism or the 92Ala allele affects protein translation or stability.

The objective of this study was to try to elucidate a potential role for D2 in skeletal metabolism and BMD by evaluating the relationship between the D2-Thr92Ala polymorphism, BMD, and bone turnover markers in cured thyroidectomized differentiated thyroid carcinoma patients receiving thyroid hormone substitution. This human model has the advantage of strictly regulated serum thyroid hormone levels that are kept in a relatively narrow range.

Patients and Methods

Patients

Patients included in the study were all under control of the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center. All patients had a diagnosis of differentiated thyroid carcinoma, for which they had been treated by near-total thyroidectomy followed by standard postoperative [131I] radioiodine ablation therapy. All patients were cured as defined by the absence of 131-Iodine accumulation at diagnostic scintigraphy, serum thyroglobulin (Tg) concentrations below 2 mg/L after TSH stimulation, in the absence of Tg antibodies, a normal neck ultrasound, and no other indication for disease (32). Patients with tumor relapse were included only if they were subsequently cured. None of the patients used any drug or had a disease known to influence bone metabolism. The Leiden University Medical Center Local Ethics Committees approved the study, and written informed consent was obtained from all subjects.

Study design

On the day of the study, patients underwent a full clinical examination, including height (meters) and weight (kilograms). Blood was collected after an overnight fast and measured for TSH, serum free T4 (FT4), T3, calcium, parathyroid hormone (PTH), 25-hydroxyvitamin D [25(OH)D], bone-specific alkaline phosphatase (BAP), cross-linking terminal C-telopeptide of type I collagen (CTX), and procollagen type 1 amino-terminal propeptide (P1NP). A second-morning-void urine was measured for excretion of cross-linked N-telopeptide of type I collagen (NTX). Plasma, serum, and urine samples were handled immediately and stored at -80°C in Sarstedt tubes. BMD (expressed in grams per square centimeter) was measured at the femoral neck and

lumbar spine (vertebrae L2–L4) by dual-energy X-ray absorptiometry (DXA, NHANES III–adjusted; Hologic 4500, Hologic, Inc., Bedford, MA, USA). Following World Health Organization (WHO) criteria, osteopenia was defined as a T-score between -1 and -2.5 and osteoporosis as a T-score below -2.5. The following data also were recorded: smoking habits, alcohol use, physical activity, calcium intake, medications (including self-prescription drugs) or vitamin or mineral supplements, and daily calcium intake and for females: date of first menstruation (menarche), date of last menstruation, cycle regularity, and estrogen substitution if applicable.

Biochemical parameters

Serum free T4 (FT4) and TSH were measured using a chemoluminescence immunoassay with a Modular Analytics E-170 system (intra-assay CV of 1.6-2.2 % and 1.3-5.0 % respectively (Roche, Almere, The Netherlands). Serum T3 was measured with a fluorescence polarization immunoassay, CV 2.5-9.0 %, on an ImX system (Abbott, Abbott Park, IL, USA). Thyroglobulin was measured by Dynotest TG-s (Brahms Diagnostica GmbH, Germany). Plasma PTH was measured using an immunoradiometric assay (Nichols Diagnostic Institutes, Wijchen, The Netherlands). Calcium was measured by colorimetry and 25(OH)-vitamin D by RIA (Incstar/DiaSorin, Stillwater, MN, USA). Serum BAP was measured by RIA (Hybritech Europe, Liege, Belgium). Serum CTX and P1NP were measured by chemoluminescence immunoassay using the Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands). NTX was measured by ELISA (Ostex International Inc., Seattle, WA, USA). NTX was expressed as the ratio between NTX and urine creatinine excretion (NTX/creatinine) to correct for differences in creatinine excretion. Insulin sensitivity was estimated by homeostasis model assessment [HOMA: fasting insulin (milliunits per milliliter) - fasting glucose (millimoles per liter)/22.5].

Genetic analyses

DNA was isolated from peripheral leukocytes by the salting-out procedure. Genotypes were determined using 5 ng of genomic DNA by a 5′ fluoregenic TaqMan assay, and reactions were performed in 384-well format on an ABI9700 2x384-well PCR machine with endpoint reading on the ABI 7900HT TaqMan machine (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). Primer and probe sequences were optimized using the SNP assay-by-design service of Applied Biosystems.

Statistical Analyses

Values are presented as mean ± SE, median (range), or as numbers or proportions of patients. Nonnormally distributed data (TSH and PTH) were log-transformed before analyses. Comparisons between groups were analyzed by ANOVA or chi-square tests. The relation between the three D2-Thr92Ala genotypes [Thr/Thr (wild type), Thr/Ala (heterozygote), and Ala/Ala (homozygote)], BMD, and markers of bone turnover were studied by a stepwise univariate regression analysis. After correction for age, gender, and estrogen status (ie, estrogen deplete or replete), the following covariables were entered: BMI, serum levels of calcium (corrected for an albumin concentration of 42 g/L), 25(OH)-vitamin D, lnPTH, FT4, T3, and lnTSH. We calculated that to detect an effect size of 0.15 (corresponding to an r2 of 0.13), adopting an alpha value of 0.05 and a beta value of 0.80, the number of subjects needed is 108. Because it has been documented that the D2-Thr92Ala polymorphism is associated with insulin resistance (28), we also compared insulin sensitivity (HOMA) in the three genotypes. Deviation from Hardy-Weinberg equilibrium was analyzed using a chi-square test. All calculations were performed using SPSS 12.0 for windows (SPSS, Inc., Chicago, IL, USA). Differences were considered statistically significant at $p<0.05$.

Results

Patient characteristics

Of a potential of 330 patients with cured differentiated thyroid carcinoma, 105 were excluded for various reasons (**Figure 1**). Sixty-nine patients were not willing or able to participate in the study for different reasons. A total of 156 patients thus were included in the study. Two patients were left out from the analyses because of incomplete data. Thirteen patients had postoperative hypoparathyroidism for which they were adequately supplemented with active vitamin D metabolites and calcium as required. Additional analyses were performed leaving out these patients (see below and **Table 2**). In addition, serum PTH levels were included as a covariable in the analyses (see below) to correct for the potentially confounding effects of hypoparathyroidism. The basal characteristics of the 154 patients included in the study are shown in **Table 1**. All patients were receiving L-thyroxine treatment at a mean dose of 183 ± 4 µg/day.

The D2 Thr92Ala polymorphism, BMD and biochemical parameters of skeletal metabolism

Genotype frequencies of the D2-Thr92Ala polymorphism [Thr/Thr = 60 (39%), Thr/Ala = 66 (43%), and Ala/Ala = 28 (18%)] did not deviate from Hardy-Weinberg

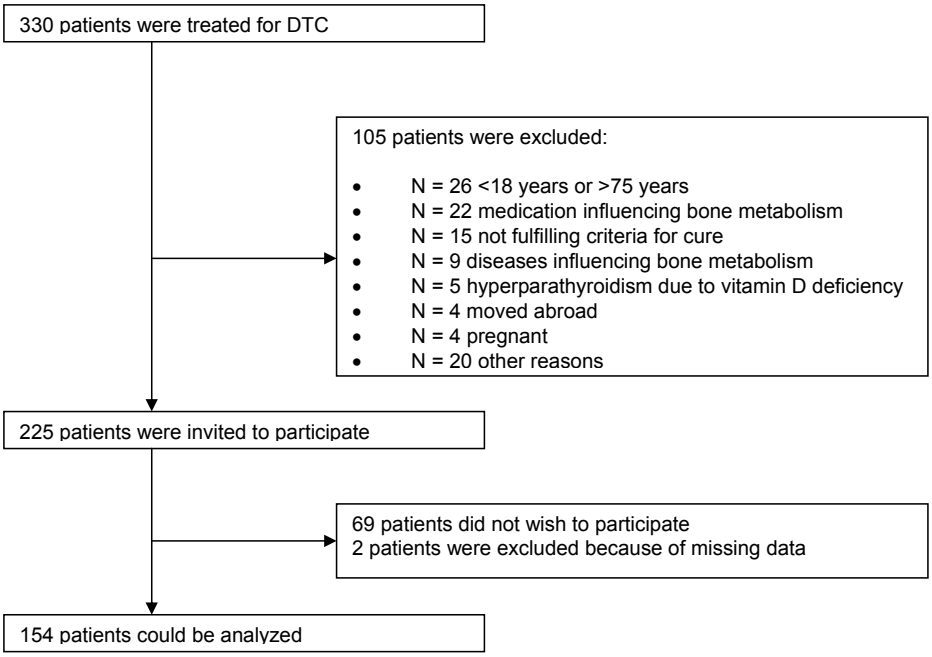

Figure 1
Flowchart of the study

Table 1: Patient characteristics

	Total (n=154)
Age (years)	49.2 ± 1.0
Males	29 (18.8 %)
Females: Estrogen Replete / Deplete	79 (51.3 %) / 46 (29.9 %)
Age at diagnosis	36.6 ± 1.1
Histology	
Papillary Thyroid Carcinoma (PTC)	107 (69 %)
Follicular Thyroid Carcinoma	25 (16 %)
Follicular variant PTC	21 (14 %)
Hürthle cell Thyroid Carcinoma	1 (1 %)
Total Activity Radioiodine	8067 ± 699 MBq
Lymph Node Surgery	14 (9 %)
TNM Stage	
T1-3 N0 M0	90 (63 %)
T1-3 N1 M0	30 (21%)
T4 or M1 n=143	23 (16 %)
Relapse DTC *(all were cured after relapse)*	20 (13 %)

Table 2: Characteristicsw of patients by D2-Thr92Ala Genotype

	Thr / Thr (60)	Thr / Ala (66)	Ala / Ala (28)	P
Men (n)	13	11	5	0.861[1]
Women (n) Estrogen Replete / Deplete	32 / 15	33 / 22	14 / 9	0.894[1]
Age (years)	47.2 ± 1.6	51.2 ± 1.7	48.3 ± 1.9	0.148[1]
Height (m)	1.72 ±0.01	1.70 ± 0.01	1.71 ± 0.02	0.307[1]
BMI (kg/m2)	25.6 ± 0.6	26.2 ± 0.4	25.8 ± 1.1	0.773[1]
Sports (hrs/week)	3.1 ± 1.1	5.0 ± 1.6	4.5 ± 2.3	0.654[1]
Smoking (n)	12 (9%)	7 (5%)	5 (1%)	0.092[1]
Menarche (age)	13.4 ± 0.2	13.1 ± 0.2	13.6 ± 0.3	0.399[1]
Menopause (age)	48.2 ± 1.5	47.7 ± 1.1	50.1 ± 1.5	0.484[1]
Follow-up duration (years)	13.1 ± 1.2	10.5 ± 1.0	11.3 ± 1.5	0.241[1]
Hypoparathyroidism (n)	5 (3%)	6 (4%)	2 (1%)	0.952[1]
Vertebral fractures (n)	1 (1%)	2 (1%)	1 (1%)	0.832[1]
HOMA (mmol*22.5/L)	1.75 ± 0.20	2.16 ± 0.21	1.86 ± 0.32	0.361[1]
Calcium (mmol/L)	2.39 ± 0.02 (59)	2.38 ± 0.01	2.39 ± 0.02	0.943[1]
25 OH vitD (nmol/L)	64.5 ± 3.9 (59)	60.4 ± 2.9	69.9 ± 4.8	0.277[1]
PTH (pmol/L)	4.88 ± 0.36 (58)	5.27 ± 0.43 (65)	6.19 ± 0.83	0.250[1]
TSH (mU/L)	0.051 (0.003-4.620)	0.031 (0.003-4.910)	0.051 (0.003-6.830)	0.753[1]
Dose thyroxine (µg/kg)	2.09 ± 1.04	2.23 ± 0.87	2.19 ± 1.03	0.398[1]
Free T4 (pmol/L)	22.7 ± 0.1	22.4 ± 0.1	21.6 ± 0.2	0.562[1]
T3 (nmol/L)	1.49 ± 0.04 (54)	1.47 ± 0.05 (59)	1.40 ± 0.07 (23)	0.624[1]
T3/T4 ratio * 10	6.6 ± 0.2 (54)	6.7 ± 0.2 (59)	6.6 ± 0.4 (23)	0.903[1]
BMD femoral neck (g/cm^2)	0.90 ± 0.02	0.84 ± 0.01	0.85 ± 0.03	0.028 /0.015[2]
BMD total hip (g/cm^2)	0.97 ± 0.02	0.92 ± 0.02	0.92 ± 0.03	0.064 /0.049[2]
BMD lumbar spine (g/cm^2)	1.08 ± 0.03	1.04 ± 0.02	1.07 ± 0.04	0.741 /0.094[2]
NTX / Creatinine * 1/1000	44.0 ± 4.1	56.5 ± 5.8	67.7 ± 10.6	0.008 /0.002[2]
BAP (ng/mL)	12.5 ± 0.5	13.5 ± 0.6	13.9 ± 0.7	0.063 /0.085[2]
P1NP (ng/mL)	40.0 ± 2.6	42.9 ± 3.4	50.9 ± 5.5	0.028 /0.032[2]
CTX (mg/mL)	0.28 ± 0.02	0.28 ± 0.02 [#]	0.37 ± 0.05	0.043 /0.036[2]

Values are presented as mean ± standard error (SE), median (range) or as numbers or proportions of patients. PTH= Parathyroid hormone, BAP= Bone Specific Alkaline Phosphatase, P1NP= Procollagen type 1 Aminoterminal Propeptide, CTX= C-crosslinking Terminal Telopeptide of Type I collagen, NTX/Creatinine= Ratio of Urinary N-Telopeptide of Collagen Cross-links and Creatinine Concentration; [1] = One-way ANOVA, [2] = general linear model, univariate with age, gender, estrogen state, BMI, Ca, lnPTH, 25-OHvitD, lnTSH and Free T4 as covariables, second value= patients with postoperative hypoparathyroidism left out.

equilibrium proportions. The 92Ala allele had a frequency of 45%, which is similar to previous studies in Caucasians (33,31). The characteristics of the three genotype subgroups are given in **Table 2**. The three groups were comparable with respect to age, gender, estrogen state (including ages at menarche and menopause), and BMI.

Physical activity and smoking habits did not differ either. Biochemical covariables for bone metabolism [ie, serum calcium, 25(OH)-vitamin D and PTH] were not different, as were serum FT4 and T3 levels, serum T3/T4 ratio, and TSH levels.

Because it has been documented that the D2-Thr92Ala polymorphism is associated with insulin resistance (28), we also compared insulin sensitivity by HOMA in the three genotypes, which again did not differ ($p = 0.361$). We also calculated whether HOMA was a significant determinant of BMD and of biochemical parameters of skeletal metabolism (corrected for age, gender, estrogen state, and BMI). Univariate analyses revealed that p values for HOMA as an independent variable were, respectively, 0.912 for femoral neck BMD, 0.583 for lumbar vertebral BMD, 0.826 for NTX/creatinine, 0.575 for BAP, 0.798 for P1NP, and 0.906 for CTX. HOMA therefore was not a determinant of BMD or bone turnover markers.

The relation between the three D2-Thr92Ala genotypes, BMD, and biochemical parameters of skeletal metabolism were studied by a stepwise univariate regression analysis. After correction for age, gender, estrogen status, and BMI, the following covariables were entered subsequently: serum levels of calcium, 25(OH)-vitamin D, lnPTH, FT4, and lnTSH. We found a significant independent relationship between the Thr92Ala genotypes and femoral neck BMD ($p = 0.022$) (**Table 2, Figure 2**) with a 6% lower BMD in homozygotes than in wild-type patients. This relationship was also present when total-hip BMD was measured. We also found independent relationships between the D2-Thr92Ala genotypes and biochemical parameters of skeletal metabolism: P1NP ($p = 0.028$), CTX ($p = 0.043$), and NTX/creatinine ($p = 0.008$), which were higher in homozygotes than in wild-type patients. Data for analyses leaving out patients with postoperative hypoparathyroidism did not influence these results (**Table 2**). The largest difference was observed for NTX/creatinine, which was 54% higher in homozygotes than in wild types.

Discussion

The main objective of this study was to investigate a potential role for the deiodinase D2 in bone metabolism in humans by studying the relationship between the D2-Thr92Ala polymorphism, BMD, and bone turnover. The D2-Thr92Ala polymorphism is associated with a lower D2 Vmax and therefore may lead to decreased local availability of T3 (28), which, in turn, may affect skeletal metabolism. We studied this relationship in a human model of thyroidectomized patients cured from differentiated thyroid carcinoma receiving thyroid hormone substitution. The advantage of this model is that study subjects have more uniform FT4 levels, which fell between the 25^{th} and 75^{th} percentiles for FT4 (19.5 and 24.9 pmol/L) in our group of patients.

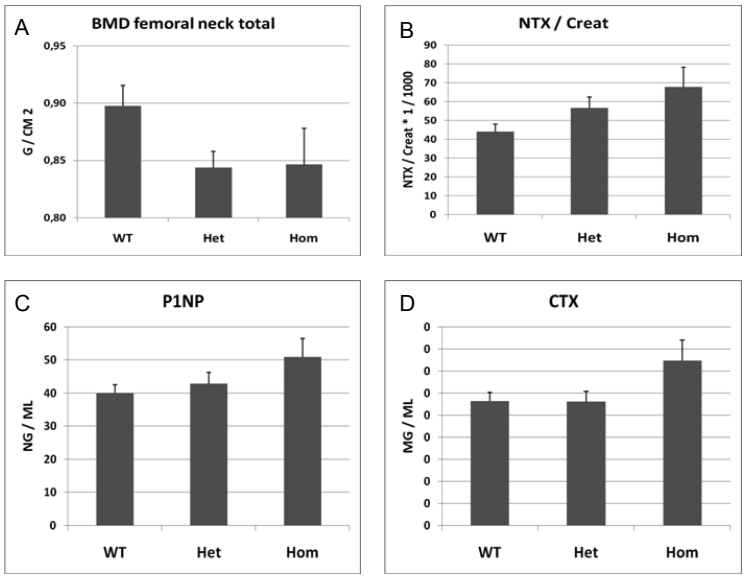

Figure 2
Relationships between D2 Thr92ALA genotypes and indicators of bone turnover.
(A) Femoral neck BMD.
(B) Ratio of urinary N-telopeptide of collagen cross-links and creatinine concentration.
(C) Procollagen type 1 amino-terminal propeptide (P1NP) levels.
(D) Cross-linking terminal C-telopeptide of type I collagen.
For levels of significance, see text and Table 2.

In support of the involvement of D2 in bone metabolism was the observation of a 6% decrease in femoral neck BMD and increased levels of P1NP (32%), CTX (27%), and NTX/creatinine (54%) in the Ala/Ala subgroup compared with wild-type subgroup. These effects were independent of factors known to influence BMD and bone metabolism, such as age, gender, BMI, estrogen state, PTH, and vitamin D. These effects were also independent of circulating levels of T3 and TSH and thus were indicative of an independent role of D2 in bone metabolism. We did not find an association of the D2 polymorphism with lumbar spine BMD, possibly owing to a differential effect of the polymorphism on predominantly trabecular bone at the lumbar spine *versus* predominantly cortical bone at the femoral neck. Our data did not confirm earlier observations of an association of the D2-Thr92Ala polymorphism with insulin sensitivity (28,29). This discrepancy may be explained by differences in the populations studied, with a low prevalence of obesity or insulin resistance in our subjects. Our data, however, are in keeping with the Framingham Offspring Study, which found no relation between the D2-Thr92Ala polymorphism and insulin resistance (31). We did not observe differences in height, indicating no difference in skeletal development among the three genotype subgroups. This is in line with

recent observations in C3H/HeJ D2-/- compound mutant mice with D1 deficiency and deletion of D2, which were shown to maintain normal growth (26). This notion is supported by a recent study suggesting that D2 may not play a physiologic role in growth plate chondrocytes (27).

The observed effects of the D2-Thr92Ala polymorphism on femoral neck BMD are in line with the importance of local availability of T3 for bone formation. D2 activity has been found on mature osteoblasts (34), which are the primary target cells for T3 regulatory effects on bone formation (1,2,10–12).

The effects of the D2-Thr92Ala polymorphism on bone turnover markers are not easy to explain. It is conventionally accepted that higher rather than lower circulating thyroid hormone levels result in higher bone turnover and decreased bone mass. However, the model we used is unique in the sense that circulating T3 levels were similar among the three D2 genotypes, allowing us to specifically study the consequences of the polymorphism for local T3 availability in the bone microenvironment. Williams and colleagues (27) showed no D2 activity in osteoclasts. The effects of the polymorphism on the markers of bone degradation (NTX/creatinine and CTX) therefore may not be explained by direct effects on osteoclasts but are more likely to result from changes in the interaction between osteoblasts and osteoclasts, possibly by alterations in the RANK/RANKL/OPG signaling pathway, which potentially can be modulated by local T3 availability in the bone microenvironment. In the context of conflicting data on a functional role for TSH in skeletal development, our data, which were corrected for serum TSH levels, outline the importance of local T3 for bone metabolism (13–17,35–38). Two recent papers by Bassett and colleagues (18,19), who studied mice with complete or haploinsufficiency of TRalpha and -beta, concluded that TRalpha regulates both skeletal development and adult bone maintenance.

Whereas a limitation of our study may be its relatively small size and its cross-sectional design, one of its clear strengths is that all subjects were phenotyped for factors other than thyroid status known to modulate bone metabolism. This design enabled us to use regression models, including relevant covariables, the feasibility of which is difficult in large cohort studies. In addition, according to the power calculation, the study had sufficient power, which was confirmed by a post hoc power analysis revealing that the power was 97% or higher for the dependent variables studied. A potential further limitation of our study is that thyroid hormone parameters measured at one point in time may not reflect the overall thyroid status over time. To address this issue, we calculated the slope of all TSH measurements routinely obtained after initial therapy in every patient participating in the study to verify the stability over time. An average of 15 TSH measurements were obtained per patient, and the slope of TSH values was -0.0001 (range -0.004 to 0) mU/L per year, thus indicating stable TSH levels over time.

In summary our data suggest that a decrease in local availability of T3 potentially owing to a D2 polymorphism may result in increased bone turnover and decreased bone mass at the predominantly cortical femoral neck. We believe that our study provides additional information on the role of D2 in bone metabolism and the functional consequences of the D2-Thr92Ala polymorphism, supporting a role for D2 in mature bone cells (27).

References

1. Bassett JH, Williams GR. The molecular actions of thyroid hormone in bone. Trends in Endocrinology and Metabolism 2003 14 356-364
2. Bassett JH, Williams GR Critical role of the hypothalamic-pituitary-thyroid axis in bone. Bone Bone. 2008 43 418-426.
3. Rivkees SA, Bode HH, Crawford JD.Long-term growth in juvenile acquired hypothyroidism: the failure to achieve normal adult stature. New England Journal of Medicine 1988 318: 599-602
4. Segni M, Gorman CA. The aftermath of childhood hyperthyroidism. Journal of Pediatric Endocrinology and Metabolism 200114 Suppl 5 1277-1282; discussion 1297-1298
5. Heemstra KA, Hamdy NA, Romijn JA, Smit JW. The effects of thyrotropin-suppressive therapy on bone metabolism in patients with well-differentiated thyroid carcinoma. Thyroid 2006 16 583-591
6. Kim DJ, Khang YH, Koh JM, Shong YK, Kim GS. Low normal TSH levels are associated with low bone mineral density in healthy postmenopausal women. Clinical Endocrinology (Oxford) 2006 64 86-90
7. Bauer DC, Ettinger B, Nevitt MC, Stone KL. Risk for fracture in women with low serum levels of thyroid-stimulating hormone. Annals of Internal Medicine 2001 134 561-568
8. Lee WY, Oh KW, Rhee EJ, Jung CH, Kim SW, Yun EJ, Tae HJ, Baek KH, Kang MI, Choi MG, Yoo HJ, Park SW. Relationship between subclinical thyroid dysfunction and femoral neck bone mineral density in women. Archives of Medical Research 2006 37 511-516
9. Britto JM, Fenton AJ, Holloway WR, Nicholson GC. Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. Endocrinology 1994 134 169-176
10. Basset P, Okada A, Chenard MP, Kannan R, Stoll I, Anglard P, Bellocq JP, Rio MC. Matrix metalloproteinases as stromal effectors of human carcinoma progression: therapeutic implications. Matrix Biololgy 1997 15 535-541
11. Kanatani M, Sugimoto T, Sowa H, Kobayashi T, Kanzawa M, Chihara K. Thyroid hormone stimulates osteoclast differentiation by a mechanism independent of RANKL-RANK interaction. Journal of Cell Physiology 2004 201 17-25
12. Miura M, Tanaka K, Komatsu Y, Suda M, Yasoda A, Sakuma Y, Ozasa A, Nakao K. A novel interaction between thyroid hormones and 1,25(OH)(2)D(3) in osteoclast formation. Biochemical and Biophysical Research Communication 2002 291 987-994
13. Abe E, Marians RC, Yu W, Wu XB, Ando T, Li Y, Iqbal J, Eldeiry L, Rajendren G, Blair HC, DaviesTF, Zaidi M. TSH is a negative regulator of skeletal remodeling. Cell 2003 115 151-162
14. Morimura T, Tsunekawa K, Kasahara T, Seki K, Ogiwara T, Mori M, Murakami M. Expression of type 2 iodothyronine deiodinase in human osteoblast is stimulated by thyrotropin. Endocrinology 2005 146 2077-2084
15. Galliford TM, Murphy E, Williams AJ, Bassett JH, Williams GR. Effects of thyroid status on bone metabolism: a primary role for thyroid stimulating hormone or thyroid hormone? Minerva Endocrinology 2005 30 237-246
16. Heemstra KA, van der Deure WM, Peeters RP, Hamdy NA, Stokkel MP, Corssmit EP, Romijn JA, Visser TJ, Smit JW. Thyroid hormone independent associations between serum TSH levels and indicators of bone turnover in cured patients with differentiated thyroid carcinoma. European Journal of Endocrinology 2008 159 69-76

17. Mazziotti G, Sorvillo F, Piscopo M, Cioffi M, Pilla P, Biondi B, Iorio S, Giustina A, Amato G, Carella C. Recombinant human TSH modulates in vivo C-telopeptides of type-1 collagen and bone alkaline phosphatase, but not osteoprotegerin production in postmenopausal women monitored for differentiated thyroid carcinoma. Journal of Bone Mineral Research 2005 20 480-486
18. Bassett JH, Nordstrom K, Boyde A, Howell PG, Kelly S, Vennstrom B, Williams GR. Thyroid status during skeletal development determines adult bone structure and mineralization. Molecular Endocrinology 2007 21 1893-1904
19. Bassett JH, O'Shea PJ, Sriskantharajah S, Rabier B, Boyde A, Howell PG, Weiss RE, Roux JP, Malaval L, Clement-Lacroix P, Samarut J, Chassande O, Williams GR. Thyroid hormone excess rather than thyrotropin deficiency induces osteoporosis in hyperthyroidism. Molecular Endocrinology 2007 21 1095-1107
20. van der Deure WM, Uitterlinden AG, Hofman A, Rivadeneira F, Pols HA, Peeters RP, Visser TJ. Effects of serum TSH and FT4 levels and the TSHR-Asp727Glu polymorphism on bone: the Rotterdam Study. Clinical Endocrinology (Oxford). 2008 68 175-181.
21. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. Journal of Clinical Investigation 2006 116 2571-2579
22. Gouveia CH, Christoffolete MA, Zaitune CR, Dora JM, Harney JW, Maia AL, Bianco AC. Type 2 iodothyronine selenodeiodinase is expressed throughout the mouse skeleton and in the MC3T3-E1 mouse osteoblastic cell line during differentiation. Endocrinology 2005 146 195-200
23. LeBron BA, Pekary AE, Mirell C, Hahn TJ, Hershman JM. Thyroid hormone 5'-deiodinase activity, nuclear binding, and effects on mitogenesis in UMR-106 osteoblastic osteosarcoma cells. Journal of Bone and Mineral Research 1989 4 173-178
24. Miura M, Tanaka K, Komatsu Y, Suda M, Yasoda A, Sakuma Y, Ozasa A, Nakao K. Thyroid hormones promote chondrocyte differentiation in mouse ATDC5 cells and stimulate endochondral ossification in fetal mouse tibias through iodothyronine deiodinases in the growth plate. Journal of Bone and Mineral Research 2002 17 443-454
25. Shen S, Berry W, Jaques S, Pillai S, Zhu J. Differential expression of iodothyronine deiodinase type 2 in growth plates of chickens divergently selected for incidence of tibial dyschondroplasia. Animal Genetics 2004 5 114-118
26. Christoffolete MA, Arrojo e Drigo, Gazoni F, Tente SM, Goncalves V, Amorim BS, Larsen PR, Bianco AC, Zavacki AM. Mice with impaired extrathyroidal thyroxine to 3,5,3'- triiodothyronine conversion maintain normal serum 3,5,3'-triiodothyronine concentrations. Endocrinology 2007 148 954- 960
27. Williams AJ, Robson H, Kester MH, van Leeuwen JP, Shalet SM, Visser TJ, Williams GR. Iodothyronine deiodinase enzyme activities in bone. Bone 2008 43 126-134
28. Canani LH, Capp C, Dora JM, Meyer ELS, Wagner MS, Harney JW, Larsen PR, Gross JL, Bianco AC, Maia AL. The Type 2 Deiodinase A/G (Thr92Ala) Polymorphism Is Associated with Decreased Enzyme Velocity and Increased Insulin Resistance in Patients with Type 2 Diabetes Mellitus. Journal of Clinical Endocrinology and Metabolism 2005 90 3472-3478
29. Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET, Shuldiner AR, Celi S. Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3- adrenergic receptor. Diabetes 2002 51 880-883

30. Peeters RP, van der Deure WM, Visser TJ. Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. European Journal of Endocrinology 2006 55 655-662
31. Maia AL, Dupuis J, Manning A, Liu C, Meigs JB, Cupples LA, Larsen PR, Fox CS. The type 2 deiodinase (DIO2) A/G polymorphism is not associated with glycemic traits: the Framingham Heart study. Thyroid 2007 17 199-202
32. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Sherman SI, Tuttle RM. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2006 16 109-142
33. Peeters RP, van den Beld AW, Attalki H, Toor Hv, de Rijke YB, Kuiper GGJM, Lamberts SWJ, Janssen JAMJ, Uitterlinden AG, Visser TJ. A new polymorphism in the type II deiodinase gene is associated with circulating thyroid hormone parameters. American Journal of Physiology Endocrinoly and Metabolism 2005 289 E75-E81
34. Burger H, van Daele PL, Algra D, van den Ouweland FA, Grobbee DE, Hofman A, van Kuijk C, Schutte HE, Birkenhager JC, Pols HA. The association between age and bone mineral density in men and women aged 55 years and over: the Rotterdam Study. Bone and Mineral 1994 25 1-13
35. Inoue M, Tawata M, Yokomori N, Endo T, Onaya T. Expression of thyrotropin receptor on clonal osteoblast-like rat osteosarcoma cells. Thyroid 1998 8 1059-1064
36. Sun L, Davies TF, Blair HC, Abe E, Zaidi M. TSH and bone loss. Annals of the New York Academy of Science 2006 1068 309- 318
37. Davies T, Marians R, Latif R. The TSH receptor reveals itself. Journal of Clinical Investigation 2002 110 161-164
38. Sampath TK, Simic P, Sendak R, Draca N, Bowe AE, O'Brien S, Schiavi SC, McPherson JM, Vukicevic S. Thyroid-stimulating hormone restores bone volume, microarchitecture, and strength in aged ovariectomized rats. Journal of Bone Mineral Research 2007 22 849-859