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The Type 2 Deiodinase Thr92Ala Polymorphism Is Associated with Increased Bone Turnover and Decreased Femoral Neck Bone Mineral Density

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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 (T_3) 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 amino-terminal 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 triiodothyroxine (T_4) 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 ($p = .028$), 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.

KEY WORDS: BONE FORMATION AND RESORPTION; THYROID HORMONE METABOLISM; SKELETON BIOLOGY; TYPE 2 DEIODINASE; THYROID CARCINOMA

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 (T_3) 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 T_3 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 T_3 via TRα.[18,19] It also has been reported recently that in humans there is a significant association between bone mineral density

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(BMD) and serum thyroid hormone concentrations rather than TSH.[20]

Most actions of thyroid hormone are mediated by the active form of thyroid hormone, T_3. Circulating and local T_3 concentrations are regulated mainly by the iodothyronine deiodinases D1, D2, and D3.[21] D2 is essential for the local production of T_3 through deiodination of triiodothyroxine (T_4). 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 addressing 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 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 having 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 [^{131}I]radioiodine ablation therapy. All patients were cured as defined by the absence of ^{131}I accumulation at diagnostic scintigraphy, serum thyroglobulin (Tg) concentrations below 2 μg/L after TSH stimulation, 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 T_4 (FT_4), T_3, 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 L_2–L_4) 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

FT4 and TSH were measured using a chemoluminescence immunoassay with a Modular Analytics E-170 System (intraassay CV of 1.6% to 2.2% and 1.3% to 5.0%, respectively (Roche, Almere, The Netherlands). Serum T_3 was measured with a fluorescence polarization immunoassay (CV 2.5% to 9.0%) on an ImX System (Abbott, Abbott Park, IL, USA). Tg 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)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). 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 2-×384-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 analysis

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 was 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)D, lnPTH, FT_4, T_3, and lnTSH. We calculated that to detect an effect size of 0.15 (corresponding to an r^2 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 $p < .05$.

Results

Patient characteristics

Of a potential of 330 patients with cured differentiated thyroid carcinoma, 105 were excluded for various reasons (Fig. 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.

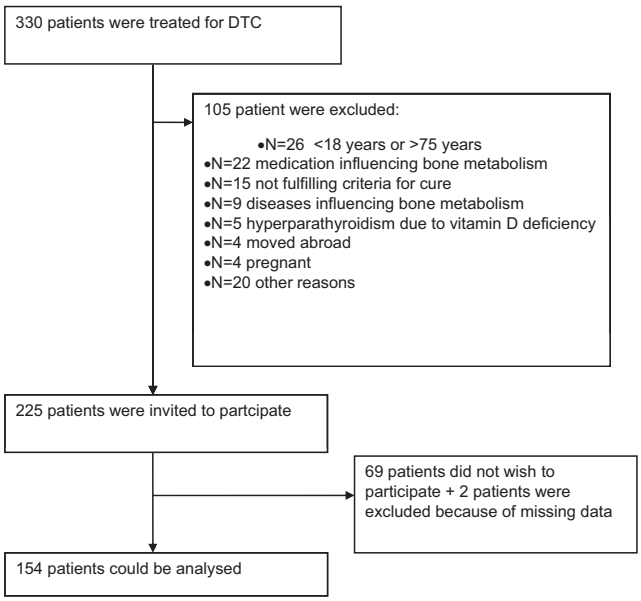

Fig. 1. Study flowchart.

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 equilibrium proportions. The *Ala92* 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)D and PTH] were not different, as were serum FT_4 and T_3 levels, serum T_3/T_4 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 ($p = .361$). 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, .912 for femoral neck BMD, .583 for lumbar vertebral BMD, .826 for NTX/creatinine, .575

Table 1. Characteristics of Patients

Characteristic	Total ($n = 154$)
Age (years)	49.2 ± 1.0
Males	29 (18.8%)
Females: Estrogen replete/depleted	79 (51.3%)/46 (29.9%)
Age at diagnosis (years)	36.6 ± 1.1
Histology	
Papillary thyroid carcinoma (PTC)	107 (69%)
Follicular thyroid carcinoma	25 (16%)
Follicular variant PTC	21 (14%)
Hurthle cell thyroid carcinoma	1 (1%)
Total activity of radioiodine	8067 ± 699 MBq
Lymph node surgery	14 (9%)
pTNM stage	
$T_{1\text{-}3}N_0M_0$	90 (63%)
$T_{1\text{-}3}N_1M_0$	30 (21%)
T_4 or M_1 ($n = 143$)	23 (16%)
Relapse DTC (all were cured after relapse)	20 (13%)

Table 2. Characteristics of Patients by the D2 Thr92Ala Genotype

	Thr/Thr (n = 60)	Thr/Ala (n = 66)	Ala/Ala (n = 28)	*p* Value
Men (n)	13	11	5	.861
Women (n)				
Estrogen replete/deplete	32/15	33/22	14/9	
Age (years)	47.2 ± 1.6	51.2 ± 1.7	48.3 ± 1.9	.148
Height (m)	1.72 ± 0.01	1.70 ± 0.01	1.71 ± 0.02	.307
BMI (kg/m^2)	25.6 ± 0.6	26.2 ± 0.4	25.8 ± 1.1	.773
Sports (hours/week)	3.1 ± 1.1	5.0 ± 1.6	4.5 ± 2.3	.654
Smoking (n)	12 (9%)	7 (5%)	5 (1%)	.092
Menarche (age)	13.4 ± 0.2	13.1 ± 0.2	13.6 ± 0.3	.399
Menopause (age)	48.2 ± 1.5	47.7 ± 1.1	50.1 ± 1.5	.484
Follow-up duration (years)	13.1 ± 1.2	10.5 ± 1.0	11.3 ± 1.5	.241
Hypoparathyroidism (n)	5 (3%)	6 (4%)	2 (1%)	.952
Vertebral fractures (n)	1 (1%)	2 (1%)	1 (1%)	.832
HOMA (mmol × 22.5/L)	1.75 ± 0.20	2.16 ± 0.21	1.86 ± 0.32	.361
Calcium (mmol/L)	2.39 ± 0.02	2.38 ± 0.01	2.39 ± 0.02	.943
25(OH)D (nmol/L)	64.5 ± 3.9	60.4 ± 2.9	69.9 ± 4.8	.277
PTH (pmol/L)	4.88 ± 0.36	5.27 ± 0.43	6.19 ± 0.83	.250
TSH (mU/L)	0.051 (0.003–4.620)	0.031 (0.003–4.910)	0.051 (0.003–6.830)	.753
Dose thyroxine (μg/kg)	2.09 ± 1.04	2.23 ± 0.87	2.19 ± 1.03	.398
Free T_4 (pmol/L)	22.7 ± 0.1	22.4 ± 0.1	21.6 ± 0.2	.562
T_3 (nmol/L)	1.49 ± 0.04	1.47 ± 0.05	1.40 ± 0.07	.624
T_3/T_4 ratio × 10	6.6 ± 0.2	6.7 ± 0.2	6.6 ± 0.4	.903
BMD femoral neck (g/cm^2)	0.90 ± 0.02	0.84 ± 0.01	0.85 ± 0.03	.022/.015[a]
BMD total hip (g/cm^2)	0.97 ± 0.02	0.92 ± 0.02	0.92 ± 0.03	.064/.049[a]
BMD lumbar spine (g/cm^2)	1.08 ± 0.03	1.04 ± 0.02	1.07 ± 0.04	.741/.094[a]
NTX/creatinine × 1/1000	44.0 ± 4.1	56.5 ± 5.8	67.7 ± 10.6	.008/.002[a]
BAP (ng/mL)	12.5 ± 0.5	13.5 ± 0.6	13.9 ± 0.7	.063/.085[a]
P1NP (ng/mL)	40.0 ± 2.6	42.9 ± 3.4	50.9 ± 5.5	.028/.032[a]
CTX (mg/mL)	0.28 ± 0.02	0.28 ± 0.02	0.37 ± 0.05	.043/.036[a]

Values are presented as mean ± SE, median (range), or as numbers or proportions of patients. PTH = parathyroid hormone; BAP = bone-specific alkaline phosphatase; P1NP = procollagen type 1 amino-terminal propeptide; CTX = cross-linking terminal C-telopeptide of type I collagen; NTX/creatinine = ratio of urinary N-telopeptide of collagen cross-links and creatinin concentration; A = one-way ANOVA.

[a]General linear model, univariate with age, gender, estrogen state, BMI, Ca, lnPTH, 25(OH)D, lnTSH, and free T_4 as covariables; second value: patients with postoperative hypoparathyroidism left out.

for BAP, .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)D, lnPTH, FT_4, and lnTSH. We found a significant independent relationship between the Thr92Ala genotypes and femoral neck BMD ($p = .022$) (Table 2, Figure 2) with a 6% lower BMD in homozygotes than in wild-type patients. This relationship a 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 ($p = .028$), CTX ($p = .043$), and NTX/creatinine ($p = .008$), which were higher in homozygotes than in wild-type patients. Data for analyses leaving out patients with postoperativel 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 V_{max} and therefore may lead to decreased local availability of T_3,[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 FT_4 levels, which fell between the 25th and 75th percentiles for FT_4 (19.5 and 24.9 pmol/L) in our group of patients.

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

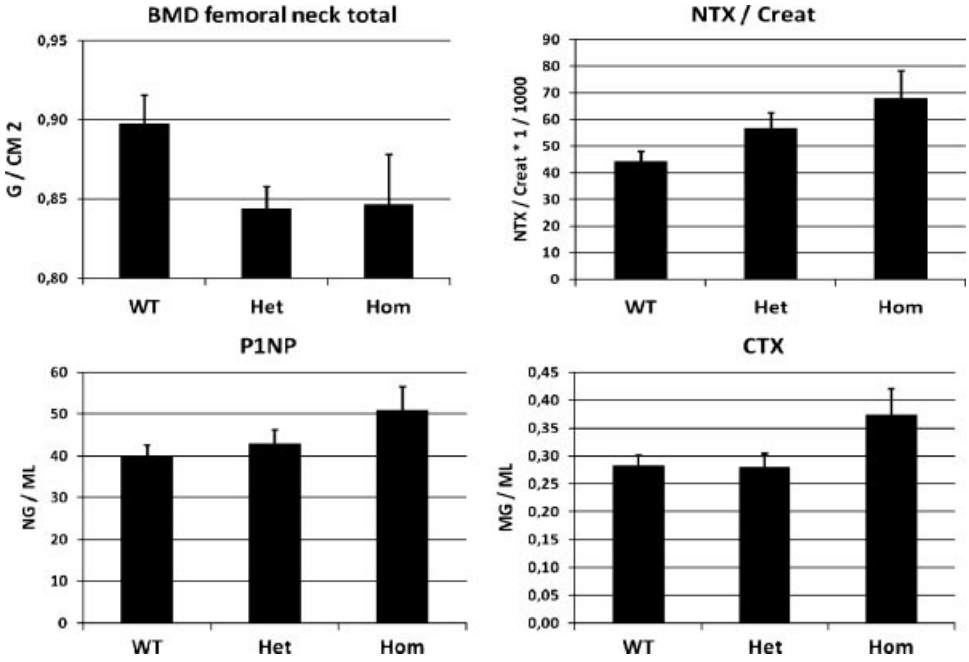

Fig. 2. Relationships between D2 Thr92ALA genotypes and indicators of bone turnover. (*A*) Femoral neck BMD. (*B*) Ratio of urinary N-telopeptide of collagen cross-links and creatinine concentration. (*C*) Procollagen type 1 amino-terminal propeptide (P1NP) levels. (*D*) Cross-linking terminal C-telopeptide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

to influence BMD and bone metabolism, such as age, gender, BMI, estrogen state, PTH, and vitamin D. These effects also were independent of circulating levels of T_3 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 T_3 for bone formation. D2 activity has been found on mature osteoblasts,[34] which are the primary target cells for T_3 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 T_3 levels were similar among the three D2 genotypes, allowing us to specifically study the consequences of the polymorphism for local T_3 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 T_3 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 T_3 for bone metabolism.[13–17,35–38] Two recent papers by Bassett and colleagues,[18,19] who studied mice with complete or haploinsufficiency of TRα and -β, concluded that TRα 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 T_3 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]

Disclosures

All the authors state that they have no conflicts of interest.

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