THYROID
Volume 22, Number 2, 2012

DOI: 10.1089/thy.2011.0245

A Large-Scale Population-Based Analysis of Common Genetic Variation in the Thyroid Hormone Receptor Alpha Locus and Bone

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Dear Editor:

Thyroid hormone (TH) is essential for normal bone development and the maintenance of adult bone mass. Childhood hypothyroidism leads to growth retardation and delayed bone age, whereas hyperthyroidism accelerates growth and advances bone age. In adults, hyperthyroidism leads to osteoporosis and increased fracture risk. TH receptor alpha (TRα), encoded by the *THRA* gene, is the predominant TR in bone. No patients with mutations in *THRA* have yet been described. Mice with inactivating mutations in *THRA* show a delay in bone development and osteosclerosis in adulthood (1). On the opposite chromosomal strand of *THRA*, the circadian clock gene *NR1D1* (REV-ERBα) is located. *THRA* and *NR1D1* partially overlap, and *NR1D1* expression influences splicing and expression of *THRA* (2). To date, limited data exist on the role of the *THRA/NR1D1* locus in human bone physiology. Previous candidate gene studies analyzing *THRA* in relation to bone mineral density (BMD) had limited sample sizes, and analyses were restricted to a subgroup of the population (i.e., older men; see Supplementary Data [available online at www.liebertonline.com/thy] for an overview of these studies). Therefore, we studied the effects of genetic variation in the *THRA/NR1D1* locus on BMD, BMD change, fracture risk, and bone geometry.

A tagging set of 14 polymorphisms was selected to cover the genetic variation in the *THRA/NR1D1* locus (see Supplementary Data). Serum TSH and FT4 levels were determined in 1350 subjects from the Rotterdam Study 1 (RS1). Femoral neck and lumbar spine BMD were measured in 19,195 subjects from the Genetic Factors for Osteoporosis (GEFOS) consortium (3). In RS1, femoral neck BMD was measured at baseline and at the second follow-up visit (follow-up [mean (SD)]: 6.51 (0.38) years) in 2366 subjects, and BMD loss rates were calculated. Four geometric outcomes measured at the femoral narrow-neck region in 4131 subjects were used: narrow-neck width, narrow-neck cortical thickness, buckling ratio (index of bone instability), and section modulus (index of bending strength). Thoracolumbar spine radiographs from 2994 subjects were scored for the presence of vertebral fractures ($n = 371$). Information on incident osteoporotic fractures was available for 5974 RS1 subjects (follow-up: 7.79 (3.04) years), and 2157 RS2 subjects (follow-up: 3.95 (0.84) years). The associations of the selected *THRA/NR1D1* polymorphisms with baseline characteristics, BMD, fracture risk, and bone geometry were studied using linear, logistic, and Cox regression analyses. See Supplementary Data for detailed information on materials and methods.

The studied *THRA/NR1D1* polymorphisms were not associated with baseline characteristics, including serum TSH and FT4. None of the polymorphisms were associated with neither BMD, BMD change, vertebral or incident osteoporotic fractures (see Supplementary Table S1), nor narrow-neck width, narrow-neck cortical thickness, buckling ratio, or section modulus.

The lack of effects of *THRA/NR1D1* polymorphisms on bone parameters was unexpected, considering the essential role of TH in bone physiology. *THRA* is expressed in both osteoblasts and chondrocytes. Mouse models with inactivating *THRA* mutations, as well as $THRA^{0/0}$ mice (lacking all *THRA* transcripts), display delayed bone development and osteosclerosis in adulthood (1). Furthermore, patients with TH resistance due to *TRβ* mutations have increased levels of TH and an increased risk of osteoporosis, which is thought to result from overstimulation of TRα. It has been shown that core circadian clock transcription factors (including REV-ERBα) and multiple metabolic bone homeostasis pathways display a circadian expression profile in bone, and that mice

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lacking circadian clock components display abnormal bone remodelling (4). Previous genome-wide association studies for BMD and fracture risk have not identified as significant associations with the *THRA/NR1D1* locus (3; see Supplementary Data for a review of these studies). However, these hypothesis-free approaches tested >300,000 variants, requiring stringent multiple-testing correction, and resulting in very low *p*-values to declare statistical significance (i.e., $p < 5 \times 10^{-8}$). Since the *THRA/NR1D1* locus is a plausible candidate locus for bone abnormalities, we performed a focused analysis of this locus on various bone parameters, thereby covering various aspects of bone (patho) physiology. Due to the large sample size, we were powered to detect at least small-to-moderate effects on the studied bone parameters (see Supplementary Data), but still did not find significant associations. However, it is important to note that the apparent absence of (common) functional variation in this locus does not negate its importance in bone.

Although TRα is the major TR in bone, mediating important effects of TH on bone development and turnover, our study excludes an important contribution of genetic variation in the *THRA/NR1D1* locus to variations in BMD, fracture risk, and bone geometry in the elderly population.

Acknowledgments

See Supplementary Data.

Disclosure Statement

The authors declare that no competing financial interests exist and there are no conflicts of interest.

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