Nephrol Dial Transplant (2001) 16: 691

Editorial Comments

Nephrology
Dialysis
Transplantation

Nephrol Dial Transplant (2001) 16: 691–694

The renal tubular Na–Cl co-transporter (NCCT): a potential genetic link between blood pressure and bone density?

Dinna N. Cruz

Yale University School of Medicine

Keywords: Na–Cl co-transporter; hypertension; osteoporosis

Correspondence and offprint requests to: Dinna N. Cruz MD, Assistant Professor of Medicine, Department of Medicine, Yale University School of Medicine, 333 Cedar St. 2071 LMP, New Haven, CT 06520-8029, USA.

Introduction

Hypertension and osteoporosis are both significant health problems. Hypertension affects 15–20% of the adult population in industrialized societies and is one of the principal independent risk factors for stroke, myocardial infarction, and end-stage renal disease.

Osteoporosis is the main cause of bone fractures in post-menopausal women and the elderly, and causes pain, deformity, and loss of independence. Recognition that these diseases are in part genetically determined has motivated studies to identify mutations that confer either susceptibility or protection from these diseases. In the case of hypertension, all of the monogenic forms of high blood pressure described to date involve a defect in the kidney's ability to excrete salt. As such, these forms of hypertension are aggravated by a high sodium intake [1]. Similarly, there are reported associations between bone mineral density and vitamin D receptor polymorphisms in the setting of osteoporosis [2]. In complex diseases such as these, a polygenic model is more likely, in which multiple genes act in conjunction with environmental exposures to produce disease. Therefore, studying the effects of very rare genetic mutations can provide important insights into mechanism of disease. Furthermore, there may be a link between these two genetically determined diseases.

Link between sodium and calcium homeostasis

A number of studies in both humans as well as animal models of hypertension have suggested an association between essential hypertension and osteoporosis [3–6]. For example, disturbances in calcium metabolism have been described in patients with essential hypertension. These include a higher urinary calcium excretion for a given salt intake, an elevated parathyroid hormone level, an increase in urinary cyclic AMP, a tendency for low serum ionized calcium level, an elevated $1,25(OH)_2$ vitamin D3 level, and increased intestinal calcium reabsorption [3,4]. It has been proposed that these alterations in calcium homeostasis are secondary to a genetic defect in the ability of the kidney to excrete sodium, although the specific mechanism(s) is (are) unclear [7]. This renal tendency to retain sodium results in volume expansion and a subsequent increase in urinary calcium excretion. As a result, a negative calcium balance develops, and a compensatory response to restore normal calcium balance occurs. Under this hypothesis, one would predict that hypertensive individuals would be more likely to develop bone demineralization, as demonstrated in certain models of animal hypertension [5,6]. This predicted consequence is corroborated by a recent study in elderly women, which reported an association between high blood pressure and bone loss at the femoral neck [8].

Conversely, an inherited renal defect could impair sodium reabsorption and induce sodium wasting. Relative volume depletion would develop and cause hypotension, or at least, protect against the development of hypertension. This in turn would potentially enhance urinary calcium reabsorption and result in a slightly positive calcium balance, perhaps either increasing bone mass or reducing bone loss. Thiazide diuretics represent one such clinical example. These drugs are commonly employed to treat hypertension and hypercalciuric nephrolithiasis. Its major antihypertensive effect occurs through salt excretion and a reduction in intravascular volume status. Ultimately, urinary calcium excretion is decreased and positive calcium balance occurs. In fact, several studies support this notion by documenting an association between chronic thiazide therapy and a reduction in some types of fractures [9,10]. Along the same line, treatment with thiazides increased bone mineral density in both men and women [11–15]. Longitudinal studies demonstrate a slower rate of bone loss with thiazide therapy when compared to age- and sex-matched normotensive controls. Importantly, these effects are independent of age, sex, or dietary calcium intake. The reductions in bone loss rate in thiazide-treated men range from 28.8 to 49.2% [11]. In women who have used these drugs for more than 10 years, appendicular bone mass is 7–10% greater than that of women who have never used thiazide diuretics [12]. Finally, thiazides employed for hypercalciuria in osteoporotic men are associated with an improvement in bone density at the hip (1.5–3%) and the spine (4–8%) after 0.7–2.2 years of follow-up [13,14].

Mutations of the sodium–chloride co-transporter: Gitelman's syndrome

It appears that a possible link between hypertension and osteoporosis resides in the gene which encodes for the thiazide-sensitive sodium–chloride co-transporter (NCCT). This allele links both renal sodium and calcium handling, whereby alterations in this gene could potentially connect the variations that develop in blood pressure, urinary calcium excretion, and bone mass. This co-transporter is responsible for sodium reabsorption in the distal convoluted tubule [16]. Furthermore, it is the site of action of thiazide diuretics and, as noted above, disturbances in transporter expression or function could either increase or decrease calcium excretion.

Gitelman's syndrome (GS), first described by Gitelman *et al.* in 1966, is an autosomal recessive renal tubular disorder in which NCCT function is impaired. As such, it is clinically analogous to a genetic version of thiazide diuretic therapy. Simon *et al.* [16] identified the molecular basis for this syndrome, demonstrating inactivating mutations in *NCCT*. The clinical features of GS include hypotension, hypokalaemia, hypomagnesaemia, metabolic alkalosis, and hypocalciuria. Mutations in the *NCCT* gene lead to loss of function of the Na–Cl co-transporter, salt wasting in the distal convoluted tubule, and volume depletion, with stimulation of the renin–angiotensin–aldosterone axis [16]. As noted previously, calcium reabsorption is probably enhanced by volume depletion. Conversely, activating mutations in *NCCT* would be predicted to result in enhanced renal sodium reabsorption and an increase in central blood volume, which MacGregor and Cappuccio [7] postulate is the direct cause of increased urinary calcium excretion.

Potential role of NCCT in hypocalciuria and bone density

Considering the similarities between the biochemical phenotype of patients with GS and patients taking thiazide diuretics, we hypothesized that *NCCT* mutations might have effects comparable to those of thiazides on bone. This hypothesis was validated in our study of 24 individuals with GS and 31 of their unaffected family members. Our data showed that despite a lower oral supplemental calcium intake and less physical exercise (Table 1), GS patients had significantly higher bone mineral density than unaffected family members at the lumbar spine and hip (Figure 1) [17]. Since an increase of 1 standard deviation in mean bone mineral density reduces the risk for fracture by approximately twofold, the changes in the subjects with GS should confer a significant protective effect on the risk for fragility fracture at sites such as the spine and hip. In terms of calcium metabolism, significantly lower urinary calcium excretion was noted, a previously known phenotype of this syndrome, but interestingly, lower $1,25(OH)_2$ vitamin D_3 levels and a trend towards a slightly higher total serum calcium level, albeit still within normal limits, were demonstrated (Table 1). It is notable that some of these findings are completely opposite to some of the abnormalities described in patients with essential hypertension [3,4,7]. However, in this small study we did not detect any difference in levels of parathyroid hormone or biochemical markers of bone turnover. Perhaps of greater interest for the general population, however, is that one copy of a *NCCT* mutation also has a positive effect on bone density. Subjects who were heterozygous for an inactivating *NCCT* mutation had significantly higher Z-scores at the lumbar spine and total femur than those subjects who carried no mutations (Figure 1). The GS heterozygotes also had significantly lower urinary calcium/ creatinine ratios (Table 1). This is the first report of a phenotype described in the more common heterozygous state. Although the true prevalence of the heterozygous state is not known, we infer that mutations in *NCCT* are relatively common in the population, based on the high proportion of compound heterozygosity in GS [16]. For example, a disease frequency of 1/40 000 would result in a heterozygous carrier frequency of 1%.

Potential role of NCCT in bone cells

Although it would be intellectually satisfying to infer that induction of hypocalciuria and subsequent positive calcium balance alone are responsible for these effects, in reality the mechanism or mechanisms by which thiazides (and GS) achieve their effects on bone are unclear. There are *in vitro* data which support the notion of direct effects of thiazide diuretics on bone cells. The NCCT is expressed in human osteoblasts and osteoblast-like cells [18,19]. In addition, thiazides at high doses directly inhibited bone resorption by isolated rat osteoclasts [20]. These studies indicate that bone cells are targets for the actions of thiazides, and that this action is specific for thiazide derivatives. In the aggregate, these mechanisms may in part explain the altered bone homeostasis in subjects with *NCCT* mutations. Studies directly examining these possibilities are currently under way.

Conclusion

Studies in GS demonstrate that the NCCT modulates renal sodium and calcium handling, and loss of function of this transporter, like a thiazide diuretic, induces sodium wasting and hypocalciuria. This raises the intriguing possibility that common variants in this gene might underlie blood pressure and bone mineral

Table 1. Clinical and biochemical characteristics of study population

Characteristic	*NCCT* genotype			*P* value
	−/− ($n=24$)	+/− ($n=18$)	+/+ ($n=13$)	
Age (years)	39.5 ± 2.0	49.8 ± 3.1	48.2 ± 4.9	0.04
Sex (M/F)	8/16	7/11	4/9	NS
Regular exercise* – (%)	8 (33)	15 (83)	11 (85)	<0.001
Estimated oral supplemental calcium intake (mg/day)	474 ± 122	801 ± 107	655 ± 126	0.04
Serum calcium (mg/dl)	9.7 ± 0.1	9.4 ± 0.1	9.4 ± 0.1	0.08
Urine calcium/creatinine (mmol/mmol)	0.14 ± 0.03	0.26 ± 0.03	0.39 ± 0.06	<0.001
Serum $1,25(OH)_2$ vitamin D_3 (pg/ml)	41.1 ± 4.3	49.5 ± 3.5	54.0 ± 4.2	0.04

Plus-minus values are means ± SEM. Genotype −/− is GS; +/− is Gitelman's heterozygote; +/+ is normal. *P* values for chi-square and Kruskal–Wallis test among different genotype classes are indicated.
*Self-reported regular exercise.
Normal values: calcium, 8.8–19.2 mg/dl. To convert values to mmol/l, multiply by 0.2495. $1,25(OH)_2$ vitamin D3, 20–65 pg/ml.

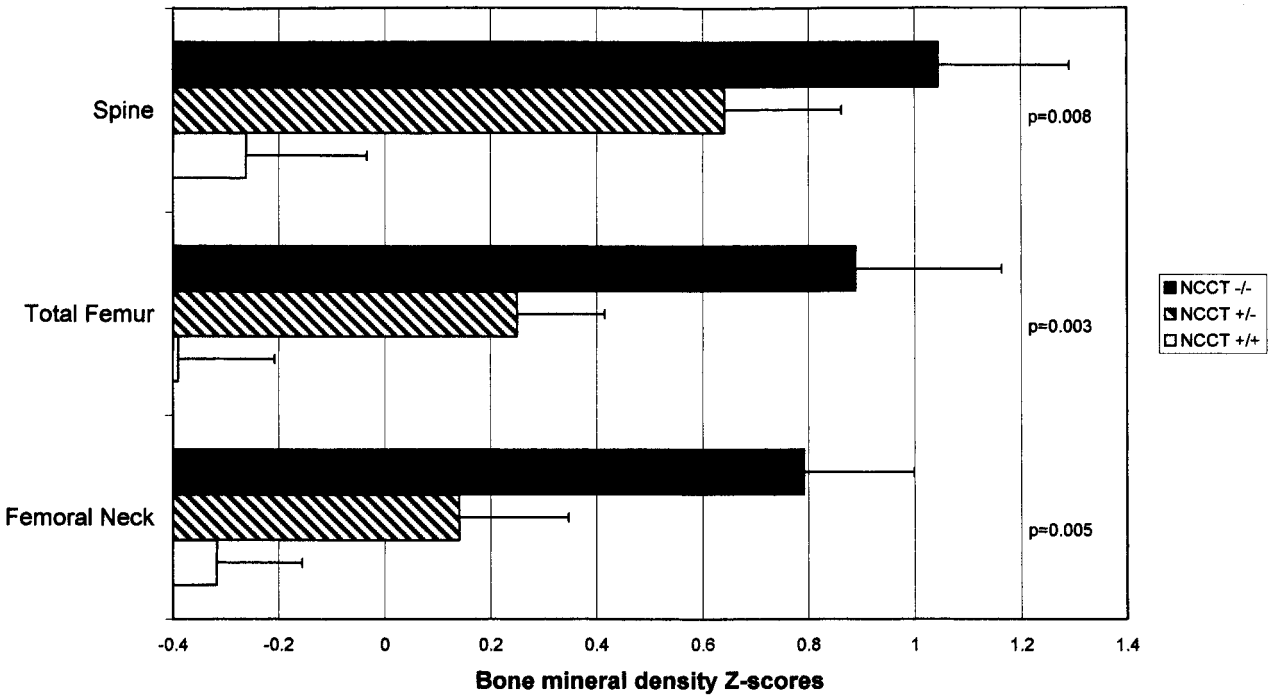

Fig. 1. Bone mineral density Z-scores in the *NCCT* genotype groups at the various skeletal sites. Mean (±SEM) for the *NCCT* genotype groups. Genotype −/− is GS; +/− is Gitelman's heterozygote; +/+ is normal. *P* values for Kruskal–Wallis test among different genotype classes are indicated.

density variation in the general population. Furthermore, these studies also demonstrate the utility of molecular genetic approaches to the understanding of hypertension and osteoporosis.

References

1. Lifton RP. Molecular genetics of human blood pressure variation. *Science* 1996; 272: 676–680
2. Morrison NA, Qi JC, Tokita A *et al.* Prediction of bone density from vitamin D receptor alleles. *Nature* 1994; 367: 284–287. Correction, *Nature* 1997; 387: 106
3. McCarron DA, Pingree PA, Rubin RJ, Gaucher SM, Molitch M, Krutzik S. Enhanced parathyroid function in essential hypertension: a homeostatic response to a urinary calcium leak. *Hypertension* 1980; 2: 162–168
4. Brickman AS, Nyby MD, von Hungen K, Eggena P, Tuck ML. Calciotropic hormones, platelet calcium, and blood pressure in essential hypertension. *Hypertension* 1990; 16: 515–522
5. Izawa Y, Sagara K, Kadata T, Makita T. Bone disorders in spontaneously hypertensive rats. *Calcif Tissue Int* 1985; 37: 605–607
6. Cirillo M, Galletto F, Strazullo P, Torielli L, Melloni MC. On the pathogenetic mechanism of hypercalciuria in genetically hypertensive rats of the Milan strain. *Am J Hypertens* 1989; 2: 741–746
7. MacGregor GA, Cappuccio FP. The kidney and essential hypertension: a link to osteoporosis? *J Hypertens* 1993; 11: 781–785
8. Cappuccio FP, Meilahn E, Zmuda JM, Cauley JA. High blood pressure and bone-mineral loss in elderly white women: a prospective study. *Lancet* 1999; 354: 971–975
9. LaCroix AZ, Weinpahl J, White LR *et al.* Thiazide diuretic agents and the incidence of hip fracture. *N Engl J Med* 1990; 322: 286–290
10. Felson DT, Slutskis D, Anderson JJ, Anthony JM, Kiel DP. Thiazide diuretics and the risk of hip fracture: results from the Framingham study. *JAMA* 1991; 265: 370–373
11. Wasnich R, Davis J, Ross P, Vogel J. Effect of thiazides on rates of bone mineral loss: a longitudinal study. *BMJ* 1990; 301: 1303–1305
12. Cauley JA, Cummings SR, Seeley DG *et al.* Effects of thiazide diuretic therapy on bone mass, fractures, and falls. The Study of Osteoporotic Fractures Research Group. *Ann Intern Med* 1993; 118: 666–673
13. Adams JS, Song CF, Kantorovich V. Rapid recovery of bone mass in hypercalciuric, osteoporotic men treated with hydrochlorothiazide. *Ann Intern Med* 1999; 130: 658–660
14. Laroche M, Mazieres B. Beneficial effect of a thiazide diuretic on bone mineral density in male osteoporosis with hypercalciuria. *Clin Exp Rheumatol* 1998; 16: 109–110
15. LaCroix AZ, Ott SM, Ichikawa L, Scholes D, Barlow WE. Low-dose hydrochlorothiazide and preservation of bone mineral density in older adults: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 2000; 133: 516–526
16. Simon DB, Nelson-Williams C, Bia MJ *et al.* Gitelman's variant of Bartter's syndrome, inherited hypokalemic alkalosis, is caused by mutations in the thiazide-sensitive Na–Cl cotransporter. *Nature Genet* 1996; 12: 24–30
17. Cruz D, Simon D, Lifton R. Inactivating mutations in the Na–Cl co-transporter is associated with high bone density. *J Am Soc Nephrol* 1999; 10: 597A
18. Aubin R, Menard P, Lajeunesse D. Selective effects of thiazides on the human osteoblast-like cell line MG-63. *Kidney Int* 1996; 50: 1476–1482
19. Barry ELR, Gesek FA, Kaplan MR, Hebert SC, Friedman PA. Expression of the sodium-chloride cotransporter in osteoblast-like cells: effects of thiazide diuretics. *Am J Physiol* 1997; 41: C109–116
20. Hall TJ, Schaueblin M. Hydrochlorothiazide inhibits osteoclastic resorption *in vitro*. *Calcif Tissue Int* 1994; 55: 266–268