BsmI Polymorphism of the Vitamin D Receptor Gene in Hyperparathyroid or Hypoparathyroid Dialysis Patients

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Key Words: Vitamin D receptor gene; End-stage renal disease; Hyperparathyroidism; Hypoparathyroidism

Abstract

Since bone mineral density may be influenced by the polymorphisms of the vitamin D receptor (VDR) gene, we studied whether VDR genotypes might drive the progression toward hyperparathyroidism or hypoparathyroidism in patients with end-stage renal disease. On the basis of their parathyroid hormone (PTH) levels, we divided 99 patients undergoing dialysis into 2 groups: 56 patients with hypoparathyroidism (PTH < 104 pg/mL [< 11 pmol/L]) and 43 with hyperparathyroidism (PTH > 261 pg/mL [> 27.5 pmol/L]). The BB polymorphism was more frequent in patients with hypoparathyroidism (34%) than in patients with hyperparathyroidism (16%), but the difference did not reach statistical significance. Patients with the B allele and BB genotype had a significantly lower dialytic age and serum PTH and alkaline phosphatase levels than patients with the b allele and bb genotype. These results suggest that in end-stage renal disease, the BB genotype may mark a higher risk of developing hypoparathyroidism and diminished bone turnover.

Bone mineral density is influenced by environmental factors, such as dietary calcium intake and physical activity, and by genetic determinants. Since vitamin D is important in calcium metabolism and bone turnover, it has been suggested that polymorphisms for the vitamin D receptor (VDR) gene may be one of these factors. In a large population of healthy whites, Morrison and coworkers1-2 described an association between the VDR-BB genotype and higher levels of osteocalcin, an index of bone turnover. They also found that postmenopausal women with the BB genotype had low bone mineral density in the lumbar spine and femoral neck, resulting in a lower fracture threshold. They suggested that the presence of the B allele of the VDR gene influenced the bone turnover and, therefore, could be considered a genetic marker of osteoporosis. Although the specific molecular role was not described fully, they suggested that the B allele might be associated with increased transcriptional activity of the VDR gene and prolonged messenger RNA (mRNA) stability. These results were not confirmed fully in further studies,3-6 probably because of differences in sample size and ethnic background of the populations.

Vitamin D works at different sites, including the intestine, kidney, bone, and parathyroid glands; in these sites, calcitriol interacts with the nuclear VDR, causing inhibition of the parathyroid hormone gene transcription and up-regulation of the VDR itself.

In renal failure, the long-term phosphorus retention and the reduced vitamin D production induce secondary hyperparathyroidism, which includes elevated serum parathyroid hormone (PTH) levels, calcium-phosphorus balance alterations, and high bone turnover osteodystrophy, the so-called osteitis fibrosa (OF).9 However, an increasing number of patients undergoing dialysis have been seen with low levels of circulating PTH (hypoparathyroidism) and abnormalities of bone remodeling, leading to low turnover osteodystrophy, named aplastic bone disease (ABD). The biochemical
markers of these 2 diseases, although not specific, are well established: OF is associated with high levels of PTH and alkaline phosphatase (ALP), while ABD is associated with low levels of PTH and ALP. Between these situations, there is a wide spectrum of renal bone lesions, namely “mixed forms,” osteomalacia and osteoporosis, that have less clear-cut biochemical patterns. Only osteomalacia, a low turnover bone disease, may manifest the same findings as ABD, but if there is no aluminum bone intoxication, this form can be excluded.10–12

We studied whether VDR polymorphism influenced the parathyroid endocrine status of patients with end-stage renal disease.

Materials and Methods

Patients

From a large multicentric white population (northwest Italy) of patients undergoing dialysis, we selected 99 with end-stage renal disease (89 patients receiving hemodialysis and 10 patients receiving continuous ambulatory peritoneal dialysis); all had abnormal parathyroid function and biochemical findings of pathologic bone metabolism. None of the patients were diabetic. To control phosphatemia, all were receiving oral calcium carbonate or calcium acetate or magnesium carbonate as phosphate binders. Patients with hyperparathyroidism were treated with calcitriol, orally or intravenously. To avoid hypercalcemia, we reduced the calcium concentration in the dialysate as needed (from 1.75 to 1.5 and finally to 1.25 mmol/L for patients undergoing hemodialysis if necessary).

The study group comprised 60 men and 39 women. The characteristics of the group, given as mean ± SD, were as follows: age, 56.5 ± 13.2 years; dialytic age, 69.8 ± 66.3 months; PTH level, 260 pg/mL (27.4 ± 30.8 pmol/L; reference range, 10–67 pg/mL [1.1–7.1 pmol/L]); ALP level, 216 ± 140 U/L (reference range, 80–220 U/L); serum calcium level, 9.80 ± 0.08 mg/dL (2.45 ± 0.2 mmol/L; reference range, 8.4–10.4 mg/dL [2.1–2.6 mmol/L]); and serum phosphorus 4.5 ± 1.0 mg/dL (1.47 ± 0.33 mmol/L; reference range, 2.5–4.4 mg/dL [0.8–1.44 mmol/L]). On the basis of their PTH levels, the 99 patients were divided into 2 groups: 56 with hypoparathyroidism (PTH < 104 pg/mL [<11 pmol/L]) and 43 with hyperparathyroidism (PTH 261 pg/mL [>27.5 pmol/L]).

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<td><strong>Main Demographic, Clinical, and Biochemical Variables for Patients With Hypoparathyroidism and Hyperparathyroidism</strong></td>
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<td><strong>Age (y)</strong></td>
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* Data are given as mean ± SD. P calculated by logistic regression.

Methods

After DNA extraction from peripheral leukocytes by a standard method with phenol-chloroform, the polymorphisms of the VDR gene were analyzed by amplification with the polymerase chain reaction (Eppendorf Mastercycler 5330, Eppendorf, Hamburg, Germany) of an 800-base-pair DNA fragment, between exons 8 and 9 of the gene. The polymerase chain reaction was done using the following temperature scheme for 30 cycles: 95°C for 1 minute, 52°C for 2 minutes, and 72°C for 3 minutes.13 The amplified fragments were digested with Bsml restriction enzyme (Roche, Italy) and fractionated by electrophoresis in a 1.5% agarose gel, detecting 3 genotypes: Bb, bb, BB, where b means the presence of the restriction site and B the absence of the site.

Serum PTH was measured by a 2-site immunoradiometric assay (Allegro IRMA PTH kit, Nichols Institute, San Juan, Capistrano, CA); serum calcium, phosphorus, and ALP levels were measured by standard automated techniques.

Statistical Analysis

We used logistic regression analysis and 1-way analysis of variance followed by the Student t test (Statistical Package for the Social Sciences for MS WINDOWS, release 6.1, SPSS, Chicago, IL) for comparison of the biochemical data, which are expressed as mean ± SD. Differences between groups for the frequencies of genotypes and alleles were examined by using the χ2 test (SPSS for MS WINDOWS release 6.1).

Results

In our study population, the genotype frequencies were under genetic (or Hardy-Weinberg) equilibrium: 53 patients

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had the Bb genotype (54%), 26 patients had the BB (26%), and 20 patients had the bb (20%). In the group with hypoparathyroidism, the B allele was overrepresented (60% vs 44%, \( P < .05 \)), and the BB genotype was more frequent (71% vs 37%, \( P < .05 \)); similar results were found for the b allele and bb genotype in the hyperparathyroidism group (56% vs 40%, \( P < .05 \); 63% vs 29%, \( P < .05 \)). When the \( \chi^2 \) test was used to compare the frequencies of the 3 genotypes, including the heterozygous condition, there was only a trend toward statistical significance (\( P = .07 \)) Table 2.

Comparison of the clinical and biochemical variables in the hypoparathyroidism and hyperparathyroidism groups showed a significant difference for the dialytic age, for serum ALP levels, and, obviously, for PTH levels (\( P < .001 \)). These data suggest that patients with lower PTH levels tend to have a lower dialytic age and a low turnover bone disease, as confirmed by lower levels of serum ALP.

We also considered whether the 3 \( VDR \) genotypes might account for differences in clinical and biochemical data. This analysis Figure II revealed a significant difference between bb vs BB and Bb for the mean dialytic age (107.3 vs 59.5 and 61.9 months; \( P < .05 \)), serum PTH level (433 vs 184 and 230 pg/mL [45.6 vs 19.4 and 24.2 pmol/L]; \( P < .05 \)), and serum ALP level (295 vs 173 and 207; \( P < .05 \)). Interestingly, patients with the BB and Bb genotype were older than those with the bb genotype (59.6 and 60.1 vs 52.9 years), even though this difference was not significant. In our population, the prevalence of hypoparathyroidism was higher among patients with the BB genotype than those with the bb genotype (73% vs 40%, \( P = .05 \)) or Bb genotype (73% vs 54.7%, \( P = .57 \)).

**Discussion**

We studied patients with altered parathyroid gland function secondary to renal failure. Several factors influence the
parathyroid status in the uremic patient. Extreme parathyroid
dysfunction in a patient undergoing dialysis does not arise
suddenly, and its appearance seems to be related to the dura-
tion of renal failure and dialytic age. The hyperparathy-
roidism was, in fact, related to a longer time on dialysis. The
etiopathogenic mechanism is not fully understood and is
probably multifactorial. Increased PTH levels are a conse-
quence of an initial transient hyperphosphatemic and hypo-
calcemic status concomitant with pathologic PTH degra-
dation and lower absolute or relative serum calcitriol levels.
The origin of hypoparathyroidism might be linked to hyper-
calcemia or hypophosphatemia, aluminum intoxication, or
inappropriate vitamin D treatment. It also has been demon-
strated that calcitriol can influence parathyroid cell function
and differentiation, accounting for one of the possible factors
involved in the parathyroid dysfunction.9 In consideration of
these factors, our interest was focused on whether the poly-
morphisms of the VDR gene influence the development of
hypoparathyroidism or hyperparathyroidism in end-stage
renal disease.

The relationship between the VDR genotype and para-
thyroid function is controversial. Carling et al14 observed that
in women with primary hyperparathyroidism, the VDR geno-
types modulated PTH secretion; the bb genotype and b allele
were overrepresented in patients with this disease compared
with age-matched controls, confirming the hypothesis proposed by Morrison et al12 that the b allele is associated
with lower mRNA stability and activity. Therefore, the b
allele is a genetic variant leading to a lesser functioning VDR
without adequate suppression of PTH secretion.

In a group of dialysis patients with low serum PTH
levels, Fernandez et al15 found a higher frequency of the BB
genotype than in the control dialysis population. They found
no real difference in genotype distribution between patients
with hypoparathyroidism and hyperparathyroidism.

Tsukamoto et al16 found that, compared with the bb
genotype, the VDR-BB genotype may be related to lower
PTH levels in uremic patients with secondary hyperparathy-
roidism. However, McCarey et al17 and Schmidt et al18 did
not confirm this correlation.

The 99 patients undergoing dialysis who were included
in the present study were divided into 2 groups according to
their PTH levels. Those with hypoparathyroidism had lower
ALP activity, which means less bone turnover. This inade-
quate bone turnover, also defined by the low PTH and ALP
levels, usually is associated with ABD. The patients with
hyperparathyroidism, instead, had higher bone turnover and
a late-onset endocrine alteration. The higher ALP levels,
which trigger accelerated bone turnover, and the increased
PTH levels are established characteristics of OF. Although
we did not have bone biopsy specimens for all patients, it
has been demonstrated that hypoparathyroidism correlates
with the histologic pattern of ABD and hyperparathyroidism
with OF.11

The hypoparathyroidism group had a significantly
higher prevalence of the B allele and the hyperparathy-
roidism group of the b allele (P < .05). The BB genotype
was more represented in the hypoparathyroidism group than
in the hyperparathyroidism group, but there was only a trend
in favor of statistical significance (33.9% vs 16.3, z² test; P =
.07). These results seem to agree with the notion that the B
allele is related to greater mRNA activity and stability.

When we compared the clinical and biochemical vari-
bles for the different genotypes independently of the PTH
status, patients with the B allele and the BB genotype,
compared with those with the b allele and bb genotype, had a
lower dialytic age and lower serum PTH and ALP levels.
Therefore, a patient with end-stage renal disease with the BB
genotype has a greater likelihood of developing hypoparathy-
roidism and the related ABD. As regards the lower dialytic
age in the hypoparathyroidism group, since time influences
parathyroid function, these results might have been influ-
cenced by the wide range of dialytic ages in the study group.

The B allele and BB genotype seem somewhat more
frequent in patients with hypoparathyroidism, although the
statistical significance is documented only for the allele
frequency. This finding might help identify a genetic risk of
hypoparathyroidism associated with diminished bone
turnover, in accordance with the hypothesis proposed by
Morrison et al1,12

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