Yonago Acta medica 2000;43:27–38

Association between Vitamin D Receptor Gene Polymorphisms and Renal Osteodystrophy in Patients on Maintenance Hemodialysis

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We examined the possible involvement of vitamin D receptor (VDR) gene polymorphisms in patients on maintenance hemodialysis and further investigated the relation between VDR genotypes and bone histology. Two hundred and nine patients undergoing regular hemodialysis (male/female ratio, 124/85) were included in this study. DNA was extracted from peripheral blood leukocytes. VDR genotypes were analyzed as restriction fragment length polymorphisms, by using *Bsm*I, *Apa*I, *Taq*I and *Fok*I. Lumbar bone mineral density was measured by dual-energy X-ray absorptiometry, and expressed as a Z-score. Serum $1,25(OH)_2D_3$, osteocalcin and intact-parathyroid hormone (i-PTH) were determined by an immunoradiometric assay. In 97 patients, bone biopsy was performed and the histology was divided into osteitis fibrosa, mild lesion, adynamic bone disease and osteomalacia. Serum levels of osteocalcin, i-PTH and bone mineral density were significantly lower in the presence of *B*, *A*, *t* and *f* alleles. However, in this study, we did not find any association between bone histology and the four VDR genotypes. We concluded that renal osteodystrophy in dialysis patients was modified by environmental factors such as medication with active vitamin D, age, gender and duration of chronic renal failure, and that the impact of the VDR allelic effect may play a small role in determining on bone histology.

Key words: bone mineral density; hemodialysis; renal osteodystrophy; vitamin D receptor gene polymorphism

Children obviously bear a striking likeness to their parents, and bone density has been considered a genetic determinant in the inheritance of appearance from parents to children. Smith and colleagues (1973) assessed bone density on the midshaft radius in monozygotic twins and dizygotic twins to prove for the first time that a significantly larger variation in intrapair differences was observed in dizygotic twins compared to that in monozygotic twins. Later investigations based on twin studies and epidemiologic surveys all favored this finding, indicating that there is an inheritable component related to bone mass which may be associated with 50 to 80% of bone mass features in individuals. However, the search for a gene which determines bone mass has long been unsuccessful.

In 1994, Morrison and others (1992) demonstrated that common allelic polymorphisms in the gene encoding the vitamin D receptor (VDR) were significantly correlated with bone density in healthy individuals. Since then, a considerable number of studies were reported to confirm this interesting finding. However, the results have been controversial to date.

Recently, VDR has become a focus of research interest not only as a candidate gene controlling bone density, but also as a gene associ-

Abbreviations: bp, base pair; BMD, bone mineral density; IGF, insulin-like growth factor; i-PTH, intact-parathyroid hormone; kb, kilobase; nVDRE, negative vitamin D responsive element; RFLP, restriction fragment length polymorphism; VDR, vitamin D receptor

Table 1. Patient characteristics

	Number of patients		Age (year)	Z-score
Chronic glomerulonephritis	123	(74/49)	55 ± 11*	–0.105 ± 1.341
Diabetes mellitus	48	(30/18)	63 ± 11*	0.331 ± 1.042
Rheumatoid arthritis	12	(2/10)	58 ± 9	–0.233 ± 0.681
Polycystic kidney disease	8	(5/ 3)	59 ± 19	0.167 ± 1.150
Others	18	(13/ 5)	56 ± 24	–0.750 ± 1.666

Values are expressed as mean ± SD.
( ), male/female ratio.
* $P < 0.05$; chronic glomerulonephritis versus diabetes mellitus.

ated with bone turnover. Patients with chronic renal failure are generally accompanied by renal osteodystrophy caused by anomalies in bone turnover. Due to the successful spread of maintenance hemodialysis, patients with chronic renal failure have attained considerable improvement in survival time, but at the same time patients are increasingly left confronted with renal osteodystrophy. Thus, renal osteodystrophy is an important factor influencing the quality of life of patients on maintenance hemodialysis. To date, however, there have been a few reports describing the relationship between the prevalence of renal osteodystrophy and VDR polymorphisms. In the present study, we assessed a possible correlation between VDR polymorphisms and renal osteodystrophy in patients undergoing maintenance hemodialysis by measuring bone mineral density (BMD) and markers for bone turnover. We also assessed such correlation in patients on maintenance hemodialysis by performing a bone biopsy.

Materials and Methods

Patients

The subjects consisted of 209 patients (124 males and 85 females) with chronic renal failure on maintenance hemodialysis. Primary diseases included chronic glomerulonephritis, diabetes mellitus, rheumatoid arthritis, autosomal dominant polycystic kidney disease and other miscellaneous diseases in 123, 48, 12, 8 and 18 patients, respectively (Table 1).

RFLP analysis of VDR locus

Prior to trial, informed consent was individually obtained from patients and their relatives by the attending physicians following full explanations of the aim of the research, and guarantees of privacy. Two-milliliter aliquots of peripheral blood samples were collected from the patients and stored in the presence of EDTA. Genomic DNA was extracted from leukocytes obtained from the blood samples with the conventional extraction method using phenol/chloroform followed by ethanol precipitation.

So far, the VDR gene, mapped to the long arm of human chromosome 12, was considered to contain 9 exons. In 1997, Miyamoto and coworkers (1996, 1997) identified two previously unreported exons located 20 kilobase (kb) upstream from the originally reported exon 1; subsequently designated them exon 1A and exon 1B, and renamed exon 1 as exon 1C. Thus, the VDR gene is comprised of 11 exons which, together with intervening introns, span approximately 75 kb, where the translation start codon is located in exon 2 as reported by Baker and others (1998) (Fig. 1). A restriction fragment length polymorphism (RFLP) involving the loss of an initiation codon (ATG) by a single base substitution from T to C (ACG) starts translation from 9-base pair (bp) downstream, raising the possibility that the polypeptides translated from the two alleles differ by three amino acid residues.

To amplify a 265-bp sequence in exon 2, a 10-μL reaction mixture containing 50 ng of template DNA, 1 μmol/L each of the primers VDR2a: 5'-AGCTGGCCCTGGCACTGA

CTCTGCTCT-3' and VDR2b: 5'-ATGGAA ACACCTTGCTTCTTC TCCCTC-3', 10-mmol/L Tris-HCl (pH 8.3), 50-mmol/L KCl, 1.5-mmol/L $MgCl_2$, 0.2 mmol/L each of deoxyribonucleotide triphosphates and 0.25 U of *Taq* DNA polymerase (Perkin-Elmer Co., Foster City, CA) were incubated in a microtube using a thermal cycler (Touch Down, Hybaid Ltd., Ashford, Middlesex, United Kingdom) (Gross et al., 1996). A reaction cycle consisting of sequential incubations for denaturation at 94°C for 45 s, for annealing at 60°C for 45 s, and for extension at 72°C for 45 s was repeated 35 times, except that denaturation at 94°C for an additional 7 min was included in the first cycle.

Five-microlitter aliquots of the polymerase chain reaction (PCR) product was incubated at 37°C for 3 h in a 10-μL reaction mixture containing 50-mmol/L NaCl, 10-mmol/L Tris-HCl (pH 7.5), 10-mmol/L $MgCl_2$, 1-mmol/L 1,4-dithiothreitol and 4 units of the restriction endonuclease *Fok*I (Nippon Gene, Toyama, Japan).

Aliquots of the *Fok*I digest of PCR products were electrophoresed for 30 min at 100 V through a gel (1% agarose S plus 1% agarose X) containing 0.4-μg/mL ethidium bromide prepared in a buffer containing 89-mmol/L Tris-borate and 2-mmol/L EDTA. As DNA size markers, ϕX174/*Hae*III digest and ϕX174/*Hinc*-II digest (Nippon Gene) were used.

DNA bands on the gel were visualized under 312-nm UV lamp (ATTO Bioinstrument, Tokyo, Japan) and fluorescent DNA bands were photographed with a Polaroid camera for RFLP assessment. Upon digestion by *Fok*I, the 165-bp PCR products derived from an allele having a *Fok*I site in exon 2, designated *f*, were split into two bands, 69 bp and 196 bp, respectively, while those derived from an allele not having a *Fok*I site in the corresponding sequence, designated *F*, remained as a single band. Thus, genotypes of the PCR products derived from individual subjects were presented as *ff*, *Ff* or *FF*.

Genotyping was similarly performed regarding RFLPs involving *Bsm*I and *Apa*I sites in intron 8, and RFLP involving the *Taq*I site in exon 9 (Tokita et al., 1996), using PCR primers described by Morrison and others (1992). The 825-bp PCR products derived from an allele having a *Bsm*I site in intron 8, designated *b*, were split into two bands, 650 bp and 175 bp, respectively, upon digestion with *Bsm*I, while those derived from an allele not having a *Bsm*I site in the corresponding sequence, designated *B*, remained a single band.

The 740-bp PCR products derived from an allele having both an *Apa*I and a *Taq*I site between intron 8 and exon 9, designated *a*, were split into two bands, 529 bp and 211 bp, respectively, upon digestion with *Apa*I, while those

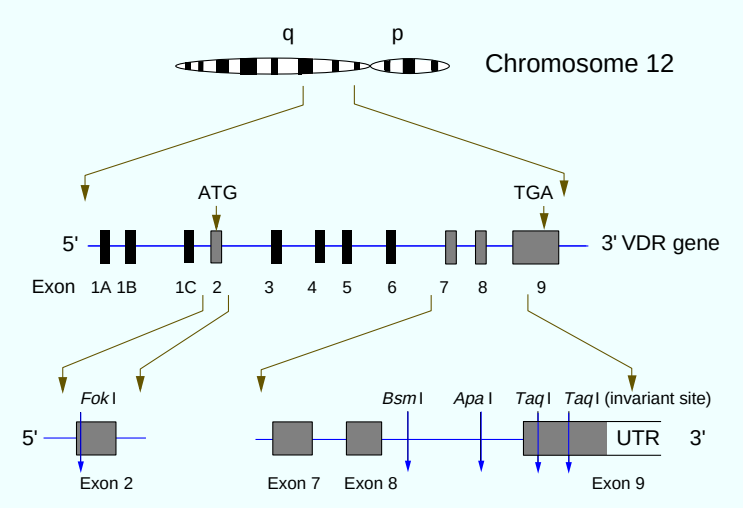

Fig. 1. Genomic organization and restriction map of the vitamin D receptor (VDR) gene.

derived from an allele not having an *Apa*I site in the corresponding sequence, designated *A*, remained a single band.

In exon 9, there is an additional invariant *Taq*I site that is not involved in generating RFLP. Thus, the above 740-bp PCR products derived from an allele having an RFLP-associated *Taq*I site in exon 9, designated *t*, were split into three bands, 291 bp, 247 bp and 202 bp, respectively, upon digestion with *Taq*I, while those derived from an allele not having an RFLP-associated *Taq*I site in the corresponding sequence, designated T, were split into two bands, 493 bp and 247 bp, respectively (Fig. 2).

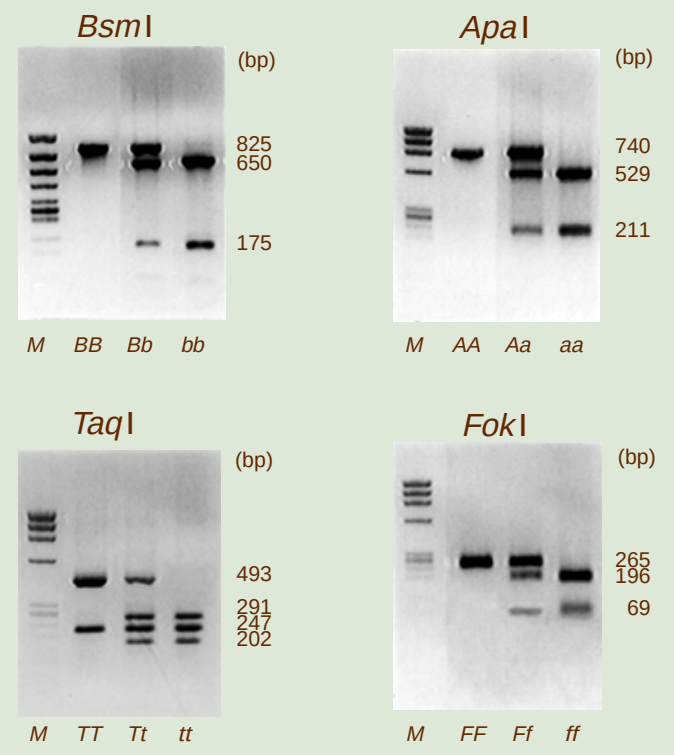

Fig. 2. Restriction fragment length plymorphism analysis of polymorphic vitamin D receptor (VDR) alleles. bp, base pair.

Measurements of bone turnover markers and BMD

Serum $1,25(OH)_2D_3$, osteocalcin and intact-parathyroid hormone (i-PTH) were determined as bone turnover markers. Patients with fracture, scoliosis or lumbar osteoarthritis upon X-ray examination were excluded from evaluation of BMD, since bones with these anomalies could provide abnormal BMD values. BMD at the lumbar spine (L2–L4) was measured by dual-energy X-ray absorptiometry (model XR-26, Norland, Fort Atkinson, WI). For age- and gender-matched comparisons, BMD values were expressed as Z-scores.

Bone biopsy

Bone biopsy was performed in 97 patients with chronic renal failure on maintenance hemodialysis. To determine the mineralization rate, bones were subjected to tetracycline double-labeling: tetracycline hydrochloride was given at a dose of 750 mg/24 h for 2 days, and after a cessation for the following 10 days, the drug was further given during the next 4 days. Bone biopsy was performed within 7 days after administration of the second label. Before bone biopsy was performed, diazepam and ketamine were given. Under local anesthesia with lidocaine, bone specimens were taken vertically from the right iliac crest with a manually driven drill attached to Kitasato's trephine. During this biopsy procedure, no patients complained of severe pain. None of the serious complications were experienced. Biopsy specimens were fixed in dehydrated ethanol, embedded in methylmethacrylate and left for 7 days for complete solidification. Sections (4–5 μm) were cut on a microtome, mounted on glass slides and stained with Villanueva-Goldner and aluminon. The bone turnover rate was quantitatively assessed by bone histomorphometry. According to the classification described by Sherrard and others (1993), bone tissues were histopathologically diagnosed as osteitis fibrosa, osteomalacia and mild lesion and adynamic bone disease (Table 2).

Table 2. Histologic classification of renal osteodystrophy

	Area of osteoid (%)	Area of fibrosis (%)	Bone formation rate ($\mu m^2/mm^2$ of tissue area/day)
Osteitis fibrosa	< 15	> 0.5	Increased
Osteomalacia	> 15	< 0.5	Decreased
Mixed*	> 15	> 0.5	Normal
Mild lesion	< 15	< 0.5	Normal (> 108)
Adynamic bone disease	< 15	< 0.5	Decreased (< 108)

Area of osteoid, osteoid volume/bone volume; area of fibrosis, fibrosis volume/tissue volume; bone formation rate, bone formation rate/bone surface.
* Mixture of osteitis fibrosa and osteomalacia.
The table is cited from Sherrard et al. (1993).

Statistical analysis

The results were assessed by the analysis of variance, contingency table test and regression analysis. P values < 0.05 were regarded as significant.

Results

Regarding patient characteristics (Table 1), average age and BMD values did not significantly differ among patients with regard to primary diseases, except that the average age was approximately 8 years older in the diabetes mellitus group than in the chronic glomerulonephritis group.

Allele and genotype frequencies for *Bsm*I-RFLP were 1.0%, 18.2% and 80.9% for *BB*, *Bb* and *bb*, respectively (Table 3). Compared to the value of approximately 20% reported for *BB* in the study of a sample of European population (Uitterlinden et al., 1996), the present value of 1.0% was significantly lower, suggesting an ethnic variation in allele frequencies. The above allele frequencies for the *Bsm*I-RFLP did not significantly differ among patients of different ages, or among those showing different serum values for Ca, P, alkaline phosphatase or active vitamin D. Values for serum osteocalcin were 43.5 ± 3.5 mg/dL, 54.9 ± 30.6 mg/dL and 78.0 ± 66.4 mg/dL for *BB*, *Bb* and *bb*, respectively, indicating that the values in individuals possessing the *b* haplotype were significantly higher ($P < 0.05$). Individuals possessing the *b* haplotype also showed significantly higher values for i-PTH: 130 ± 55 pg/mL, 149 ± 144 pg/mL and 278 ± 260 pg/mL for *BB*, *Bb* and *bb*, respectively ($P < 0.05$). The age- and gender-matched Z-scores for BMD were also significantly higher in individuals possessing *b* haplotype ($P < 0.01$): –2.50 ± 0.14, –0.50 ± 1.44 and

Table 3. Relationship between bone turnover markers and *Bsm* I genotypes of VDR

		Genotype			*P**
		BB	*Bb*	*bb*	
Number of subjects		2 (1.0%)	38 (18.2%)	169 (80.9%)	
Age	(year)	77.5 ± 2.1	56.0 ± 15.8	57.0 ± 13.9	NS
Ca	(mg/dL)	6.1 ± 3.3	7.4 ± 2.1	7.8 ± 2.2	NS
P	(mg/dL)	6.5 ± 1.6	5.6 ± 2.0	5.7 ± 1.6	NS
Alkaline phosphatase	(IU/L)	158 ± 102	149 ± 96	186 ± 307	NS
1,25 $(OH)_2D_3$	(pg/mL)	6.9 ± 5.7	8.9 ± 5.8	8.0 ± 5.6	NS
Osteocalcin	(mg/dL)	43.5 ± 3.5	54.9 ± 30.6	78.0 ± 66.4	< 0.05
i-PTH	(pg/mL)	130 ± 55	149 ± 144	278 ± 260	< 0.05
Z-score		–2.50 ±0.14	–0.50 ± 1.44	0.43 ± 1.58	< 0.01

* Significant difference in P values among the three genotypes.
i-PTH, intact parathyroid hormone; NS, not significant; VDR, vitamin D receptor.

Table 4. Relationship between bone turnover markers and *Apa* I genotypes of VDR

		Genotype			*P**
		AA	*Aa*	*aa*	
Number of subjects		23 (11.0%)	79 (37.8%)	107 (51.2%)	
Age	(year)	55.9 ± 16.7	56.7 ± 14.5	57.7 ± 13.0	NS
Ca	(mg/dL)	7.4 ± 2.2	7.3 ± 2.2	8.1 ± 2.2	NS
P	(mg/dL)	5.7 ± 2.0	5.8 ± 1.7	5.6 ± 1.5	NS
Alkaline phosphatase	(IU/L)	164 ± 120	194 ± 375	170 ± 217	NS
1,25 $(OH)_2D_3$	(pg/mL)	6.1 ± 1.6	10.6 ± 7.3	6.7 ± 3.8	NS
Osteocalcin	(mg/dL)	56.5 ± 30.0	65.4 ± 60.4	82.9 ± 58.7	NS
i-PTH	(pg/mL)	129 ± 118	262 ± 207	273 ± 258	< 0.05
Z-score		–0.68 ± 1.50	0.32 ± 1.73	0.36 ± 1.50	< 0.05

* Significant difference in *P* values among the three genotypes.
i-PTH, intact parathyroid hormone; NS, not significant; VDR, vitamin D receptor.

Table 5. Relationship between bone turnover markers and *Taq* I genotypes of VDR

		Genotype			*P**
		TT	*Tt*	*tt*	
Number of subjects		169 (80.9%)	35 (16.7%)	5 (2.4%)	
Age	(year)	57.7 ± 13.6	54.3 ± 17.0	57.4 ± 19.8	NS
Ca	(mg/dL)	7.9 ± 2.2	7.3 ± 2.4	6.7 ± 2.5	NS
P	(mg/dL)	5.8 ± 1.6	5.5 ± 1.9	5.5 ± 1.8	NS
Alkaline phosphatase	(IU/L)	185 ± 309	144 ± 103	201 ± 87	NS
1,25 $(OH)_2D_3$	(pg/mL)	7.5 ± 4.5	11.0 ± 8.6	6.2 ± 0.6	NS
Osteocalcin	(mg/dL)	77.1 ± 66.0	58.3 ± 37.1	55.4 ± 26.9	NS
i-PTH	(pg/mL)	273 ± 98	182 ± 172	129 ± 123	< 0.05
Z-score		0.45 ± 1.58	–0.56 ± 1.39	–1.55 ± 1.58	< 0.01

* Significant difference in *P* values among the three genotypes.
i-PTH, intact parathyroid hormone; NS, not significant; VDR, vitamin D receptor.

0.43 ± 1.58 for *BB*, *Bb* and *bb*, respectively. Regarding *Apa*I-RFLP, allele and genotype frequencies were 11.0%, 37.8% and 51.2% for *AA*, *Aa* and *aa*, respectively (Table 4). Again, the frequencies differed from those reported in the European population, but less significantly than in cases of *Bsm*I-RFLP. Allele frequencies for *Apa*I-RFLP did not significantly differ among

Table 6. Relationship between bone turnover markers and *Fok*I-genotypes of VDR

		Genotype			*P**
		FF	*Ff*	*ff*	
Number of subjects		82 (39.2%)	93 (44.5%)	34 (16.3%)	
Age	(year)	58.3 ± 14.8	56.4 ± 14.5	56.5 ± 13.2	NS
Ca	(mg/dL)	8.4 ± 2.1	7.4 ± 2.3	7.4 ± 2.2	NS
P	(mg/dL)	5.6 ± 1.6	5.8 ± 1.6	5.7 ± 1.7	NS
Alkaline phosphatase	(IU/L)	179 ± 257	197 ± 344	131 ± 84	NS
1,25 $(OH)_2D_3$	(pg/mL)	9.1 ± 6.4	6.7 ± 3.0	8.4 ± 6.7	NS
Osteocalcin	(mg/dL)	80.8 ± 52.3	75.7 ±74.3	49.2 ± 34.5	< 0.05
i-PTH	(pg/mL)	278 ± 236	260 ± 214	172 ± 112	NS
Z-score		0.40 ± 1.60	0.29 ± 1.66	–0.37 ± 1.42	< 0.05

* Significant difference in *P* values among the three genotypes.
i-PTH, intact parathyroid hormone; NS, not significant; VDR, vitamin D receptor.

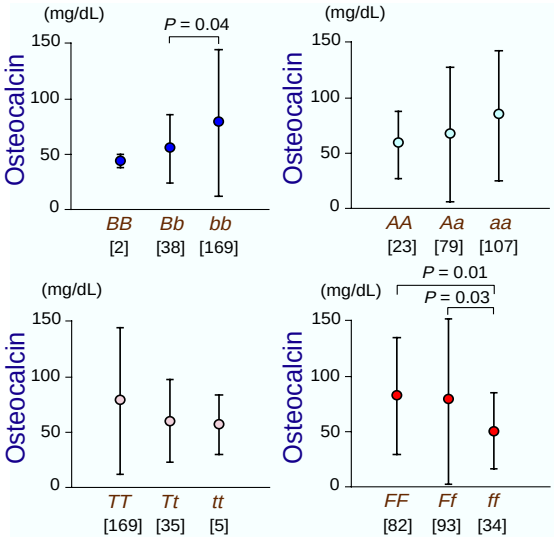

Fig. 3. Serum osteocalcin levels compared among polymorphic vitamin D receptor (VDR) genotypes. Values shown are mean ± SE. [ ], number of subjects.

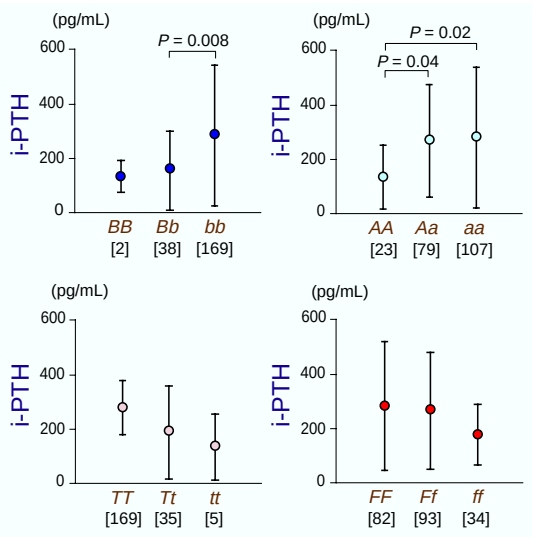

Fig. 4. Serum intact-parathyroid hormone (i-PTH) levels compared among polymorphic vitamin D receptor (VDR) genotypes. Values shown are mean ± SE. [ ], number of subjects.

patients of different ages, or among those showing different serum values for Ca, P, alkaline phosphatase, $1,25(OH)_2D_3$ or osteocalcin. However, values for i-PTH and Z-scores representing BMD were significantly higher in individuals possessing the *a* haplotype.

Allele and genotype frequencies for *Taq*I-RFLP were 80.9%, 16.7% and 2.4% for *TT*, *Tt* and *tt*, respectively (Table 5), indicating that genotype frequency for *tt* was extremely low. Z-scores for BMD significantly varied among patients having different genotypes; that is, the highest score was obtained for *TT*, followed by those for *Tt* and *tt* in that order.

Allele and genotype frequencies for the *Fok*I-RFLP were 39.2%, 44.5% and 16.3% for *FF*, *Ff* and *ff*, respectively (Table 6). Values for osteocalcin and Z-scores significantly varied among patients having different genotypes; i.e., the highest value was obtained for *FF*, followed by those for *Ff* and *ff* in that order.

It is noted that allele and genotype frequencies for RFLPs obtained with chronic renal failure in the present study did not differ from those reported for healthy subjects.

Distribution of serum osteocalcin levels were presented with respect to genotyping for *Bsm*I, *Apa*I, *Taq*I and *Fok*I RFLPs (Fig. 3). Distribution of serum i-PTH levels (Fig. 4) and Z-scores (Fig. 5) were similarly presented. Low levels of osteocalcin and i-PTH were notably correlated with *B*, *A*, *t* and *f* haplotypes,

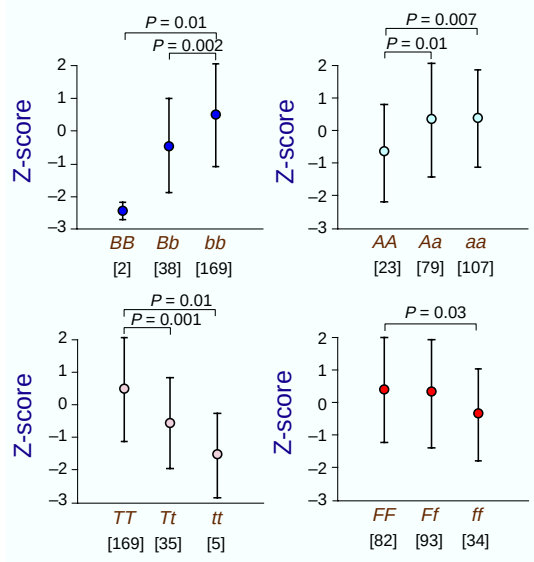

Fig. 5. Z-scores compared among polymorphic vitamin D receptor (VDR) genotypes. Values shown are mean ± SE. [ ], number of subjects.

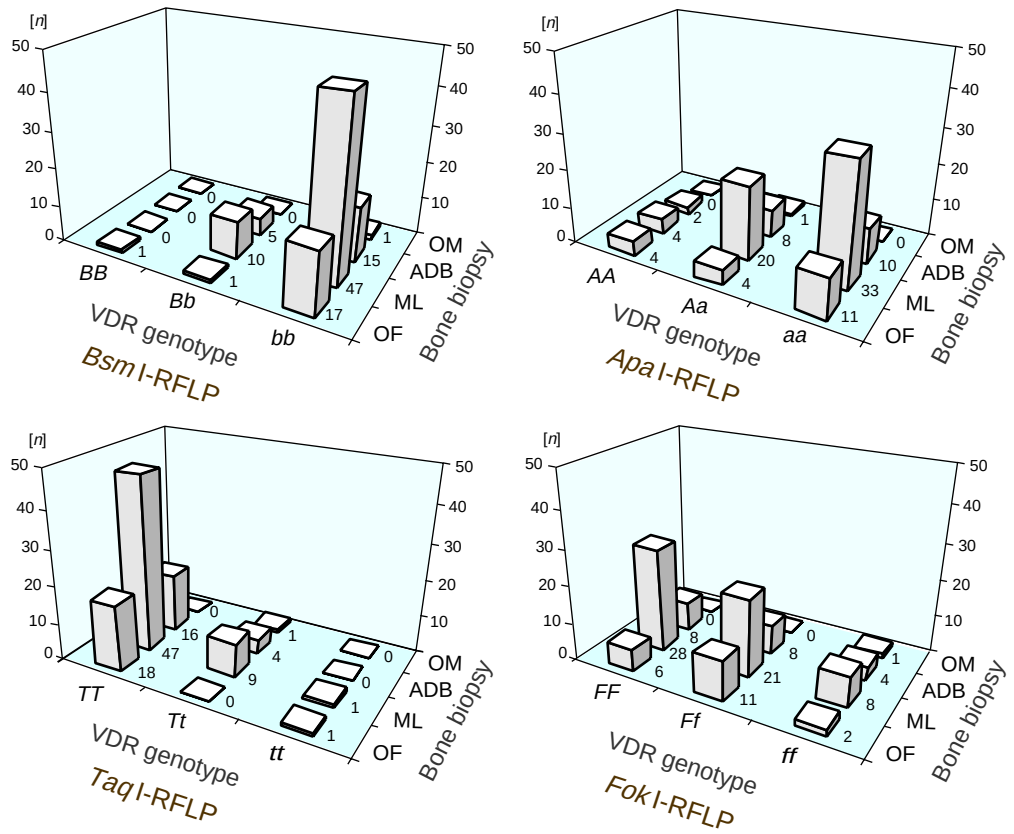

Fig. 6. Histologic comparison of bone biopsy in terms of polymorphic vitamin D receptor (VDR) genotypes. ADB, adynamic bone disease; ML, mild lesion; OF, osteitis fibrosa; OM, osteomalacia; RFLP, restriction fragment length polymorphism.

suggesting that VDR polymorphisms are correlated with BMD via bone turnover markers.

Histopathological diagnosis of bone biopsy specimens revealed osteitis fibrosa, mild lesion, adynamic bone disease and osteomalacia in 19, 57, 20 and 1 cases, respectively. Distribution of the incidences of these bone diseases among RFLP genotypes did not differ by the restriction endonuclease used: in incidence, mild lesion was the highest, osteitis fibrosa and adynamic bone disease were almost equal, and osteomalacia was the lowest in all studies. For example, in *Bsm*I-RFLP, 17 osteitides fibrosa, 47 mild lesions, 15 adynamic bone diseases and 1 osteomalacia were found among patients having genotype *bb*, and 1 osteitis fibrosa, 10 mild lesions and 5 adynamic bone diseases in those having genotype *Bb*. These constant distribution patterns of bone diseases among RFLPs generated by different restriction endonucleases were consistently demonstrated by the contingency table analysis (Fig. 6).

With respect to a possible correlation between serum levels of bone turnover markers and histologic types of bone diseases, both osteocalcin and i-PTH levels were the highest in osteitis fibrosa, followed by mild lesion, and the values were lowest in adynamic bone disease. There was a positive correlation between osteocalcin and i-PTH, indicating that these two markers were both useful in predicting renal osteodystrophy (Fig. 7).

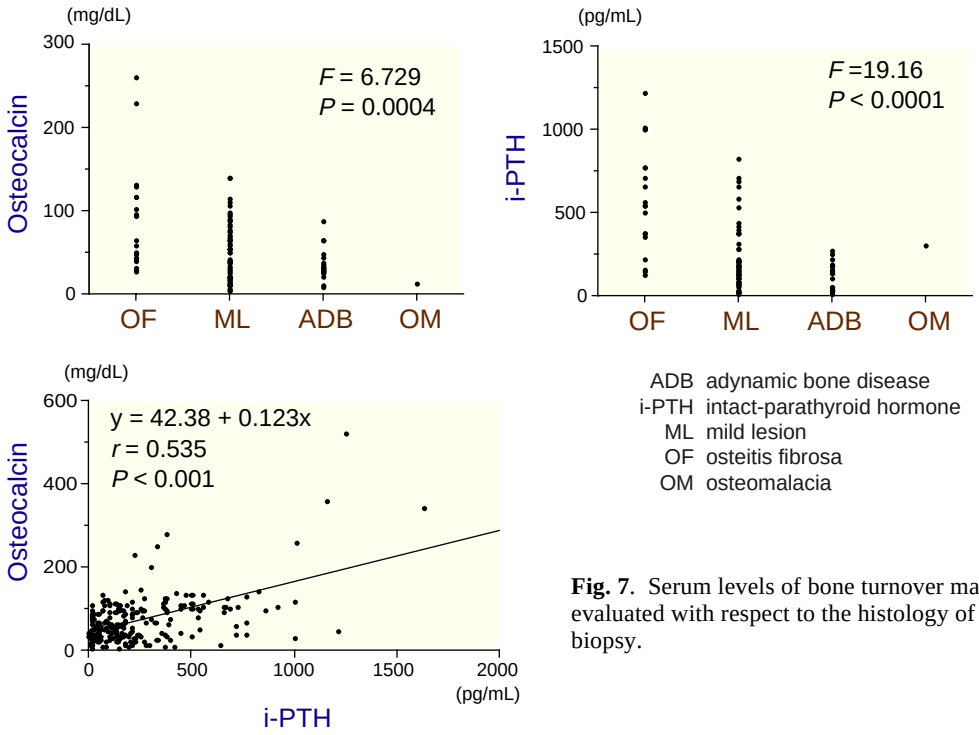

Fig. 7. Serum levels of bone turnover markers evaluated with respect to the histology of bone biopsy.

Discussion

In the present study, a significant correlation was demonstrated between patients with chronic renal failure having the RFLP haplotypes *B*, *A*, *t* and *f* and patients showing lower Z-scores for BMD. This fact suggests that particular RFLP haplotypes can be used as a risk factor predictive of osteoporosis. Indeed, RFLP haplotypes *B*, *A*, *t* and *f* were associated with low levels of serum osteocalcin and i-PTH; polymorphic VDR genes could determine dynamic features of bone remodeling via actions of these bone turnover markers.

Carling and colleagues (1997) assessed extracellular Ca^{2+}-mediated suppression of i-PTH secretion in 62 patients with primary hyperparathyroidism due to parathyroid adenoma using dispersed adenoma cells in vitro, and showed that Ca^{2+}-mediated PTH inhibition produced a higher median effective dose and reduced suppression in adenoma cells from patients who were homozygous for *b*, *a* and *T* alleles. These findings were consistent with the positive correlation observed between particular VDR polymorphic alleles and serum i-PTH in patients with chronic renal failure in the present study. There is a negative vitamin D responsive element (nVDRE) on the PTH gene to which $1,25(OH)_2D_3$ acts negatively by binding to the nVDRE (Demay et al., 1992). Taken together, it seems that VDR polymorphisms affect nVDRE-mediated regulation of the PTH gene expression.

Vitamin D and PTH primarily act on osteoblasts by binding to respective cellular receptors. Subsequently, the activated osteoblasts stimulate bone resorption through the monocyte-macrophage colony-stimulating factor and the osteoclast differentiation factor. At the same time, however, vitamin D stimulates normal calcification of bone matrix including type I collagen, alkaline phosphatase and osteocalcin produced by osteoblasts. PTH also enhances calcification by stimulating the release of insulin-like growth factor (IGF)-I and -II from osteoblast cells, and stimulates the accumula-

tion of IGF-I and transforming growth factor-β in the bone. Thus, PTH seems to be one of the potent regulators of dynamic bone remodeling (Christakos et al., 1989; Linkhart et al., 1989), and VDR polymorphism may be associated with the mechanism of bone remodeling.

Since a reduction in BMD is due to a failure in normal bone remodeling, a VDR polymorphism could be associated with BMD at the level of the bone remodeling process.

There remains the important question of how *Bsm*I- and *Apa*I-RFLPs, due to a nucleotide change in introns, and *Taq*I-RFLP, due to a synonymous codon change (ATC/ATT) in exon 9, lead to a functional change in VDR proteins. Although an answer to the question of the involvement of changes in 3'UTR, poly(*A*) tail, transcription efficiency and messenger RNA stability has been proposed (Gross et al., 1998), there has been no conclusive evidence which can explain this problem.

Regarding *Fok*I-RFLP, the nucleotide change in the VDR gene was reported to alter not only the translation initiation site but also transcription efficiency as demonstrated by luciferase assay, and change the translation efficiency determined in vitro (Arai et al., 1997). These changes should all affect BMD.

As Sainz and coworkers (1997) clearly demonstrated, VDR gene polymorphisms and bone density were significantly correlated with each other in 100 prepubertal American girls of Mexican descent in California. The success of their findings may be due to the recruitment of females at puberty when bone density reaches the maximum; during the puberty, genetic factors may most predominantly affect bone density control, while environmental factors such as nutrition and exercise may play more influential roles at later ages (Cooper et al., 1996).

Histopathological diagnosis of bone biopsy revealed osteitis fibrosa, mild lesion, adynamic bone disease and osteomalacia in 19, 57, 20 and 1 cases, respectively. The incidence distribution of these bone diseases did not vary when different restriction endonucleases were used for VDR genotyping. A cyclical process, called remodeling, maintains a dynamic steady state of bone structure density without changing the size and shape, through sequential resorption and formation of a small amount of bone at the same site. Renal osteodystrophy can be defined as a failure of normal bone remodeling (Hruska, 1997). Upon the introduction of hemodialysis, patients with end stage renal failure tend to have complications with hyperphosphatemia, hypocalcemia, acidosis and a moderate increase in i-PTH due to the retarded elimination of serum phosphates and impaired activation of vitamin D. These signs are mainly associated with mild lesion. Complication with severe secondary hyperthyroidism is associated with osteitis fibrosa. When patients are medicated with calcium carbonate as a precipitant for aluminum accumulation or for hypocalcemia, or have complications with diabetes mellitus, adynamic bone disease develops. In the case of $1,25(OH)_2D_3$ deficiency and hypocalcemia, osteomalacia develops due to insufficient calcification. A spectrum of bone diseases developing into renal osteodystrophy may be partly influenced by various factors including duration of chronic renal failure, primary disease, age, complications, nutrition, extent of exercise, aluminum accumulation and effectiveness of medication. In the present study, although the pathogenesis of renal osteodystrophy may well be affected by VDR polymorphism at the stage of i-PTH secretion and activation of osteoblasts associated with remodeling, the incidence distribution of bone diseases did not vary depending on different restriction endonucleases used for VDR genotyping. This discrepancy may be explained by assuming that the haplotype-dependent genetic consequences may have been obscured by the prominent improvement of bone disease induced by medication with active vitamin D and calcium pharmaceuticals.

Bone turnover markers such as i-PTH and osteocalcin have proven useful in predicting renal osteodystrophy. Malluche and others (1984) first claimed osteocalcin as a useful marker reflecting the state of bone formation by demonstrating that there was a significant correlation between osteocalcin and cellular and noncellular parameters of bone formation including bone turnover. In general, i-PTH values below 60–65 pg/mL reflect adynamic bone disease,

while those above 450–460 pg/mL reflect osteitis fibrosa. Clearly, bone turnover markers are thus useful as noninvasive diagnostic markers of renal osteodystrophy. However, bone biopsy is necessary when i-PTH values between 65 and 460 pg/mL reflect various histologic features of renal osteodystrophy as reported by Faugere and others (1994).

Conclusion

Possible correlations between VDR polymorphic genotypes and factors involving BMD were evaluated in 209 patients with chronic renal failure by assessing allele and genotype frequencies of VDR polymorphisms, bone turnover markers and BMD. Individuals having *B*, *A*, *t* and *f* haplotypes showed low levels of serum osteocalcin and i-PTH and low BMD. The histologic spectrum of bone diseases as assessed by biopsy did not significantly vary depending on different restriction endonucleases used for VDR genotyping. VDR polymorphism was shown to be one of the factors determining BMD. Histologic features of bone in renal osteodystrophpy failed to correlate with VDR polymorphisms, probably due to bias generated by clinical improvement due to medication. Further studies that exclude the distorting effects of active vitamin D and calcium supplementations are warranted to clarify this matter.

Acknowledgments: We are grateful to Prof. Keisuke Satoh, Dept. of Pharmacology and to Prof. Ikuo Miyagawa, Dept. of Urology, Faculty of Medicine, Tottori University, for their useful comments on the manuscript.

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(Received December 20, Accepted December 27, 1999)