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Persistent secondary hyperparathyroidism after renal transplantation

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Background. The persistence of secondary hyperparathyroidism after renal transplantation is frequent and often complicated by overt hypercalcemia. Recent investigations have shown an effect of the different vitamin D receptor (VDR) genotypes on parathyroid hormone (PTH) secretion in both primary and secondary hyperparathyroidism. The aims of this study were (*i*) to assess whether persistent secondary hyperparathyroidism after renal transplantation is characterized by any change in calcium-controlled PTH secretion, and (*ii*) whether different VDR allelic distributions might play any role on this setting.

Methods. Eighty-one cadaveric renal transplantation recipients, followed-up for at least 12 months, were checked for PTH, other primary metabolic and clinical variables, and VDR B/b alleles (BsmI). In 22 of these the following parameters were evaluated: (*a*) kinetics parameters of the Ca-PTH relation curve; (*b*) vertebral mineral density; (*c*) calcitriol serum levels; (*d*) PTH-related peptide serum levels; and (*e*) urinary hydroxyproline.

Results. According to the stabilised PTH levels (reached by the third month), the patients were divided in two groups: group A ($N = 40$, PTH < 80 pg/ml) and group B ($N = 41$, PTH > 80 pg/ml). Group B differed from group A in that patients had higher PTH levels at the time of transplantation, were older in age, and spent more time on dialysis. Group B had increased maximal and minimal PTH levels, and higher set-point levels than Group A. The patients with the *BB* pattern of VDR genotype were characterized by the lowest PTH levels both at time of transplantation and after stabilization, and lower set point values than patients with *Bb* and *bb* patterns.

Conclusions. Our study suggests that (*i*) the severity of preexisting secondary hyperparathyroidism is the main factor determining its persistence after renal transplantation, (*ii*) persistent secondary hyperparathyroidism is characterized by an autonomous pattern of PTH secretion, (*iii*) the VDR *BB* genotype seems to be related to lower PTH levels.

Key words: VDR genotype, parathyroid hormone kinetics, calcium, bone mineral density, hypercalcemia.

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Persistent secondary hyperparathyroidism (SHP) is quite common even after successful renal transplantation (RTx) [1–3]. A retained control of parathyroid hormone (PTH) secretion was first found in transplanted patients by McCarron and co-workers [4], and an autonomous pattern of secretion, accompanied by overt hypercalcemia and often requiring parathyroidectomy (PTX), has been subsequently reported by many authors [3, 5, 6]. The first aim of the present investigation was to assess whether the persistence of SHP after RTx is characterized by any change in PTH calcium-controlled secretion.

Following the report by Morrison and co-workers of an association between vitamin D receptor (VDR) gene polymorphism in intron 8 (*Bb* allele) and mineral bone density [7], a large number of studies have addressed the issue of the potential role, if any, of VDR allele polymorphism on other targets of vitamin D activity. In particular, as in Morrison's study [7], it was suggested that the *b* allele was linked with reduced VDR gene transcription and/or mRNA stability, and the possibility that a consequent different regulatory action of vitamin D might play a role on parathyroid cell proliferation has recently stimulated research on this issue. In fact, the VDR *bb* genotype was found to be associated with sporadic primary hyperparathyroidism by Carling and co-workers [8, 9]. In addition, the same authors were able to demonstrate a relationship between the *bb* genotype and half-maximal inhibition of Ca-controlled PTH secretion *in vitro* [10].

On the other hand, conflicting results on the possible association between VDR genotype and SHP of uremia have emerged. In fact, some studies demonstrated no relationship between the VDR *B/b* allele polymorphism and the level of SHP in uremic patients [9, 11]. At variance with these results, in a recent investigation performed on a group of hemodialyzed patients, Fernandez and co-workers found that patients with VDR *BB* genotype have consistently lower PTH levels [12]. In the only published paper (to our knowledge) addressing the issue of the possible link between VDR genotype and SHP after RTx, Torres and

co-workers, studying 34 RTx patients, found lower values of PTH in the VDR *bb* genotype group [13].

The second aim of our study was to investigate the possible role of VDR *B/b* gene polymorphism on the evolution of SHP after RTX in a larger group of patients, and to address the issue of a link, if any, with Ca-controlled PTH secretion *in vivo*.

METHODS

Patients

Among the patients transplanted in our center from July 1993 to July 1996, 81 consecutive recipients (59 males, age 18 to 63 years) of a kidney transplant from cadaveric donors were selected by the following criteria: (*1*) before RTx none had been parathyroidectomized or had a history of diabetes mellitus, (*2*) after RTx the patients had a stable renal function (plasma creatinine < 2.0 mg/dl) for at least 12 months, and no significant liver involvement.

The causes of their chronic renal failure were: chronic glomerulonephritis in 35, interstitial nephritis in 9, polycystic kidney disease in 7, Alport's syndrome in 4, hypertensive nephropathy in 4, reflux nephropathy in 3, familiar form of nephritis different from Alport's syndrome in 3, lupus erythematosus syndrome, vasculitis, and post-traumatic renal insufficiency each in 1, and unknown cause in 13.

At the time of RTx, 65 of the patients were on extracorporeal dialysis treatment and the other 16 were on CAPD.

Immunosuppressive therapy consisted of cyclosporine and prednisone in 70 and cyclosporine, prednisone and azathioprine or mycophenolate in 11 patients. The mean prednisone dose in the first six months was 0.4 mg/kg body wt/day and thereafter the dose was tapered to 0.12 mg/kg of body wt/day. Cyclosporine was administered in two doses in order to maintain a trough blood level of about 250 ng/ml in the first three months, and thereafter about 150 ng/ml (monoclonal assay). Acute rejection was treated with three boluses each of 3 mg/kg of i.v. methylprednisolone. Steroid resistant episodes were treated with OKT3 (5 mg/day over 10 days).

Most of these patients had been treated with vitamin D derivatives at some time before transplantation; however, in the three months preceding RTx, 23 patients were on some vitamin D therapy. All these patients used calcium salts (carbonate and/or acetate) as phosphate binders. At the time of RTx, it was possible to assess the serum aluminum concentration in only 34 of the patients and in them it was less than 50 μg/liter.

Sixty-eight of these patients were hypertensive after RTx and their treatment consisted of diltiazem plus clonidine and/or ramipril. None of them received calcium supplementation, vitamin D derivatives, or other drugs potentially interfering with calcium metabolism and calciotropic hormones after RTx.

The patients gave their informed consent to the study, which was approved by the local Ethics Committee.

Study design

In all these patients intact PTH, serum Ca, phosphate (Pi), total alkaline phosphatase, and body mass index were checked at time of RTx and after 3, 6 and 12 months. According to their stabilized PTH serum levels reached after the third month, the patients were divided into two groups. Group A had stabilized PTH levels < 80 pg/ml, and group B had PTH levels > 80 pg/ml (the value of 80 pg/ml represents the mean $\pm$ 2 SD of a population of renal non-transplanted patients with a comparable degree of renal function). The two groups were then compared with regards to the main clinical and metabolic parameters.

All the patients and 80 normal Caucasian subjects, matched for gender and living in the northeast regions of Italy, were genotyped for VDR alleles by the BsmI restriction enzyme.

In 22 of the RTx patients (11 in group A and 11 in group B), after one year of follow-up the following parameters were evaluated: (*a*) kinetic parameters of the Ca-PTH relation curve, using hypocalcemic and hypercalcemic infusional tests; (*b*) vertebral mineral density; (*c*) 1,25-dihydroxy-vitamin D serum levels; (*d*) PTH-related peptide serum levels; and (*e*) hydroxyproline, measured in a urine sample when patients were in a fasted state. These patients were chosen for their acceptance to be submitted to the extended protocol. However, great attention was paid to their metabolic and clinical variables being very representative of both the larger Groups A and B (**Results**).

Kinetic parameters of RTx patients were compared with those studied in the 11 control subjects (C). In addition, two other control groups were considered: the first group, composed of 16 renal patients (RP1) who were matched with RTx patients with regards to renal function, was considered as a proper control group, owing to the even slightly reduced glomerular filtration rate (GFR) of RTx patients; the second group, comprised of 16 renal patients on conservative treatment (RP2) who were matched with RTx patients with regards to basal PTH levels, was considered for a better comparison of the possible changes in PTH secretory parameters in the two groups with a comparable degree of SHP.

Parathyroid hormone kinetic parameters

Parathyroid hormone secretion parameters were evaluated with a previously described infusion method [14]. Briefly, the PTH stimulation test was performed by an i.v. infusion of Na_2-EDTA added to 5% dextrose solution plus 20 ml of 1% lidocaine, in an amount of about 37 mg/kg of body wt, at a constant rate of 4.2 ml/min of solution for 120 minutes. Blood samples were taken from the controlateral

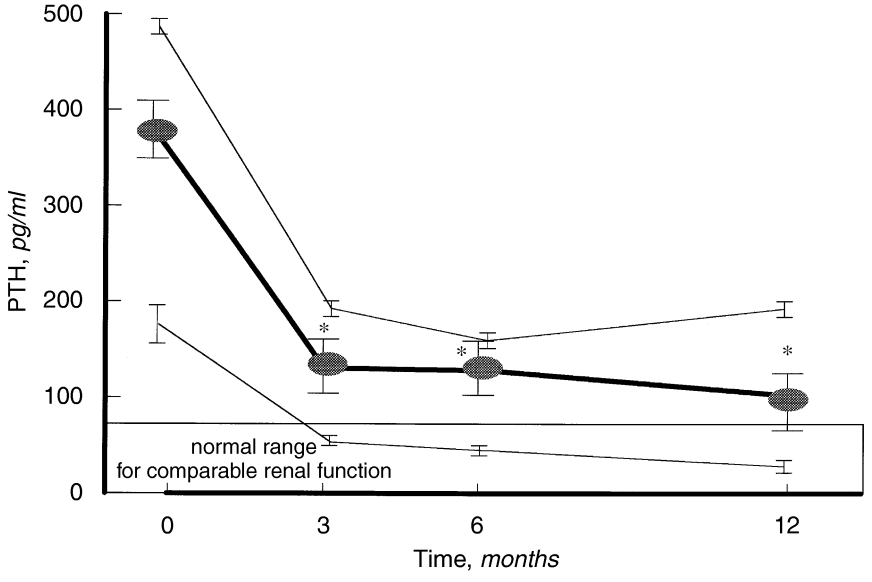

Fig. 1. Behavior of parathyroid hormone (PTH) after renal transplantation (RTx). The thicker line represents the behavior in the total number of patients; the two thinner lines represent respectively group A (lower line) and group B (upper line). PTH levels fell rapidly during the first three months and thereafter remained substantially stable (mean ± SE; $^{**}P < 0.01$ vs. basal values; the differences between the two groups were all statistically significant by definition).

arm at times 0, 5, 10, 15, 30, 45, 75, 105, 120 minutes to evaluate ionized calcium and intact PTH. The PTH suppression test was performed between two or five days from the stimulation test, by infusing i.v. calcium gluconate in an amount of 8 mg/kg of body wt of calcium element during 120 minutes, for the evaluation of ionized calcium and intact PTH. The calculation of PTH secretory parameters was performed according to the four-parameter model described by Brown [15], as previously described [16].

Vitamin D receptor genotype assessment

Genomic DNA was extracted from blood using a fast protocol by Talent (Micromix-200). Genomic DNA is amplified with TaqPolymerase (GIBCO) and gene specific primers of 30 and 28 bp during cycles. Each cycle consisted of denaturation at 95°C for one minute and an extension at 72°C for two minutes. The reaction mixture contained 1 × PCR buffer [67 mM Tris-HCl, pH 8.8, at 25°C, 16 mM $(NH_4)_2SO_4$, 0.01% Tween-20], $MgCl_2$ (2 mM). DNTP (200 μM of each), primers (0.5 μM of each) and 2.0 units of TaqDNA-Polymerase. Amplified DNA was analyzed on a 2% agarose gel, and a single 870 bp was obtained. Then 10 μl of the amplified DNA were restricted with BsmI (3 U) in the appropriate reaction buffer 1× provided by Boehringer Mannheim (Mannheim, Germany) according to the supplier's specification. Restricted DNA was still analyzed on 2% agarose gel. Three different patterns of bands were obtained and genotype polymorphism was defined as: *BB* (absence of restriction site on both alleles), *bb* (presence of restriction site on both alleles), or *Bb* (heterozygous pattern).

Other measurements and statistical analysis

Measurement of bone mineral density was performed by dual energy X-ray absorptiometry (HOLOGIC 2000) of lumbar spine (L1-L4) and expressed as grams of hydroxyapatite divided by the surface of the projected area in cm^2 and as the number of SD from the mean of an age and sex-matched population (Z score).

PTH was measured by an intact-PTH immunoradiometric assay (IRAM; Nichols Institute Diagnostics, San Diego, CA, USA) giving a normal range of between 7 and 55 pg/ml, an intra-assay coefficient of variation of 2.4% and an inter-assay coefficient of 5.7%.

For 1,25-dihydroxyvitamin D determination a radioreceptorial method (RRA; Nichols) was utilized, which gives an intra-assay coefficient of variation of 10.0% and an inter-assay coefficient of 14.0%, with a range in our normal population of 18 to 54 pg/ml.

PTH-related peptide was measured in plasma, treated with a protease inhibitor cocktail, by a two-site immunoradiometric assay (n.v. <1.3 pmol/liter; Nichols). Hydroxyproline in urine was determined by liquid chromatography (Beckman System Gold) after acid hydrolysis. Serum ionized calcium was evaluated using an ICA 1 ionized calcium analyzer (n.v. 1.22 to 1.36 mmol/liter; Radiometer, Copenhagen, Denmark). Electrolytes and creatinine in serum and urine were measured by standard methodology.

Statistics were calculated utilizing a *t*-test for paired data, ANOVA, regression analysis, parametric and non-parametric tests, and chi-square, with Yates correction, using a statistic package (BMDP) implemented on an IBM 150 MX computer.

RESULTS

Clinical characteristics of parathyroid hormone

The behavior of PTH serum levels after RTx in the patients studied is shown in Figure 1. PTH levels fell significantly during the first three months, and remained

Table 1. Main metabolic and clinical parameters at the time of renal transplantation (RTx) in patients with (group B) and without (group A) persistent secondary hyperparathyroidism (SHP)

	N	Gender M/F	Age years	Dialysis time months	S_{Ca} mg/dl	S_{Pi} mg/dl	PTH pg/ml	VitD t/nt	CAPD/HD
Group A	40	29/11	39.9 ± 11.5	23.8 ± 14.4	9.81 ± 1.04	5.1 ± 1.64	168.1 ± 141	9/31	10/30
Group B	41	30/11	48.7 ± 11.7	44.6 ± 37.2	9.96 ± 1.27	5.36 ± 1.73	538.3 ± 375.6	13/28	6/35
P		NS	< 0.01	< 0.01	NS	NS	< 0.01	NS	NS

Abbreviations are: VitD t/nt, ratio between patients treated and not treated with vitamin D derivatives in the 3 months preceding RTx; CAPD/HD, ratio between patients on CAPD and HD at the time of RTx (mean ± SD); S_{Ca}, serum calcium; S_{Pi}, serum phosphate; PTH, parathyroid hormone.

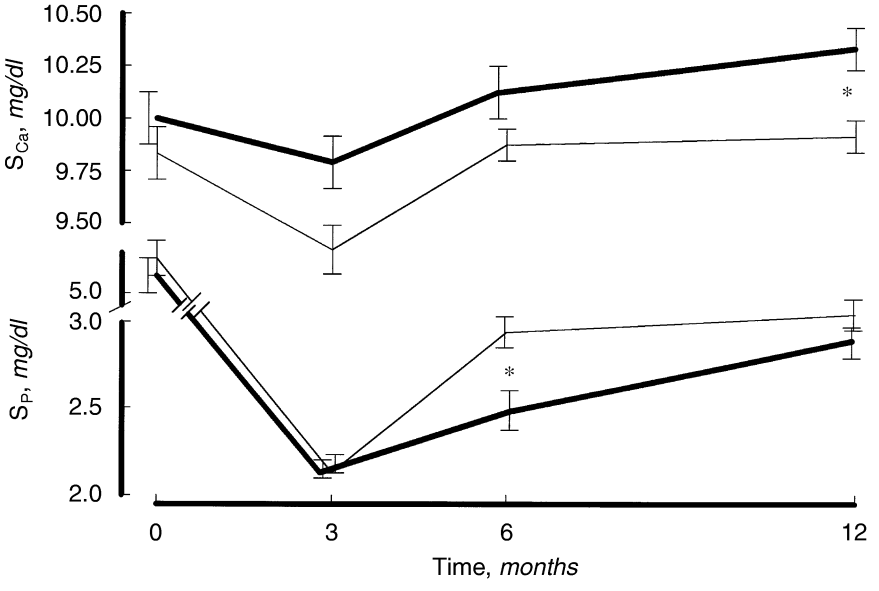

Fig. 2. Behavior of total serum calcium and phosphate in group A (thinner lines) and group B (thicker lines) in the follow-up period (mean ± SE; *$P < 0.05$ group A vs. group B).

substantially stable thereafter. However, great variability was observed among the patients, with 40 of them (group A) reaching stable PTH levels lower than 80 pg/ml (value representing the mean ± 2 SD of a population of renal patients with a comparable degree of renal function) and the other 41 (group B) still maintaining elevated serum levels. Table 1 shows the main metabolic and clinical parameters in the two groups of patients at the time of RTx. The patients with higher stabilized PTH levels were characterized by higher PTH levels at the time of RTx, greater time spent on dialysis and older age. No significant difference was present in the serum levels of both calcium and phosphate, in gender distribution, and the number of patients on CAPD treatment at the time of RTx between the two groups. The behavior of total serum calcium and phosphate during the follow-up period is shown in Figure 2. The values of calcium tended to be higher and phosphate lower in group B for the overall follow-up period. However, statistically significant differences were reached only at twelve and six months for calcium and phosphate respectively. During the follow-up period, 10 of the latter group gradually developed a high degree of hypercalcemia (total serum calcium higher than 11.5 mg/dl), requiring parathyroidectomy in eight of them, performed between 12 and 18 months.

Table 2 shows the main metabolic and clinical parameters in the two groups after 12 months of follow-up. By definition, the two groups showed different PTH levels. No other major difference was observed. In particular, no significant differences in serum calcium, phosphate, and creatinine levels, cumulative steroid dose, presence of hypertension and the number of episodes of rejection were evident.

Results of the extended protocol

Table 3 shows the main basal metabolic and clinical parameters of the 22 patients (11 for each group) submitted to the extended protocol. The data show that the two groups were well representative of the larger original groups A and B.

Table 4 shows the main parameters in these 22 patients at the time of the completion of the follow-up period. Group B patients had, by definition, higher PTH levels. In addition, group B had a higher hydroxyproline excretion in

Table 2. Main metabolic and clinical parameters after 12 months of follow-up, in the renal transplanted patients with (group B) and without (group A) persistent SHP

	N	PTH	S_{Ca}	S_{Pi}	Creatinine	Steroid	Rejection	Hypertension	VDR geneotype
		pg/ml	*mg/dl*			*g total*	*Yes/No*		BB/Bb/bb
Group A	40	55.9 ± 21.1	9.84 ± 0.54	3.06 ± 0.74	1.40 ± 0.32	3.10 ± 2.31	20/20	32/8	10/17/13
Group B	41	163.4 ± 93.9	10.1 ± 1.06	2.84 ± 0.73	1.68 ± 0.62	2.70 ± 1.72	22/18	36/5	3/23/15
P		dbd	NS	NS	NS	NS	NS	NS	0.06

Data are mean ± SD; dbd is different by definition. Abbreviations are in Table 1.

Table 3. Main metabolic and clinical parameters at the time of renal transplantation (RTx) in the 22 patients (11 of group A and 11 of group B) submitted to the extended protocol

	N	Gender *M/F*	Age *years*	Dialysis time *months*	Total S_{Ca}	S_{Pi}	PTH *pg/ml*
					mg/dL		
Group A	11	8/3	40.1 ± 11.1	23.6 ± 18.6	9.7 ± 0.50	5.0 ± 1.83	134 ± 140
Group B	11	9/2	50.2 ± 8.8	55.8 ± 32.3	10.0 ± 0.88	5.3 ± 2.01	445 ± 380
P		NS	< 0.05	< 0.05	NS	NS	< 0.05

Data are mean ± SD. Abbreviations are in Table 1.

Table 4. Main parameters of the extended protocol performed 12 months after renal transplantation (RTx), in 11 RTx patients of group A and 11 of group B

	N	PTH	CTR	Ionized Ca	OHP/Cr	PTHrP	BMD	Z score
		pg/ml		*mmol/liter*	*mg/mg*	*pmol/l*	*g/cm²*	
Group A	11	52.8 ± 13.5	35.3 ± 11.9	1.33 ± 0.07	12.2 ± 3.33	0.309 ± 0.081	0.924 ± 0.117	−1.05 ± 0.94
Group B	11	149.8 ± 63.5	33.9 ± 15.8	1.46 ± 0.15	29.6 ± 22.1	0.353 ± 0.077	1.011 ± 0.159	−0.56 ± 1.44
P		dbd	NS	< 0.05	< 0.05	NS	NS	NS

Data are mean ± SD. Abbreviations are: CTR, calcitriol; dbd, different by definition; Ca, calcium; OHP, urinary hydroxyproline; Cr, creatine; PTHrp, parathyroid hormone related protein; BMD, bone mineral density.

Table 5. Secretory parameters of PTH in the two groups of renal transplant (RTx) patients, in 16 renal patients matched for renal function (RP1), in 16 renal patients matched for basal PTH levels (RP2), and in 11 control subjects (C)

	N	Ionized Ca	Basal PTH	Set point	Sensitivity	PTH_{max}	PTH_{min}	PTH basal/max	PTH min/max
		mmol/liter	*pg/ml*	*mmol/liter*	*%/mmol/liter*	*pg/ml*		*%*	
Group A	11	1.33 ± 0.07	52.8[h] ± 13.5	1.25 ± 0.06	370.4[e] ± 138.1	239.7[d,f] ± 72.4	20.7 ± 9.9	23.6 ± 9.1	8.51 ± 3.45
Group B	11	1.46[b] ± 0.15	149.8[f] ± 63.5	1.39[b] ± 0.15	336.1[e] ± 128.3	527.6[d,f] ± 385.2	68.5[b] ± 54.5	38.6 ± 19.9	17.8[c] ± 14.5
RP1	16	1.27 ± 0.05	42.5 ± 22.1	1.24 ± 0.05	651.6 ± 349.6	138.0 ± 56.8	17.5 ± 12.3	30.4 ± 8.4	12.8 ± 7.5
RP2	16	1.25 ± 0.09	111.1 ± 85.6	1.23 ± 0.08	567.5 ± 338.2	224.3[c] ± 127.0	31.4[c] ± 41.0	45.5[c] ± 12.9	12.4 ± 9.2
C	11	1.26 ± 0.04	32.82 ± 17.9	1.23 ± 0.04	453.6 ± 376.7	117.1 ± 54.8	8.9 ± 5.2	28.2 ± 10.3	8.04 ± 3.48

Data are: mean ± SD.
[a] $P < 0.05$ vs. all the other groups
[b] $P < 0.001$ vs. all the other groups
[c] $P < 0.05$ vs. C
[d] $P < 0.01$ vs. C
[e] $P < 0.05$ vs. RP1 + RP2
[f] $P < 0.01$ vs. C + RP1
[g] $P < 0.05$ A vs. B
[h] $P < 0.001$ A vs. B

fasting urine and ionized calcium serum levels than group A. Although total serum calcium had a tendency to be higher in group B, it was not significantly higher. No significant difference was found in serum creatinine, calcitriol and PTH-related peptide serum levels. The mean values of both bone mineral density and Z score were also not different between the two groups.

Table 5 shows the PTH secretory parameters in the two

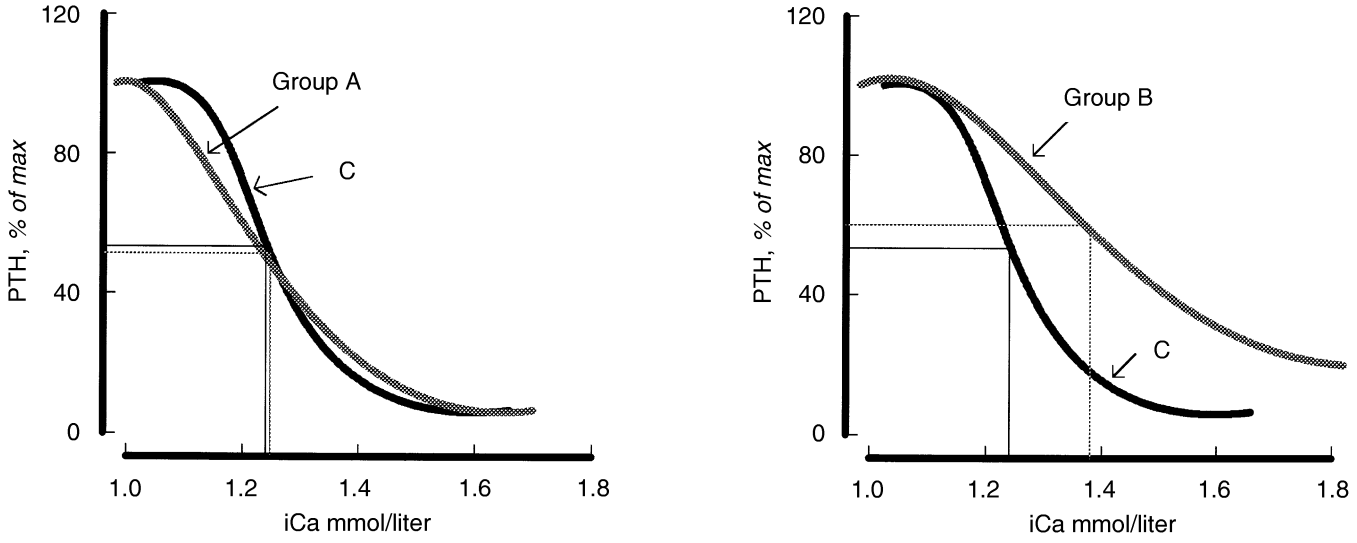

Fig. 3. Calcium-parathyroid hormone (PTH) sigmoidal relationship in RTx patients without (group A, left panel) and with persistent SHP (group B, right panel), compared with controls (C). In group B patients a shift of the curve to the right and upward, compared to the controls, is evident.

groups studied, compared with 16 renal patients with comparable renal function, 16 renal patients with comparable PTH basal levels, and 11 control subjects (C). Group B patients had increased maximal and minimal PTH levels, as compared to all the other groups. Furthermore, group B patients only had consistently higher set point levels.

Figure 3 depicts the Ca-PTH sigmoidal relation curve in the two groups of RTx patients, compared with controls. A shift to the right and upward in group B patients is evident.

In addition, the set points were well related to the basal PTH levels only in RTx (Fig. 4A), but such a relationship was not evident in the other renal patients and controls (Fig. 4B).

Vitamin D receptor genotype and PTH behavior

The VDR allele distribution in the 81 RTx patients was comparable with that found in the 80 control Caucasian subjects (Table 6).

When the distribution of the three different VDR genotypes in the two groups of RTx was considered, the patients with lower stabilized PTH levels tended to segregate BB genotype (Table 2), even if the difference of allele distribution between the two groups only approached the level of statistical significance ($P = 0.06$).

When the RTx patients were grouped according to their VDR genotype, patients with *BB* pattern were characterized by the lowest PTH levels both at the time of transplantation and after stabilization (Table 7). Of the 10 patients who subsequently developed severe hypercalcemia, eight of whom were then submitted to PTX, none had the *BB* genotype (6 had the *Bb* and 4 the *bb* patterns).

In addition, of the patients who were assessed, the set-point values for Ca-regulated PTH secretion were significantly lower in *BB* patients (Table 7).

DISCUSSION

Renal transplantation is effective in correcting the main causal factors of SHP of uremia, namely phosphate retention and calcitriol deficiency [17]. Nevertheless, the persistence of SHP after RTx has been reported to occur in a large proportion of patients [1–3]. In the present series the rate of occurrence of persistent SHP was about 50%, which is not different from that reported in other series [18, 19].

As previously reported in the literature [1, 18, 20], the RTx patients with persistent SHP were characterized by higher levels of PTH at the time of RTx and a greater time spent on dialysis. These results support the notion that the degree of SHP after RTx is largely dependent on the parathyroid glandular volume reached during the course of uremia [21], and that the involution of parathyroid gland hyperplasia, if this really occurs, takes a very long time [20].

Among the other factors potentially affecting the degree of SHP at the time of RTx, in our patients we can rule out a role for a different distribution of previous CAPD treatment and previous vitamin D therapy, since only a small number of patients in both groups were consuming vitamin D derivatives during the period of time immediately preceding RTx. With regards to serum aluminium levels, in most of our patients it was not possible to obtain its assessment before RTx. However, in those patients who could be checked, the aluminium levels were in the normal range, independent of the presence or otherwise of persistent SHP after RTx.

About two decades ago, McCarron and co-workers demonstrated, by infusional methodology, that post-transplantation hyperparathyroidism was characterized by a retained

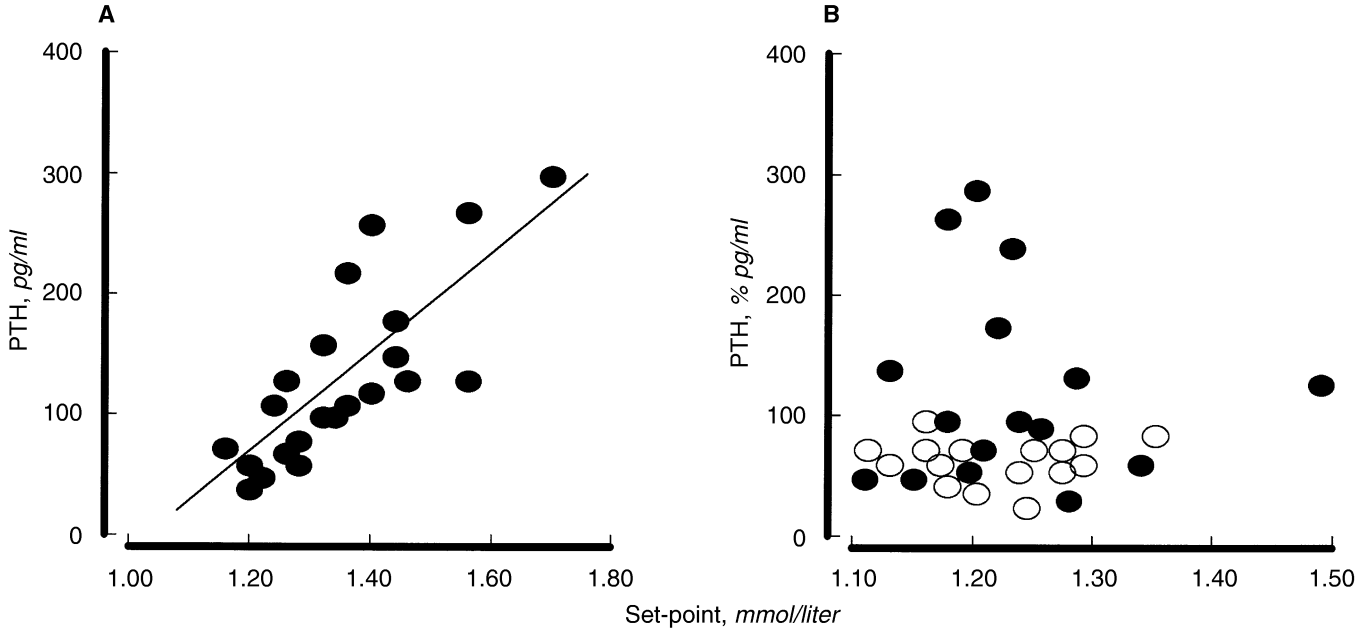

Fig. 4. Relationship between set point values and PTH basal levels in RTx patients (*A*) and the RP1 (○) and RP2 (●) (*B*). A direct linear relationship is evident in RTx patients (PTH = −406.5 + 384.8 × set point; $r = 0.69$, $P < 0.001$), but not in the other studied groups.

Table 6. Vitamin D receptor (VDR) allele distribution in 81 renal transplant (RTx) patients and 80 control subjects

	BB	*Bb*	*bb*
VDR genotype		*N (%)*	
RTx patients	13 (16.1)	40 (49.4)	28 (34.5)
Controls	17 (21.3)	36 (45.0)	27 (33.7)

Table 7. Vitamin D receptor (VDR) genotype pattern and parathyroid hormone (PTH) serum levels, both at baseline and after 12 months after renal transplantation (RTx), and set point (SP) values

	BB 13	*Bb* 40	*bb* 27
PTH at time of RTx *pg/ml*	70.7 ± 46.8[b]	412.0 ± 617.0	388.0 ± 107.7
PTH 1 year after RTx *pg/ml*	53.7 ± 20.3[a]	127.6 ± 93.7	114.1 ± 87.9
Set point *mmol/liter*	1.24 ± 0.07[a] [5]	1.36 ± 0.14 [9]	1.33 ± 0.14 [8]

The number of patients where SP values were checked are reported between brackets.
[a] $P < 0.05$ *BB* vs. *Bb* + *bb*
[b] $P < 0.01$ *BB* vs. *Bb* + *bb*

control of the parathyroid function [4]. However, subsequent studies pointed out that the SHP of RTx patients was characterized by higher calcium levels for any given basal PTH value, in both basal conditions [22] and during calcium infusion [23]. Furthermore, the occurrence of hypercalcemia after RTx, often requiring PTX, has been widely reported [2, 5, 6], suggesting an autonomous pattern of secretion.

In our patients the rate of occurrence of hypercalcemia after RTx was consistent, since 10 of the 81 patients studied reached a stable serum calcium level above 11.5 mg/dl during the follow-up period and 8 of them were submitted to PTX. In all eight subjects a nodular form of hyperplasia in all the removed glands was found.

When we assessed PTH secretory parameters in 22 of these patients (11 for each group), the persistence of SHP was accompanied by increased values of maximal and minimal PTH secretion. This was an expected finding, because these secretory parameters are considered to be an indirect index of the glandular mass [21, 24] and, as previously discussed, a greater degree of hyperplasia is considered the main relevant cause for the persistence of SHP after RTx. The lesser sensitivity found in group B might be interpreted as the expected consequence of the increased parathyroid cell number, as sensitivity is expressed as the fractional changes of PTH (that is, normalized for PTH_{max}), the higher is the absolute value of PTH_{max}, the lower the fractional change of PTH is expected to be for a given variation in ionized calcium. The real cause for the reduced sensitivity in group A, where PTH_{max} values were substantially the same as in the RP1 patients, seems less obvious. Merely speculative explanations for this finding could be that the reduced sensitivity might reflect a reduced rate of recruitment of nonsecreting parathyroid cells or, alternatively, that the sensitivity of the single cell (CaR affinity for Ca?) might be lower for some unknown reason(s).

The most relevant finding was that the calcium-controlled PTH secretion in the patients with persistent SHP was reset around higher calcium values, with consequently higher set point and relatively lower sensitivity values. This pattern of secretion is quite different from that commonly found in uremic patients, whose set point values are still in the normal range both before [14] and after [25] dialytic treatment is started.

On the other hand, increased set point values have been described in uremic patients with increased calcium levels due to two opposite conditions: severe hyperparathyroidism or relative hypoparathyroidism with low turnover bone disease [26–28]. At variance, RTx patients with increased set points all had increased PTH values. It could be that the increase in the set point is the result of the underexpression of the calcium sensing receptor, occurring in the hyperplastic parathyroid glands of severe SHP [29], since the calcium sensing receptor is thought to be the main factor determining the set point [30]. The occurrence of hypercalcemia only after RTX, even in the case of pre-existing altered calcium-controlled PTH secretion, could be explained by the enhanced sensitivity of peripheral target organs of PTH action (particularly the bone), due to the normalization of the *mielieu interior*. This interpretation is also in agreement with Parfitt, who more appropriately defined the secretory set-point as the target value for calcium homeostatic system [31].

The clinical implication for these findings is that more effort must be given to identify patients with severe SHP and altered calcium-controlled PTH secretion before RTx, because they are more prone to develop overt and often severe hypercalcemia following renal transplantation.

The second part of our study faced the problem of a possible role for the VDR genotype in the evolution of SHP after RTx.

Many recent studies have stressed the influence of VDR genotype on bone mass in nonrenal patients [7, 32, 33] as well as in kidney transplanted patients [13]. In addition to a possible link between VDR genotype and bone mineral density, further studies pointed out a relationship between the different VDR alleles and the occurrence of primary hyperparathyroidism [8, 9], with the *bb* genotype being considered a favorable factor for the occurrence of PTH hypersecretion. Moreover, the same genotype pattern was associated with increased set point values for calcium-controlled PTH secretion in dispersed cells of parathyroid gland adenoma [10]. Taking these data into consideration, a not yet defined allele-related effect on PTH secretion has been proposed, at least in this pathological condition.

On the other hand, to the best of our knowledge, only two studies dealt with the possible relationship between VDR genotype and SHP of uremia [11, 12]. The first of the two was not able to demonstrate any influence of different VDR allele distribution on the degree of SHP in a cohort of dialyzed patients [11]. However, due to the short form of presentation of this paper (letter), much of the information about other adjunctive factors potentially affecting the degree of SHP was not given. At variance with this study, Fernandez and co-workers did find an association between *BB* genotype and reduced degree of SHP or, as an alternative explanation, between the presence of the *b* allele and increased degree of SHP in a group of dialyzed patients, even after other potentially affecting factors were taken into consideration [12]. These results were considered in keeping with the notion that *b* allele is associated with lower transcriptional activity and/or mRNA stability, possibly affecting a different degree of VDR expression and modulation of the calcitriol effects.

Regarding the possible interaction between VDR genotype and SHP after RTx, the only study that indirectly provides data about this issue is, to our knowledge, that by Torres and co-workers [13]. In the above paper, which mainly deals with the potential interactions between VDR allele distribution and bone mass, PTH serum levels at baseline, and 3 and 12 months after RTx are reported, with a lower degree of SHP found in *bb* genotype, when compared with the *Bb* and *BB* genotypes pooled in one group.

Our study is at variance with Torres et al's data [13] and in agreement with the results of Fernandez et al [12] in that the *BB* genotype is associated with lower PTH levels both before and after RTx. There is no obvious explanation for the discrepancy between these data, but the number of patients studied by Torres et al is substantially lower than that of our and Fernandez et al's series. In addition, in the former study, patients with the *BB* genotype were grouped together with the *Bb* genotype due to a particularly low frequency, and this could have introduced a confounding factor. In fact, according to our data, no difference was evident between the *bb* and *Bb* genotypes with regards to PTH levels.

As an additional finding, patients with the *BB* genotype also had lower set point levels compared to the other two genotype patterns. These results suggest that the *BB* genotype or alternatively the absence of *b* allele might increase the sensitivity of the parathyroid gland to calcitriol activity, especially regarding its inhibitory effect on cellular growth and/or calcium sensitivity. In keeping with this view is the finding that none of the 10 patients who developed the most severe form of SHP after RTx had the *BB* genotype. In eight of these 10 patients in whom PTX was performed, a nodular form of glandular hyperplasia was found. It is also known that the nodular form of parathyroid gland hyperplasia is characterized by underexpression of the VDR [34]. Thus, it must be stressed that this condition probably plays an overwhelming role not only in the development of the severe SHP, but also in the lack of its regression after RTx.

On the other hand, it is worth stressing that, when dealing with the possible influence of the genotype pattern

on the phenotypic expression of an indirectly controlled character, a very large population needs to be explored in order to reach definitive conclusions. This is especially true when one faces a multifactorial event, as is the case with the development of the SHP of uremia.

It must also be kept in mind that the *B*/*b* polymorphism of VDR gene is referred to a non-coding region, which is linked to other polymorphisms in the 3′-untranslated region of VDR [7]. Furthermore, no data are available as yet to prove whether the VDR genotype directly or indirectly, by linkage with other undefined genes, might affect a different pattern of cellular secretion and/or growth. More information is needed to better define the link between gene polymorphism and the described metabolic effects. For all these reasons, the present results need to be handled with caution and await further corroboration.

In summary, our study suggests that: (*i*) the persistence of SHP after RTx is largely caused by a more severe pre-existing SHP; (*ii*) this persistent form of SHP is characterized by an autonomous pattern of PTH secretion, mainly represented by both higher serum calcium and set point values; and (*iii*) the VDR *BB* genotype seems to be related in some way to lower PTH levels both before and after RTx, suggesting partial protection against the development of the most severe form of SHP for the patients who carry this genotype pattern.

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