NEFROLOGÍA. Volumen 27. Número 6. 2007

ORIGINALS

Changes in bone mineral metabolism in patients with recurrent urolithiasis and vitamin D receptor gene polymorphisms. Preliminary results

M.ª J. Moyano*, M.ª J. Gómez de Tejada, R. García Lozano***, R. Moruno**, R. Ortega*, V. Martí*, R. Sánchez Palencia*, M. J. Miranda****, A. Palma* and R. Pérez Cano******

Departments of *Nephrology and ****Internal Medicine, Virgen Macarena Hospital of Seville. **Department of Medicine of the University of Seville. ***Department of Immunology of Virgen del Rocío Hospital of Seville.

SUMMARY

Bone health, within calcium kidney stone disease is a matter of controversy. On the other hand, some genetic studies have shown an association between some Vitamin D receptor polymorphisms and calcium kidney stone disease. ***Main objective:*** *To study the possible association between calcium kidney stone disease with bone metabolism and some Vitamin D receptor polymorphisms.* ***Patients and methods:*** *This is a case-control study, with seventy-two subjects of both genders divided into two groups: Group I: cases, composed by 51 patients suffering from calcium kidney stone disease. Twenty-four of them had no hypercalciuria, 16 had absortive hypercalciuria and 11 had renal hypercalciuria. Group II: controls, composed by 21 people, without either urolithiasis or hypercalciuria. We performed a complete study including biochemical markers of bone mineral remodelling, bone mineral density (BMD) was estimated both in the lumbar spine (L2-L4) and femoral neck, and also VDR polymorphism for the* loci b, a *and* t. ***Results:*** *Patients with urolithiasis had lower values of BMD both in the lumbar spine and femoral neck, compared to controls. Z-score were lower in the lumbar spine and femoral neck (p = 0.045 y 0.031, respectively). Those patients with absorptive hypercalciuria had higher BMD in the femoral neck than those with renal hypercalciuria and non-hypercalciuria. Because they had more weight and height all the statistical study was performed alter adjusting by these two variables and statistical significance was then only stated between patients with hypercalciuria and without it. Patients with urolithiasis had higher values of 1.25 $(OH)_2$ vitamin D (p = 0.002), and lower of PTH (p = 0.049), without any relationship to hypercalciuria and its subtypes. Seventy six percent of the patients had a daily calcium intake lower than 800 mg/day. The distribution of VDR alleles in patients with urolithiasis was similar to controls, although after grouping genotypes, a lower distribution of* BB *and* tt *polymorphisms were observed in patients suffering from urolithiasis.* ***Conclusions:*** *Calcium kidney stone disease by itself produces a decrease in BMD, more intense in femoral neck, independently the presence or absence of hypercalciuria. Patients suffering from urolitihiasis have higher values of 1.25 $(OH)_2$ vitamin D than non-hypercalciuric patients and*

Correspondence to: Dra. María Jesús Moyano Franco
C/ Mosquera de Figueroa, 18, escalera 1, 2º D
41010 Seville (Spain)
E-mail: medicimon@yahoo.es

lower values of PTH probably due to a low dietary calcium intake. In our population studied there is no relationship between VDR polymorphisms and the presence of calcium kidney stone disease. Because the reduced number of patients of our study, more studies are needed to obtain definitely conclusions.

Key words: ***Calcium kidney stone disease. Absortive hypercalciuria. Renal hypercalciuria. Bone mineral density. Calcium intake. Vitamin D polymorphism.***

ALTERACIONES EN EL METABOLISMO MINERAL ÓSEO EN PACIENTES CON UROLITIASIS DE REPETICIÓN Y POLIMORFISMOS DEL GEN DEL RECEPTOR DE LA VITAMINA D. RESULTADOS PRELIMINARES

RESUMEN

Existe controversia sobre la afectación ósea en la litiasis renal cálcica. Por otro lado, algunos estudios genéticos han encontrado asociación entre los polimorfismos del receptor de la vitamina D (VDR) y la urolitiasis. ***Objetivo principal:*** *Relacionar la nefrolitiasis cálcica de repetición con el metabolismo óseo y los polimorfismos del gen del VDR.* ***Material y métodos:*** *Estudio de casos y controles, estando el grupo de casos formado por 51 pacientes con litiasis renal de repetición, que subdividimos en no hipercalciúricos (NHC, n = 27), hipercalciúricos absortivos (HCA, n = 10) e hipercalciúricos renales (HCR, n = 14); el grupo control, formado por 21 sujetos sin historia de litiasis renal ni hipercalciuria. Se les determinaron parámetros del metabolismo fosfocálcico, marcadores de remodelado óseo, densidad mineral ósea (DMO) en columna lumbar y en cuello femoral, y polimorfismos del gen del VDR para los* loci b, a *y* t. ***Resultados:*** *Los pacientes litiásicos presentaron frente a los controles una DMO inferior tanto en L2-L4 como en cuello femoral* (Z-score, *p = 0,045 y 0,031), niveles superiores de 1,25 $(OH)_2$ vitamina D (p = 0,002) e inferiores de PTH (p = 0,049), y una menor ingesta cálcica (p < 0,001). Los HCA mostraron una mayor DMO frente a los NHC (sólo significativo en cuello femoral). Los pacientes con LRC no mostraron diferencias en las frecuencias genotípicas estudiadas frente a los controles. Al reagrupar los alelos, sólo se apreció una menor frecuencia del genotipo BB respecto al* Bb-bb, *y del tt frente al* TT-Tt, *en los pacientes litiásicos (p = 0,098 y p = 0,051, respectivamente).* ***Conclusiones:*** *La litiasis renal cálcica parece influir en la DMO de cuello femoral. Los pacientes litiásicos mostraron niveles elevados de 1,25 $(OH)_2$ vitamina D, posiblemente relacionado con la baja dieta cálcica. Los genotipos homocigóticos* BB *y* tt *parecen ser menos frecuentes entre los pacientes con litiasis renal cálcica.*

Palabras clave: ***Litiasis renal cálcica. Hipercalciuria absortiva. Hipercalciuria renal. Densidad minera ósea. Polimorfismos del receptor de la vitamina D. Ingesta cálcica habitual.***

INTRODUCTION

The most frequent form of renal lithiasis (RL) is calcium lithiasis, predominantly that formed by calcium oxalate stones.[1] Several prospective studies have shown that calcium lithiasis is recurrent, the second episode of calculi occurring within 5-10 years from the first one.[2-4] In about 50% of the patients with calcium lithiasis hypercalciuria is present that is considered idiopathic since an underlying metabolic impairment is not found.[5] Although increased sodium and protein intake and reduced potassium increase calcium urinary excretion, these do not seem to primarily account for hypercalciuria.[6]

Some investigators have demonstrated that RL patients, particularly those presenting with hypercalciuria, may have reduced bone mass, frequently associated with an increase in bone remodeling.[7-11] However, the results are not definitively conclusive; on the other hand, there are contradictory opinions

on whether these findings may be related with all hypercalciuric patients[8, 10, 11] or only with those presenting renal hipercalciuria.[12]

Besides, several studies have concluded that the genotypes of the vitamin D receptor have an influence on intestinal absorption and/or renal calcium clearance, such as genotypes *b*, *a* and *t*.[13, 14] This could have an interest on calcium renal lithiasis (CRL), so that it could represent an important factor in its etiopathogenesis, and even be related with the type of hypercalciuria, both renal[15] and absorptive.[16] There is, however, no unanimous opinion on accepting this genetic influence since the results obtained are controversial.[17-19]

Therefore, the main objective of our study was to study the relationship between recurrent calcium oxalate renal lithiasis (CRL) with low bone mineral density (BMD) and polymorphisms of the *b, a*, and *t* loci of the VDR gene. Secondarily, we assessed whether the presence of hypercalciuria (HC) and its variants (absorptive or renal) could have an influence on these relationships. In this work we show the preliminary results, while we are increasing the number of cases and controls to achieve our goals.

PATIENTS AND METHODS

We have carried out a case-control study in which we selected 72 subjects in total, distributed as follows: 51 patients suffering from long-term recurrent CRL, objectively shown by biochemical analysis of the calculi, and that constituted the group of cases. A control group consisting of 21 randomly selected healthy volunteers, and comparable by gender and age to selected cases. None of these individuals have ever had previous nephrolithiasis episodes. Both patients and controls had normal renal function.

The following exclusion criteria were followed:

a) Those patients suffering from diseases having a likely influence on bone metabolism: endocrine diseases (hyper- or hypoparathyroidism, hyperthyroidism), AHT, renal failure, lung diseases, neurological disorders (motor deficit), inflammatory diseases of the locomotor apparatus, chronic liver disease, alcoholism, organ transplant, hypogonadism, and malabsorption.
b) Those individuals taking medication influencing bone metabolism: oral or inhaled corticosteroids for more than 3 months; anti-convulsants, anti-coagulants, GnRH analogues, anti-hypertensive drugs, thiazides, calcium, vitamin D, contraceptives, masculine sexual hormones, calcitonin, biphosphonates or isoflavones, and hormone replacement therapy in women.
c) Among the control group, subjects presenting baseline hypercalciuria or a history of renal Lithiasis were excluded.

Once the cases were selected, lithiasis patients were reassigned to the following subgroups: subjects without hypercalciuria (NHC) and those with hypercalciuria (HC), which were further classified as absorptive hypercalciuria (AHC) and renal hypercalciuria (RHC). Calciuria was measured in 24-h urine under baseline conditions. Hypercalciuria was considered when calcium output was higher than 250 mg/24 hours in women and 300 mg/24 h in men, or higher than 4 mg/Kg/day for both. Since most of lithiasic patients had low or null dairy products intake, hypercalciuria was also considered, in both genders, when the values were > 200 mg/24 h together with calcium/creatinine indexes after restriction > 0.11. To classify hipercalciuria, lithiasic patients were submitted to a low-calcium diet (approximately 400 mg of calcium/day) for one week; the diet consisted in withdrawal of milk and other diary products, with no protein restriction. At day 7, the 24-h diuresis (from 8 a.m. to 8 a.m. of the following day) was gathered again in order to determine calciuria after restriction, and the urine of the two following hours (8-10 a.m.) to establish the calcium/creatinine ratio. Then, oral calcium overload was carried out consisting in giving the patient 1 g of calcium and a diary product (147 mg of calcium in 230 mL); then, the urine of the following 4 hours was gathered (from 10 a.m. to 2 p.m.).

Absorptive hypercalciuria was considered when the urinary calcium/creatinine ratio (Ca/Cr) after a low-calcium diet was < 0.11.

Renal hypercalciuria was considered when the Ca/Cr ratio after dietary restriction was > 0.11, and after calcium overload the value was > 0.20 or doubled that without restriction.

Questionnaire

All studied subjects received a questionnaire on dietary habits regarding diary products intake, and based on that usual calcium intake was classified as: insufficient (< 800 mg de calcium per day) or sufficient (> 800 mg per day).

Laboratory determinations

Baseline 12-h fasting blood extractions were done in all subjects early in the morning, from which we determined:

Full blood count: by automated cell counter model STKS from «Coultier Científica»; general biochemical profile: by DAX-96 auto-analyzer, including serum levels of glucose, urea, uric acid, creatinine, albumin, calcium, phosphate, total alkaline phosphatase total, total bilirubin, aminotransferases, gamma-glutamil-transpeptidase, total cholesterol total, as well as urinary measurements of calcium, phosphorus and creatinine; serum levels of calcium-regulating hormones: PTH by electrochemoluminiscence, and 1-25$(OH)_2$ vitamin D by radioimmunoanalysis (RIA); serum levels of markers of bone remodeling, formation (osteocalcin — OC—) and reabsorption (β-crosslaps), both by electrochemoluminiscence.

Genetic study

Polymorphisms of the VDR gene alleles were determined from And extracted from peripheral blood leukocytes, by the polymerase chain reaction (PCR-RFLP), directed to show the presence or absence of restriction enzymes BsmI, ApaI and TaqI, the *b*, *a*, and *t* alleles being indicators of the presence of the *locus* for the corresponding enzymes, and *B*, *A*, and *T* those indicating their absence, respectively.

Bone Densitometry

BMD was measured by Dual Radiologic Absortiometry (DXA), by HOLOGIC® Densitometer, model QDR-1000, at two sites: the right proximal femur proximal and the lumbar spine (L2-L4). At the proximal femur, the area measured was the femoral neck. The variation coefficient of the device *in vivo* is 2.9% at the femoral neck, and 1.4% at the lumbar spine; *in vitro,* this coefficient was 3.41% and 0.53%, respectively. Since the age range of the study subjects was wide and they were of both genders, besides expressing the bone mass as absolute BMD values we also used the Z-*score* as a relative value for age and gender group of the individual. The T-*score* (relative value of the population peak bone mass) was not considered since there was a group of subjects not reaching the age of the peak bone mass (around 30 years). The Z-*scores* were obtained according to the normality of the local population,[20] by the following formula:

Individual BMD - Mean BMD for the age and gender group

Z-score = Standard deviation for the age gender group

Statistical analysis

The SPPS software (version 14.0) was used for the statistical procedure. A descriptive analysis was carried out by using central tendency and dispersion measures of the data assessed. The means of BMD determinations, analytical determinations and the remaining parameters studied were compared by the Student's t test for independent samples, for normally distributed variables, and the Mann-Whitney test for non-parametric variables. The different variables were correlated by the Pearson's correlation coefficient.

When necessary, regression models were used to adjust by variables. In all cases, a p value < 0.05 was considered as statistical significance. The sample size had enough statistical power to reach the main objective.

RESULTS

The group of cases was comprised by 51 subjects, with ages ranging 16-68 years. Gender distribution was 29 females and 22 males. The control group was comprised by 21 subjects with ages ranging 19-70 years, with 12 females and 9 males.

Table I shows baseline characteristics for both groups, controls and lithiasic patients: age, gender, weight, height, and body mass index (BMI). There were no significant differences between them. Table I also shows the parameters for phosphate-calcium metabolism (calcemia, calciuria, phosphatemia, phosphaturia, 1,25 $(OH)_2$ vitamin D and PTH), BMD and markers of bone remodeling between both groups. As expected, baseline calciuria was higher in lithiasic patients, being statistically significant ($p < 0.001$), as well as mean 1,25 $(OH)_2$ vitamin D levels ($p = 0.002$), which were above normal values (50-105 pmol/L). PTH values were also significantly lower in lithiasic patients ($p = 0.049$). The remaining phosphate-calcium metabolism parameters (calcemia, phosphatemia, phosphaturia) showed normal values and with no significant differences between both groups.

About markers of bone remodeling, there were no significant differences between lithiasic patients and controls, for both formation (osteocalcin) and reabsorption (b-crosslaps). We did not observe differences either between absolute BMD values at both sites, although the values were lower in the group of lithiasic patients. When considering the Z-*score*, the difference did become significant ($p = 0.045$ at the lumbar spine and $p = 0.031$ at the femoral neck).

There were no significant differences between lithiasic patients with hypercalciuria (HC) and those

Table I. Comparison of the values of the studied parameters between lithiasic patients and controls (mean ± standard deviation)

	Lithiasis (n = 51)	Control (n = 21)	p
Age (years)	45.5 ± 13.5	48.6 ± 15.4	0.400
Gender (♂/♀)	22/29	9/12	0.983
Weight (kg)	77.0 ± 16.7	77.9 ± 10.4	0.986
Height (cm)	162.3 ± 7.8	163.3 ± 12.2	0.669
BMI (kg/cm^2)	29.1 ± 5.2	29.3 ± 5.7	0.917
Dairy products intake < 800 mg/day (%)	76.0	0.0	< 0.001
Baseline calciuria (mg/24 h)	221.7 ± 122.3	122.2 ± 51.9	< 0.001
Calcemia (mg/dL)	9.4 ± 0.4	9.4 ± 0.3	0.496
Phosphaturia (mg/24 h)	629.3 ± 258.8	566.1 ± 240.6	0.348
Phosphatemia (mg/dL)	3.4 ± 0.5	3.3 ± 0.6	0.929
Hypercalciuria (%)	52.9	0.0	< 0.001
1.25 $(OH)_2$ Vitamin D (pmol/L)	121.1 ± 46.4	84.7 ± 30.2	0.002
PTH (pg/ml)	39.0 ± 15.6	52.9 ± 28.5	0.049
Osteocalcin (ng/mL)	20.8 ± 12.8	24.7 ± 11.0	0.244
β-crosslaps (mg/mL)	0.45 ± 0.29	0.47 ± 0.21	0.755
BMD L2-L4 (g/cm^2)	0.942 ± 0.144	1.001 ± 0.132	0.091
Z-*score* L2-L4	-0.5 ± 1.4	0.3 ± 1.4	0.045
BMD femoral neck (g/cm^2)	0.805 ± 0.130	0.849 ± 0.085	0.105
Z-*score* femoral neck	-0.08 ± 1.03	0.5 ± 1.0	0.031

without hypercalciuria (NHC) for any of the parameters analyzed, but logically for baseline calciuria (p = 0.000). The mean 1,25 $(OH)_2$ vitamin D levels were high in both groups (higher than the normal value) and somewhat higher in hypercalciuric patients, whereas PTH values were lower. On the other hand, BMD values at the femoral neck, lower in NHC patients, showed a trend towards statistical significance (p = 0.056).

Table II shows the results of the comparison of the 3 subgroups of lithiasic patients (NHC, AHC, and RHC) between one each other. The patients with AHC had significantly higher weight and height than patients with RHC (p = 0.042 and 0.027, respectively) and than those with NHC (p = 0.021 and 0.022, respectively), but there were no differences between NHC and RHC patients. They also showed higher baseline calciuria than RHC patients (p = 0.008). Mean 1,25 $(OH)_2$ vitamin D values were high and above the normal range (50-105 pmol/L) in both types of HC patients. Only densitometry parameters at the femoral neck were significantly higher in AHC patients as compared to RHC patients (BMD, p = 0.007; and Z-*score* = 0.025) and to NHC patients (BMD, p = 0.002; and Z-*score* = 0.007). When adjusting for weight and height the significance between AHC and RHC patients disappeared (p = 0.104 and p = 0.085, respectively), but remained between AHC and NHC patients (p = 0.021 and p = 0.032. respectively).

Finally, in order to assess whether RL itself may have an influence on the study parameters, independently of the presence of hipercalciuria, we compared non-hypercalciuric lithiasic patients with the control group (Table III). There were only significant differences in 1,25 $(OH)_2$ vitamin D levels, which still were higher in NHC lithiasic patients (p = 0.023), and in PTH levels that were low nearly reaching statistical significance (p = 0.051). BMD was lower in the group of lithiasic patients without hypercalciuria, being significant at the femoral neck only (BMD, p = 0.025; Z-*score* = 0.007).

Correlation studies were carried out between the different variables, observing only a weak positive correlation between baseline hypercalciuria basal and 1,25$(OH)_2$ vitamin D levels (r = 0.395; p = 0.001).

About usual calcium intake, this was significantly lower in the group of patients with RL as compared to the control group (Tables I and III), being similar between the different subgroups of RL patients (Table II).

The frequencies of occurrence of the different alleles corresponding to the *loci* studied of the VDR gene, *b*, *a*, and *t*, were compared between lithiasic patients and controls (Table IV). There was no significant difference in the distributions, the heterozygous alleles *(Bb, Aa, and Tt)* being more common. When regrouping the alleles, considering together those showing the presence *(Bb-bb, Aa-aa, Tt-tt),* and comparing them with homozygous showing the absence *(BB,*

Table II. Comparative study of the values of the parameters studied between NHC lithiasic patients, with AHC and RHC (mean ± standard deviation)

	NHC (n = 27)	AHC (n = 10)	RHC (n = 14)	p
Age (years)	43.9 ± 14.8	48.9 ± 11.7	46.1 ± 12.2	NS
Weight (kg)	74.5 ± 12.9	89.6 ± 24.9	72.9 ± 12.5	0.042[#] 0.021[$]
Height (cm)	161.2 ± 7.7	167.6 ± 5.1	160.6 ± 8.2	0.027[#] 0.022[$]
BMI (kg/cm^2)	28.6 ± 4.1	31.7 ± 7.2	28.4 ± 5.3	NS
Dairy products intake < 800 mg/day (%)	76.9	60.0	85.7	NS
Baseline calciuria (mg/24 h)	130.7 ± 60.1	377.4 ± 90.1	286.1 ± 63.5	0.008[#] 0.000[$] 0.000[¶]
Calcemia (mg/dL)	9.4 ± 0.5	9.6 ± 0.2	9.4 ± 0.3	NS
Phosphaturia (mg/24 h.)	577.8 ± 213.1	793.4 ± 452.4	656.7 ± 213.8	NS
Phosphatemia (mg/dL)	3.5 ± 0.3	3.2 ± 0.8	3.2 ± 0.6	NS
1.25 $(OH)_2$ Vitamin D (pmol/L)	113.4 ± 48.9	140.8 ± 42.5	121.4 ± 43.1	NS
PTH (pg/ml)	39.5 ± 17.4	36.9 ± 9.9	39.5 ± 16.0	NS
Calciuria after restriction (mg/24 h)	109.3 ± 45.5	292.0 ± 142.4	274.6 ± 139.3	NS[#] 0.001[$] 0.000[¶]
Ca/Cr ratio pre-overload	0.08 ± 0.05	0.08 ± 0.03	0.20 ± 0.06	0.000[#] 0.035[$] 0.000[¶]
Ca/Cr ratio post-overload	0.3 ± 0.18	0.27 ± 0.08	0.53 ± 0.22	0.003[#] 0.002[¶]
Osteocalcin (ng/mL)	21.4 ± 1.0	16.6 ± 14.9	23.2 ± 17.4	NS
β-crosslaps (mg/mL)	0.42 ± 0.22	0.46 ± 0.33	0.52 ± 0.40	NS
BMD L2-L4 (g/cm^2)	0.952 ± 0.168	0.972 ± 0.128	0.901 ± 0.097	NS
Z-*score* L2-L4	-0.3 ± 1.4	-0.2 ± 1.6	-1.1 ± 1.1	NS
BMD femoral neck (g/cm^2)	0.773 ± 0.125	0.921 ± 0.089	0.783 ± 0.123	0.007[#*] 0.002[$*]
Z-*score* femoral neck	-0.3 ± 0.9	0.75 ± 1.13	-0.33 ± 1.01	0.025[#*] 0.007[$*]

[#] Between AHC-RHC; [$] Between NHC-AHC; [¶] Between NHC-RHC.
[#*] When adjusting by weight and height, p values were: BMD, 0.104; Z-*score*, 0.085.
[$*] When adjusting by weight and height, p values were: BMD, 0.021; Z-*score*, 0.032.
NS: not significant.

AA, and *TT),* there was only a trend towards lower occurrence of the *BB* allele as compared with the grouped *Bb-bb*, although not reaching the significance level (P = 0.098). When grouping by the feature of absence *(BB-Bb, AA-Aa, TT-Tt),* as compared to the homozygous showing presence *(bb, aa,* and *tt),* the lower occurrence of the *tt* allele was only mildly significant (p = 0.051) as compared to the *TT-Tt* group. There were no significant differences when grouping the homozygous alleles as compared with the heterozygous alleles (Table IV). We do not show the results from comparing the frequencies of the alleles within the lithiasic group by the presence or absence of hipercalciuria, and the type of hypercalciuria, because the ensuing subgroups showed very low frequencies with no possibility of obtaining definitive conclusions from a statistical point of view.

DISCUSSION

Both calcium renal lithiasis and idiopathic hipercalciuria are conditions with multiple, yet not clearly understood, etiopathogenic mechanisms, many of which we have not considered here since they fall beyond our goals. There is a general agreement that approximately 50% of idiopathic hypercalciurias cannot be classified within the types defined by Pak;[5] This difficulty yields the diverse outcomes found in works aimed at studying it, and that lack of uniform group protocols. So that, in order to make easier and clearly define hypercalciuria types, we have simplified our classification. Besides, this is a study of which we are showing the preliminary results, so that the number of subjects in each subgroup is still low.

Table III. Comparison of the values of the studied parameters between NHC lithiasic patients and the control group (mean ± standard deviation)

	NHC (n = 27)	Control (n = 21)	p
Age (years)	44.0 ± 14.8	48.6 ± 15.4	0.294
Weight (kg)	74.5 ± 12.9	74.5 ± 12.9	0.470
Height (cm)	161.2 ± 7.7	161.2 ± 7.7	0.475
BMI (kg/cm^2)	28.6 ± 4.1	29.3 ± 5.7	0.656
Dairy products intake < 800 mg/day (%)	76.9	0.0	0.000
Baseline calciuria (mg/24 h)	130.7 ± 60.1	122.2 ± 51.9	0.611
Calcemia (mg/dL)	9.4 ± 0.5	9.4 ± 0.3	0.804
Phosphaturia (mg/24 h)	577.8 ± 213.1	566.1 ± 240.6	0.861
Phosphatemia (mg/dL)	3.5 ± 0.3	3.3 ± 0.6	0.412
1.25 $(OH)_2$ Vitamin D (pmol/L)	113.4 ± 48.9	84.7 ± 30.2	0.023
PTH (pg/ml)	39.5 ± 17.4	52.9 ± 28.5	0.051
Osteocalcin (ng/mL)	21.4 ± 1.0	24.7 ± 11.0	0.296
β-crosslaps (mg/mL)	0.42 ± 0.22	0.47 ± 0.21	0.395
BMD L2-L4 (g/cm^2)	0.952 ± 0.168	1.001 ± 0.132	0.240
Z-*score* L2-L4	-0.3 ± 1.4	0.3 ± 1.4	0.173
BMD femoral neck (g/cm^2)	0.773 ± 0.125	0.849 ± 0.085	0.025
Z-*score* femoral neck	-0.3 ± 0.9	0.5 ± 1.0	0.007

We have observed lower bone mass in lithiasic patients as compared with healthy controls, according to densitometry parameters, both at the lumbar spine and at the femur, although that difference has only been statistically significant for the *Z-scores*. These results are in agreement with those observed in previous studies,[7-11] pointing towards a negative influence of CRL on BMD. Laderdale *et al.* found lower BMD in RL patients from NHANES III study, more clearly shown in male patients (in that study, BMD measurement was done only at the proximal femur), as well as higher prevalence of vertebral and wrist fractures.[19] Melton *et al.* also found higher prevalence of vertebral fractures in urolithiasis patients. [21] Both authors point out as the cause the low-calcium diet these patients usually do. By contrast, Asplin *et al.*[22] carried out a study similar to ours in 59 subjects of both genders from 11 families, of which 22 had lithiasis; the authors did not differences in BMD (expressed as Z-*score*) nor at the spine nor the femur between both groups. They did not find either different 1,25 $(OH)_2$ vitamin D values between them, which may explain the differences with our results, as we will see later on. Tsuji *et al.*, in a study performed on 310 patients of both genders with renal lithiasis, obtained that 27% of them had low BMD, with no differences with control subjects (23.5%); although the frequency significantly increased when considering only female patients with hypercalciuria, reaching 40%, and 65% when HC had not a diabetic origin ($p < 0.01$). Besides, we should highlight that in that study patients with dietary restriction were excluded and no 1,25 $(OH)_2$ vitamin D determinations were done.[23]

However, when comparing among the group of lithiasis patients those with hypercalciuria (HC) and those without it (NHC), we obtained controversial data: the bone mass was similar at the lumbar spine, whereas higher values were observed at the femoral neck of HC patients, with a trend towards statistical significance. Vezzoli *et al.*, in a study done on lithiasic women, with and without hypercalciuria, found lower BMD at the spine but not at the femur of postmenopausal women with HC and high calcium absorption index. They did not perform a comparison with a control group.[24] However, Caudarella *et al.* performed a similar study in which thy measured BMD at the radial bone, without obtaining differences between HC and NHC.[25] Pietschmann *et al.* found lower BMD at the spine of lithiasic HC patients as compared to NHC, but no differences in BMD at the radius.[8] This may give rise to the hypothesis of different influence of hypercalciuria in bone mass from the different sites measured; we have seen how in our study significant differences in BMD are found at the femur and not at the spine. Gianini *et al.*, in a study performed in osteoporic postmenopausal women, concluded that hypercalciuria was a common finding among them but they did not observed differences in bone mass between those presenting these changes and those not, both at the spine and the hip.[26] There really exist different etiopathogenic mechanisms leading to hypercalciuria, and that may have different or

Table IV. Frequency of occurrence of the different alleles of the VDR polymorphisms studied, taken separately and pooled together, among the cases and controls

Alleles (%)	CRL (51)	Controls (21)	p
BB	9.8	25.0	
Bb	49.0	45.0	0.237
bb	41.2	30.0	
AA	21.6	35.0	
Aa	56.9	45.0	0.492
aa	21.6	21.6	
TT	30.0	41.2	
Tt	45.0	51.0	0.141
tt	25.0	7.8	
bb	41.2	30.0	0.383
BB, Bb	58.8	70.0	
aa	21.6	20.0	0.884
AA, Aa	78.4	80.0	
tt	7.8	25.0	0.051
TT,Tt	92.2	75.0	
BB	9.8	25.0	0.098
Bb, bb	90.2	75.0	
AA	21.6	35.0	0.242
Aa, aa	78.4	65.0	
TT	41.2	30.0	0.383
Tt, tt	58.8	70.0	
Bb	49.0	45.0	0.760
BB, bb	51.0	55.0	
Aa	56.9	45.0	0.367
AA, aa	43.1	55.0	
Tt	51.0	45.0	0.650
TT, tt	49.0	55.0	

contrary differences on the bone, which could explain the diversity of outcomes in these studies.

In our study, within the group of lithiasic patients, we only observed calciuria-related differences in BMD at the femoral neck in AHC patients, and only when comparing them with lithiasic NHC patients. The finding of significantly lower BMD at the femoral neck of lithiasic NHC patients as compared to controls supports our believe that renal Lithiasis, by itself and independently of the presence or absence of hypercalciuria and its types, negatively affects on BMD, and particularly the hip. Tasca *et al.* found lower BMD only at the spine, but not the femur, of patients with RHC as compared to control subjects.[27] Deutschmann *et al.* found that renal hypercalciuria is one of the main changes found in an important group of patients with idiopathic osteoporosis.[28] Again, the explanation for these diverse results may lay on the lack of homogenous criteria when classifying hypercalciurias and the diversity of pathogenic mechanisms that may cause them.

Lithiasic patients show high levels of vitamin D (above normal reference values) and in all the subgroups (NHC, AHC, and RHC) with no significant differences between one each other. The higher values of 1,25 $(OH)_2$ vitamin D in lithiasic patients as compared with controls were clearly significant. Among the causes of these high values of 1,25 $(OH)_2$ vitamin D may be hypophosphatemia, hyperparathyroidism, and idiopathic hipercalciuria itself. However, our lithiasic patients show normal mean phosphatemia and lower PTH levels than controls, likely due to the inhibition of the parathyroid gland because of high levels of 1,25 $(OH)_2$ vitamin D. Idiopathic hypercalciuria could be the cause, although the lack of a difference in 1,25 $(OH)_2$ vitamin D levels between lithiasic NHC patients and HC patients lo questions it. One possible explanation, although not demonstrable with this work, could be that calcium restriction in the diet of lithiasic patients could stimulate higher production of 1,25 $(OH)_2$ vitamin D in order to increase calcium intestinal absorption. This explanation is in agreement with Hess's, who recommends not to restrict calcium intake in subjects with renal lithiasis in order to prevent the subsequent metabolic changes.[29] Asplin and Tasca did not find differences in 1,25$(OH)_2$ vitamin D levels;[22,27] although in Asplin's study calcium intake was similar and higher than the minimum required amount (901 mg/day in lithiasic patients and 1,019 mg/day in controls), whereas in Tascas's study this parameter was not specified.

Although lithiasic patients showed lower PTH levels ælikely related with high 1,25$(OH)_2$ vitamin Dæ they fall within the normality range. In the study by Giannini *et al.*, PTH levels virtually showed no differences between both groups, although their patients did not show differences in calcium intake and 1,25 $(OH)_2$ vitamin D levels were not measured.[26] Tasca *et al.* obtained identical results: they did not find either significant differences between AHC and RHC patients, and controls but the values follow the same order in the three groups; although the vitamin D levels they report were within the normality range and subjects with low-calcium diet were excluded.[27]

The diet plays an important role in the creation of renal stones, and diet modification may reduce the risk for recurrence of new stones. Most of the patients forming stones need to increase fluid intake and be recommended to take a diet with adequate (but not low) calcium amounts and low content in animal proteins and sodium.[30]

In our lithiasic patients the low intake of dairy products observed, independently of the type of hypercalciuria, was expected since, to date, this is a

strongly recommended prescription to prevent the formation of new stones.

In the long-term, however, the negative effect of low-calcium diet has been shown. This measure leads to a negative internal calcium balance favored by other factors related with calcium lithiasis: renal hypercalciuria, hypophosphatemia, and increased levels of 1,25 $(OH)_2$ vitamin D.[31] Asplin *et al.* point out this fact as a risk factor for low bone mass in lithiasic patients, since it starts up the necessary mechanisms to prevent hypocalcaemia and that, finally, have an effect on the bone.[22] In their study, Fuss *et al.* have shown that renal lithiasic patients that had a regular low-calcium diet presented lower bone mass and higher risk for developing osteopenia.[32] This observation was confirmed by Jaeger *et al.* that studied the effect of different levels of dietary calcium intake on the bone mass in a group of lithiasic patients.[33] Trincheri *et al.* carried out a similar study[34] in which the bone mass density at the lumbar spine was significantly lower and 1,25$(OH)_2$ vitamin D levels were higher in the group with the lowest calcium intakes; these results are in agreement with those obtained by us.

The results of the studies on the influence of polymorphisms of the VDR gene on renal lithiasis are different, and even some of them contradictory. It is true that most of them are done in a very small number of subjects. Our work does not show conclusive results. Although we have not found evident statistical significance, it seems that the homozygous alleles *BB* and *tt* occur less frequently among CRL patients. This may be in agreement with the study by Mosetti *et al.*, who showed a statistically significant relationship between the *bT* haplotype of the VDR gene and early age of onset of renal lithiasis and higher family incidence of nephrolithiasis in hypercalciuric patients, as well as higher recurrence rate of CRL among them.[18] Ozkaya *et al.* studied *a, b, and t* polymorphisms in 64 children with hypercalciuric lithiasis and 90 healthy children, also finding that relationship between the TT genotype and the family history and risk for recurrence of lithiasis, as well as higher frequency of occurrence of the *AA* genotype among hypercalciuric lithiasic children.[17] Jackman *et al.* also investigated the association with these same factors in a group of 19 hypercalciuric subjects compared to 37 controls, concluding that the *TT* genotype is statistically and significantly associated with a family history of Lithiasis but not with the risk for recurrent lithiasic episodes.[13] Ruggiero *et al.* found that subjects with the *bb* allele showed higher daily excretion of urinary calcium with higher risk for presenting Lithiasis;[35] these findings have been shown in other studies.[12, 36]

However, Nishijima *et al.* found a higher proportion of the *tt* and *Tt* genotypes in 83 lithiasic patients studied as compared to 83 subjects controls, without finding any polymorphism at *locus a*.[16] On the other hand, Söylemezoglu *et al.* found a higher frequency of the *AA* genotype in absorptive hypercalciuria, as compared to the group comprising the other two genotypes (*Aa/aa*), without finding any association with polymorphisms of the *loci b* and *t*.[15] Adding to the controversy, the study done by Heilberg *et al.* suggests that the polymorphism of BsmI does not play an important role in bone mass loss or in hipercalciuria, in renal Lithiasis.[37]

To conclude, our results suggest that renal calcium lithiasis *per se*, negatively affects BMD, and more specifically at the femoral neck, independently of the presence or absence of hypercalciuria and its types. Similarly, there is increased 1,25 $(OH)_2$ vitamin D secretion that, in turn, slows down PTH secretion, the causes being unknown; it may be considered that low calcium intake could have an influence by stimulating the production of 1,25 $(OH)_2$ vitamin D, although other studies specifically designed would be necessary to draw up conclusions. Finally, although in our study lithiasic patients present a genotype distribution similar to that of controls, it seems that homozygous alleles *BB* and *tt* occur less frequently among patients with calcium renal lithiasis, which could become evident when enlarging the study sample size.

BIBLIOGRAFÍA

1. Torres A, Suria S, Balaguer G, Concepción M, Martínez M, Lorenzo V: Litiasis renal recidivante: estudio metabólico e incidencia relativa de las distintas formas. *Nefrología* 10: 362-70, 1990.
2. Johnson CM, Wilson DM, O'Fallon WM, Malek RS, Kurland LT: Renal stone epidemiology: a 25 year study in Rochester, Minnessota. *Kidney Int* 16: 624-31, 1979.
3. Sierakowsky R, Finlayson B, Lande, RR: The frecuency of urolithiasis on hospital discharge diagnosis in the Unites States. *Invest Urol* 15: 438-41, 1978.
4. Abraham PA, Smith CL: Medical evaluation and management of calcium nephrolithiasis. *Med Clin North Am* 68: 281-99, 1984.
5. Audran M, Erick L: Hypercalciuria. *Joint Bone Spine* 67: 509-15, 2000.
6. Lemann J Jr, Worcester EM, Gray RW: Hipercalciuria and stones. *Am J Kidney Dis* 17: 386-91, 1991.
7. Pacifici R, Rothstein M, Rifas L, Lau KH, Baylink DJ, Avioli LV et al. Increased monocyte interleukin-1 activity and decreased vertebral bone density in patients with fasting idiopathic hypercalciuria. *J Clin Endocrinol Metab* 71: 138-45, 1990.
8. Pietschmann F, Breslau NA, Pack CYC: Reduced vertebral bone density in hypercalciuric nephrolithiasis. *J Bone Miner Res* 12: 1383-8, 1992.
9. Borghi L, Meschi T, Guerra A, Maninetti L, Pedrazzoni M, Marcato A et al.: Vertebral mineral content in diet-dependent hypercalciuria. *J Urol* 146: 1334-8, 1991.
10. Bataille P, Achard JM, Fournier A, Boudailliez B, Westeel PF, Esper N et al.: Diet Vitamin D and vertebral mineral density in hypercalciuric calcium stone formers. *Kidney Int* 39: 1193-205, 1991.

11. Weisinger JR, Alonzo E, Bellorin-Font E, Blasini AM, Rodríguez MA, Paz-Martínez V et al.: Possible role of cytokines on the bone mineral loss in idiopathic hypercalciuria. *Kidney Int* 49: 244-50, 1996.
12. Onghiphadhanakul B, Rajatanavin R, Chanprasertyothin S, Chailurkit L, Piaseu N, Teerarungsikul K et al.: Vitamin D receptor gene polymorphisms associated with urinary calcium excretion but not with bone mineral density in postmenopausal women. *J Endocrinol Invest* 20: 592-6, 1997.
13. Jackman SV, Kibel AS, Ovuworie CA, Moore RG, Kavoussi LR, Jarrett TW: Familial calcium stone disease: TaqI polymorphism and the vitamin D receptor. *J Endourol* 4: 313-6, 1999.
14. Rendina D, Mossetti G, Viceconti R, Sorrentino M, Castaldo R, Manno G et al.: Association between vitamin D receptor gene polymophisms and fasting idiopathic hypercalciuria un recurrent stone-forming patients. *Urology* 64: 833-8, 2004.
15. Söylemezoglu O, Ozkaya O, Gönen S, Misirhoglu M, Kalman S, Buyan N: Vitamin D receptor gene polymorphism in hypercalciuric children. *Pediatr Nephrol* 19: 724-7, 2004.
16. Nishijima S, Sugaya K, Naito A, Morozumi M, Hatano T, Ogawa Y: Association of vitamin D receptor gene polymorphism with urolithiasis. *J Urol* 167: 2188-91, 2002.
17. Ozkaya O, Söylemezoglu O, Misirhoglu M, Gönen S, Buyan N, Hasanoglu E: Polymorphism in the vitamin D receptor gene and the risk calcium nephrolitiasis in children. *Eur Urol* 44: 150-4, 2003.
18. Mossetti G, Rendina D, Viceconti R, Manno G, Guadagno V, Strazullo P et al.: The relationship of 3' vitamin D receptor haplotypes to urinary supersaturation of calcium oxalate salts and to age at onset and familial prevalence of nephrolithiasis. *Nephrol Dial Transplant* 19: 2259-65, 2004.
19. Laderdale DS, Thisted RA, Wen M, Favus MJ: Bone mineral density and fracture among prevalent kidney stone cases in the Third National Health and Nutrition Examination Survey. *J Bone Miner Res* 16: 1893-8, 2001.
20. De Tejada Romero M.ªJ: Tesis Doctoral. Valores de control de Densidad Mineral Ósea en nuestra población mediante QDR.; evaluación de los factores de riesgo de Osteoporosis en mujeres perimenopáusicas. Universidad de Sevilla. 1992.
21. Melton III LJ, Crowson CS, Khosla S, Wilson DM, O'Fallon WM: Fracture risk among patients with urolithiasis: a population-based cohort study. *Kidney Int* 53: 459-64, 1998.
22. Asplin JR, Bauer KA, Kinder J, Muller G, Coe BJ, Parks JH et al.: Bone mineral density and urine calcium excretion amount subjects with and without nephrolithiasis. *Kidney Int* 63: 662-9, 2003.
23. Tsuji H, Umekawa T, Kurita T, Uemura H, Iguchi M, Kin K et al.: Analysis of bone mineral density y urolithiasis patients. *Int J Urol* 12: 335-339, 2005.
24. Vezzoli G, Rubinacci A, Bianchin C, Arcidiacono T, Giambona S, Mignogna G et al.: Intestinal calcium absorption in associated with bone mass in stone-former women with idiopathic hypercalciuria. *Am J Kidney Dis* 42: 1177-83, 2003.
25. Caudarella R, Vescini C, Buffa A, Sinicropi G, Rizzoli E, La Manna G et al.: Bone mass loss in calcium stone diseases: focus on hypercalciuria and metabolic factors. *J Nephrol* 16: 260-6, 2003.
26. Giannini S, Nobile M, Dalle L, Lodetti MG, Sella S, Vittadello G et al.: Hypercalciuria is a common and important finding in postmenopausal women with osteoporosis. *Eur J Endocrinol* 149: 209-13, 2003.
27. Tasca A, Cacciola C, Ferrarese P, Ioverno E, Visona E, Bernardi C et al.: Bone alterations in patients with idiopathic hypercalciuria and calcium nephrolithiasis. *Urology* 59: 865-9, 2002.
28. Deutschmann HA, Weger M, Weger W, Kotanko P, Deutschmann MJ, Skrabal F: Search for occult secondary osteoporosis: impact of identified possible risk factors on bone mineral density. *J Intern Med* 252: 389-97, 2002.
29. Hess B: Low calcium diet in hypercalciuric calcium nephrolithiasis: first do no harm. *Scanning Microsc* 10: 547-54, 1996.
30. Taylor EN, Curhan GC: Diet and fluid prescription in stone disease. *Kidney Int* 70: 835-9, 2006.
31. Trinchieri A: Bone mineral content in calcium renal stone formers. *Urol Res* 33: 247-53, 2005.
32. Fuss M, Pepersack T, Bergman P, Hurard T, Simon J, Corvilain J: Low calcium diet in idiophatic urolithiasis: a risk factor for osteopenia as great as in primary hyperparathyroidism. *Br J Urol* 65: 560-3, 1990.
33. Jaeger P, Lippuner K, Casez JP, Hess B, Ackermann D, Hugh C: Low bone mass in idiopathic renal stone formers: magnitude and significance. *J Bone Miner Res* 10: 1525-32, 1994.
34. Trinchieri A, Nespoli R, Ostini F, Rovera F, Zanetti G, Pisani E: A study of dietary calcium and other nutrients in idiopathic renal calcium stone formers with low bone mineral content. *J Urol* 159: 654-57, 1998.
35. Ruggiero M, Pacini S, Amato M, Aterini S, Chiarugi V: Association between vitamin D receptor gene polymorphism nephrolithiasis. *Miner Electrolite Metab* 25: 185-90, 1999.
36. Relan V, Khullar M, Singh SK, Sharma SK: Association of vitamin D receptor genotypes with calcium excretion in nephrolithiatic subjects in Northern India. *Urol Res* 32: 236-40, 2004.
37. Heilberg IP, Teixeira SH, Martini LA, Boim MA: Vitamin D receptor gene polymorphism and bone mineral density in hypercalciuric calcium-stone-forming patients. *Nephron* 90: 51-7, 2002.