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ORIGINAL ARTICLE

Two siblings with a novel nonsense mutation, p.R50X, in the *vitamin D receptor* gene

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Abstract Hereditary vitamin D-resistant rickets (HVDRR), an autosomal recessive disorder caused by inactivating mutations in the vitamin D receptor (*VDR*) gene. We identified two affected children from the same family, one at the age of 10 years and 9 months and the other at 9 months old. Mutation analysis by PCR-sequencing the entire coding region of the *VDR* gene revealed a homozygous C to T transition in exon 2 of the *VDR* gene (c.148C>T) resulting in a stop codon at amino acid position 50 (p.R50X) in the proband and his younger sister. The p.R50X has never been previously described. Both asymptomatic parents were heterozygous for the mutation. In addition to most of the clinical features of HVDRR including total alopecia, symptoms of hypocalcemia at a later onset and normophosphatemia, rarely found in HVDRR were present in the proband. This study also emphasizes an important role of genetic testing for early diagnosis and genetic counseling.

Keywords Hereditary vitamin D-resistant rickets · Hypocalcemia · Alopecia · Nonsense mutation · Vitamin D receptor

Abbreviations

HVDRR	Hereditary vitamin D-resistant rickets
VDR	Vitamin D receptor
PTH	Parathyroid hormone

Introduction

Hereditary vitamin D-resistant rickets (HVDRR), previously known as vitamin D dependent rickets type II (VDDR type II), is an autosomal recessive disorder (OMIM # 277440) caused by inactivating mutations in the vitamin D receptor (*VDR*) gene [1–3]. Deficiency of VDR leads to rickets with end-organ unresponsiveness to 1,25-dihydroxyvitamin D_3 (1,25 $(OH)_2D_3$). HVDRR is characterized by early onset rickets, hypocalcemia, secondary hyperparathyroidism, and elevated serum levels of 1,25 $(OH)_2D_3$ [3, 4]. Hypophosphatemia is generally found in patients with HVDRR. However, some patients with HVDRR have been reported with normophosphatemia [5, 6]. About 80% of patients with HVDRR have alopecia, either totalis or partialis by which the degree of alopecia is associated with severity of the resistance. Age at diagnosis of HVDRR is varied from few months to the second decade of life by the typical skeletal defects [6].

To date, at least 48 different mutations in the *VDR* gene have been reported and most are missense/nonsense

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mutations (http://www.hgmd.cf.ac.uk, accessed January 2011). Most *VDR* mutations affect the DNA binding domain (DBD) and result in inactivation or loss of protein function [3, 7]. The genotype-phenotype correlation is still unclear and further studies are required.

In this study, we report clinical and molecular characterization of two Thai children from the same family with HVDRR. A homozygous novel nonsense mutation in the DBD of the *VDR* was identified, expecting to result in a truncated protein leading to hormone resistance and total body alopecia.

Methods

Laboratory evaluation of hormonal function

The intact PTH level was analyzed by electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN) and the level of 25-hydroxyvitamin D (25-OHD) was determined by direct competitive chemiluminescence immunoassay (DiaSorin S.p.A., Saluggia, Vercelli, Italy).

Mutation analysis

After informed consent, peripheral blood samples were obtained from the patients and their parents. Total RNA and genomic DNA (gDNA) were extracted from peripheral leukocytes according to standard protocols. The study was approved by local Ethics Committee. Reverse transcription was performed using ImProm-II™ reverse transcriptase (Promega, Madison, WI), according to the manufacturer's instructions. The *VDR* cDNA was amplified by PCR using a set of primers and condition as previously described [8]. In brief, we used 50 ng of cDNA, 1XPCR buffer (Fermentas, Thermo Fisher Scientific, Inc), 1.5 mM MgCl2, 0.2 mM dNTPs, 0.2 μM of each primer and 0.5 U Taq DNA polymerase (Promega) in a volume of 20 μl using the following parameters: 35 cycles of 45 s at 94°C, 30 s at 64°C and 1 min 15 s at 72°C. PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, OH) according to the manufacturer's recommendations, and sent for direct sequencing (Macrogen Inc., Seoul, Korea). Sequence data were analyzed using Sequencher (version 4.2; Gene Codes Corporation, Ann Arbor, MI). The entire exon 2 of the *VDR* gene was amplified from gDNA of the patients and their parents by PCR using primers 5′-CCA GAA GAC AGG TCT CCG TG-3′ and 5′-TGC CCA AAC TTG CAG GAG AG-3′ at the annealing temperature of 58°C. The PCR products were sent for direct sequencing.

Results

The proband was a 10-year and 9-month-old boy who presented with intermittent cramping for 1 month due to hypocalcemia. He was born at term to consanguineous parents without complications. The parents were first cousins and phenotypically normal. There was no history of bone disease. The proband was found to have sparse and thin hair since birth and developed total alopecia at 7 months old. Teeth erupted at appropriate for age but all were pulled out from severe dental caries. He started to walk at 3 years of age. Except for delayed walking, his other developmental milestones were within normal limit. He had one long bone fracture due to falling at 2 years of age. Before being brought to our hospital, he had seizure at age 10 years and 8 months due to hypocalcemia for which he was treated at the local hospital. Before visiting us, the cause of his hypocalcemia had never been characterized as he rarely suffered from seizure despite intermittent cramping. Physical examination at age 10 years and 9 months showed a height of 90.5 cm (height age at 2-year and 6-month old) and a weight of 15.5 kg (weight age at 3-year and 6-month old). He was disproportionately short with bowed legs and total alopecia (Fig. 1a). Frontal bossing with sparse and thin eyebrows was noted. He had small thoracic cage with rachitic rosary at both sides of costochondral junctions. Widening of both wrists was found. Generalized small papular lesions were found on his face, trunk and both arms. Laboratory investigations were shown in Table 1 and revealed hypocalcemia, normophosphatemia, elevated parathyroid hormone (PTH) and alkaline phosphatase. Imaging studies revealed generalized osteopenia with severe rickets (Fig. 1c, e). The proband was initially treated with 425 mg/kg/day of calcium, 40,000 U/day of ergocalciferol and 133 ng/kg/day of calcitriol. Calcium and calcitriol were subsequently increased to 750 mg of elemental calcium/kg/day and 7 μg/day (466 ng/kg/day), respectively. Despite high-dose oral calcium therapy for 2 months, normal serum calcium levels could not be achieved. An alternate day of intravenous calcium administration (1 g/day) was therefore added to the daily oral dose of calcium to raise and maintain serum levels of calcium at a normal range. The alkaline phosphatase and PTH gradually improved (Table 1). Alopecia remained unchanged.

The proband's sister was a 9-month-old girl with sparse and thin hair since birth (Fig. 1b). She had no history of fractures or hypocalcemic symptoms. Her diet was adequate for vitamin D and calcium according to recommended daily allowance. Her dentition appeared normal with four teeth at age 1 year. She was able to stand alone without bowed legs. Her other developmental milestones remained appropriate for age. On physical examination, her

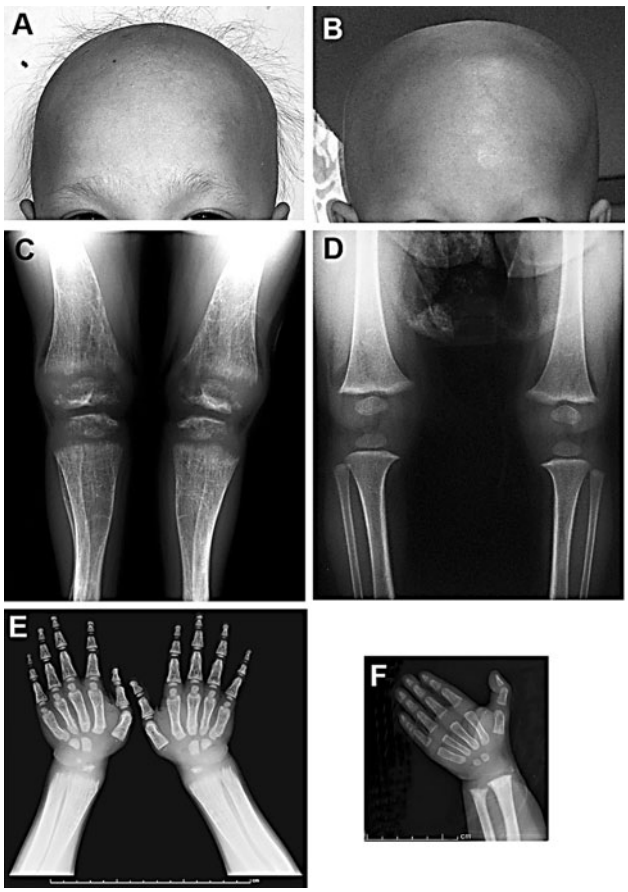

Fig. 1 The clinical features and radiological findings of the proband (**a**, **c**, **e**) and the younger sister (**b**, **d**, **f**). Photographs of both patients showing alopecia and sparse and thin eyebrows and eyelashes (**a**, **b**). X-ray of both knees (**c**, **d**) and wrists (**e**, **f**) showing findings consistent with rickets

length was 71 cm (25th percentile), weight was 8.2 kg (25th percentile), and head circumference was 43 cm (25th percentile). The upper-to-lower segment ratio was 1.36:1. Mild skeletal deformities were found at both wrists. Total alopecia with sparse and thin eyebrows was noted. Laboratory investigations revealed normal levels of calcium and phosphate with elevated levels of PTH and alkaline phosphatase (Table 1). The radiographs showed mild metaphyseal flaring of distal ends of femurs and wrists (Fig. 1d, f). She was initially treated with 40,000 units of vitamin D2 and 500 ng of calcitriol and 400 mg of elemental calcium per day. Due to persistent elevation of alkaline phosphatase and PTH levels, intermittent administration of intravenous calcium was subsequently added.

Analysis of the *VDR* gene by PCR-sequencing identified a homozygous nonsense mutation, a C to T transition in exon 2 at nucleotide position 148 (c.148C>T) from the proband and his sister (Fig. 2, upper panel). No other sequence alterations were found. Both parents were heterozygous for this substitution (Fig. 2, middle panel). The c.148C>T mutation is expected to result in changing an arginine at amino acid position 50 into a stop codon (p.R50X). The novel nonsense mutation is located in the DBD.

Discussion

We describe the clinical and genetic features of a family with two siblings affected with HVDRR. The proband had severe rickets and total alopecia, but a later onset of hypocalcemic symptoms and normophosphatemia. His 9-month-old sister had early signs of rickets and total alopecia with elevated levels of PTH and alkaline phosphatase but normal levels of calcium and phosphate.

Mutation analysis revealed that both siblings who were born to consanguineous parents harbored a homozygous nonsense mutation, c.148C>T (p.R50X), of the *VDR* gene. Although five different nonsense mutations in the *VDR* gene have previously been reported [2, 9–15], the p.R50X is novel. Their parents are heterozygous for the substitution and remain asymptomatic.

Alopecia is a feature frequently found in individuals with HVDRR with the majority of the patients having sparse body hair. Total scalp and body alopecia have been reported in some cases [2, 6, 9–19]. Our patients were found to have sparse and thin hair since birth and developed total alopecia within 12 months. Generalized small

Table 1 Laboratory findings of the proband and the younger sister

	Before treatment		After treatment		Normal range
	Proband	Sister	Proband	Sister	
Calcium (mg/dl)	4.3	8.9	9.8	8.7	8.1–10.4
Phosphate (mg/dl)	3.5	3.7	3.0	2.8	2.7–4.5
Alkaline phosphatase (U/l)	1031	747	475	906	39–117
Intact PTH (pg/ml)	100.8	381.5	84.1	188.3	15–65
25-hydroxy vitamin D (ng/ml)	5.2	N/A	23.4	109.0	>25

N/A non available

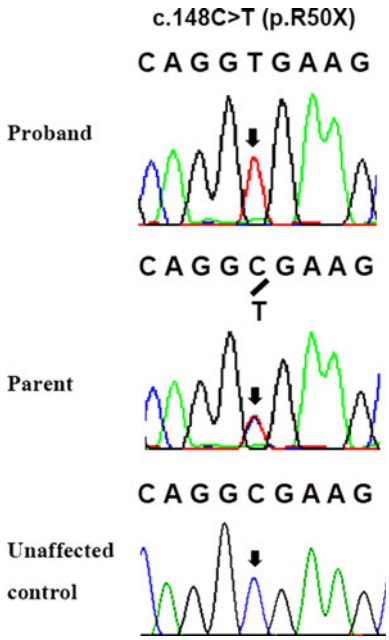

Fig. 2 Mutation analysis. An electropherogram of the proband showing a homozygous C to T transition (c.148C>T) (an *arrow*) resulting in changing an arginine at amino acid position 50 into a stop codon (p.R50X) (the *upper panel*). The parents are heterozygous for the identified mutation (the *middle panel*)

papular lesions were also detected on the proband's face, trunk, and both arms. Previous studies demonstrated striking clinical and microscopic similarities between alopecia and skin lesions associated with *VDR* mutations and with hairless gene (*hr*) mutations [19, 20]. The protein product of the *hr* gene is a corepressor of the VDR and retinoid-related orphan receptor [21]. The VDR and HR are thought to repress specific genes during the hair cycle. Mutations in *VDR* or *HR* cause alopecia by derepression of these genes leading to disturbance of the hair cycle [22, 23]. Most of *VDR* mutations associated with alopecia are located in the DBD or result in premature termination truncating the VDR [3]. Some mutations have been identified in the ligand-binding domain preventing VDR from binding to the retinoid X receptor (RXR) [11, 15, 18, 24]. A mutation in the *VDR* gene that abolished coactivator interaction and transactivation but not DNA binding or RXR heterodimerization was identified in an HVDRR patient without alopecia [25]. The findings of total alopecia observed in our HVDRR patients with the novel nonsense mutation, p.R50X, presumably leading to the formation of a truncated protein lacking parts of the DNA binding domain as well as a complete loss of the C-terminal ligand-binding domain provide evidence supporting this hypothesis.

A few variations in the laboratory investigations of HVDRR have been demonstrated. Hypophosphatemia would be expected in HVDRR patients due to decreased vitamin D action; however, some affected cases including ours were found to have normal serum phosphate levels or levels higher than expected with unclear explanation [5, 6]. Normal levels of serum 25-OHD, an indicator of vitamin D reserve, are generally observed in HVDRR individuals. Previous studies however reported HVDRR individuals with low levels of 25-OHD due to concomitant vitamin D deficiency [26, 27]. The initial 25-OHD level in our proband was low. It was corrected after treatment with ergocalciferol, calcium, and calcitriol suggesting a possible poor nutritional intake.

Individuals with *VDR* mutations causing alopecia are generally unresponsive to oral calcium and vitamin D or calcitriol therapy and the degree of alopecia is associated with the treatment outcome [28]. Intravenous calcium infusion is required to achieve normal serum calcium levels [29]. Our proband with total alopecia was initially treated with high-dose oral calcium therapy. The partial clinical responses included relief of hypocalcemic symptoms and slight improvement of serum chemistries. After two months of treatment, normal serum calcium levels however could not be achieved. Addition of intravenous calcium at a dose of 1 g every other day was therefore required. These findings indicated that the VDR defect in our proband led to an ineffectiveness of intestinal calcium absorption. Intravenous calcium infusion is necessary to restore normocalcemia and reverse secondary hyperparathyroidism.

The genetic analysis of the *VDR* is crucial for giving definite diagnosis and appropriate genetic counseling in patients suspected of HVDRR. This also leads to early diagnosis and management in other at-risk family members to prevent further bone deformities and hypocalcemic events. The proband's sister received treatment after definite diagnosis was made at 9 months of age.

In conclusion, we report a novel nonsense mutation (c.148 C>T, p.R50X) in the *VDR* gene in two siblings with HVDRR and total alopecia. This study emphasizes an important role of genetic testing for a definite diagnosis and appropriate genetic counseling. Early diagnosis and treatment could be provided to prevent complications associated with VDR defects.

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References

1. M.R. Hughes, P.J. Malloy, D.G. Kieback, R.A. Kesterson, J.W. Pike, D. Feldman, B.W. O'Malley, Point mutations in the human

vitamin D receptor gene associated with hypocalcemic rickets. Science **242**, 1702–1705 (1988)
2. H.H. Ritchie, M.R. Hughes, E.T. Thompson, P.J. Malloy, Z. Hochberg, D. Feldman, J.W. Pike, B.W. O'Malley, An ochre mutation in the vitamin D receptor gene causes hereditary 1,25-dihydroxyvitamin D3-resistant rickets in three families. Proc. Natl. Acad. Sci. USA **86**, 9783–9787 (1989)
3. P.J. Malloy, J.W. Pike, D. Feldman, The vitamin D receptor and the syndrome of hereditary 1,25-dihydroxyvitamin D-resistant rickets. Endocr. Rev. **20**, 156–188 (1999)
4. M.H. Brooks, N.H. Bell, L. Love, P.H. Stern, E. Orfei, S.F. Queener, A.J. Hamstra, H.F. DeLuca, Vitamin-D-dependent rickets type II. Resistance of target organs to 1,25-dihydroxyvitamin D. N. Engl. J. Med. **298**, 996–999 (1978)
5. H. Yagi, K. Ozono, H. Miyake, K. Nagashima, T. Kuroume, J.W. Pike, A new point mutation in the deoxyribonucleic acid-binding domain of the vitamin D receptor in a kindred with hereditary 1,25-dihydroxyvitamin D-resistant rickets. J. Clin. Endocrinol. Metab. **76**, 509–512 (1993)
6. Z. Hochberg, Vitamin-D-dependent rickets type 2. Horm. Res. **58**, 297–302 (2002)
7. M.R. Haussler, C.A. Haussler, P.W. Jurutka, P.D. Thompson, J.C. Hsieh, L.S. Remus, S.H. Selznick, G.K. Whitfield, The vitamin D hormone and its nuclear receptor: molecular actions and disease states. J. Endocrinol. **154**, S57–S73 (1997)
8. P. Katavetin, P. Katavetin, S. Wacharasindhu, V. Shotelersuk, A girl with a novel splice site mutation in VDR supports the role of a ligand-independent VDR function on hair cycling. Horm. Res. **66**, 273–276 (2006)
9. K. Kristjansson, A.R. Rut, M. Hewison, J.L. O'Riordan, M.R. Hughes, Two mutations in the hormone binding domain of the vitamin D receptor cause tissue resistance to 1,25 dihydroxyvitamin D3. J. Clin. Investig. **92**, 12–16 (1993)
10. R.J. Wiese, H. Goto, J.M. Prahl, S.J. Marx, M. Thomas, A. Al-Aqeel, H.F. De Luca, Vitamin D-dependency rickets type II: truncated vitamin D receptor in three kindreds. Mol. Cell. Endocrinol. **90**, 197–201 (1993)
11. F.J. Cockerill, N.S. Hawa, N. Yousaf, M. Hewison, J.L. O'Riordan, S.M. Farrow, Mutations in the vitamin D receptor gene in three kindreds associated with hereditary vitamin D resistant rickets. J. Clin. Endocrinol. Metab. **82**, 3156–3160 (1997)
12. J.B. Mechica, M.O. Leite, B.B. Mendonca, E.S. Frazzatto, A. Borelli, A.C. Latronico, A novel nonsense mutation in the first zinc finger of the vitamin D receptor causing hereditary 1,25-dihydroxyvitamin D3-resistant rickets. J. Clin. Endocrinol. Metab. **82**, 3892–3894 (1997)
13. W. Zhu, P.J. Malloy, E. Delvin, G. Chabot, D. Feldman, Hereditary 1,25-dihydroxyvitamin D-resistant rickets due to an opal mutation causing premature termination of the vitamin D receptor. J. Bone Miner. Res. **13**, 259–264 (1998)
14. P.J. Malloy, W. Zhu, R. Bouillon, D. Feldman, A novel nonsense mutation in the ligand binding domain of the vitamin D receptor causes hereditary 1,25-dihydroxyvitamin D-resistant rickets. Mol. Genet. Metab. **77**, 314–318 (2002)
15. Y. Zhou, J. Wang, P.J. Malloy, Z. Dolezel, D. Feldman, Compound heterozygous mutations in the vitamin D receptor in a patient with hereditary 1,25-dihydroxyvitamin D-resistant rickets with alopecia. J. Bone Miner. Res. **24**, 643–651 (2009)
16. S. Beer, M. Tieder, D. Kohelet, O.A. Liberman, E. Vure, G. Bar-Joseph, D. Gabizon, Z.U. Borochowitz, M. Varon, D. Modai, Vitamin D resistant rickets with alopecia: a form of end organ resistance to 1,25 dihydroxy vitamin D. Clin. Endocrinol. (Oxf) **14**, 395–402 (1981)
17. S.J. Marx, M.M. Bliziotes, M. Nanes, Analysis of the relation between alopecia and resistance to 1,25-dihydroxyvitamin D. Clin. Endocrinol. (Oxf) **25**, 373–381 (1986)
18. P.J. Malloy, W. Zhu, X.Y. Zhao, G.B. Pehling, D. Feldman, A novel inborn error in the ligand-binding domain of the vitamin D receptor causes hereditary vitamin D-resistant rickets. Mol. Genet. Metab. **73**, 138–148 (2001)
19. J. Miller, K. Djabali, T. Chen, Y. Liu, M. Ioffreda, S. Lyle, A.M. Christiano, M. Holick, G. Cotsarelis, Atrichia caused by mutations in the vitamin D receptor gene is a phenocopy of generalized atrichia caused by mutations in the hairless gene. J. Investig. Dermatol. **117**, 612–617 (2001)
20. R. Bergman, R. Schein-Goldshmid, Z. Hochberg, O. Ben-Izhak, E. Sprecher, The alopecias associated with vitamin D-dependent rickets type IIA and with hairless gene mutations: a comparative clinical, histologic, and immunohistochemical study. Arch. Dermatol. **141**, 343–351 (2005)
21. J.C. Hsieh, J.M. Sisk, P.W. Jurutka, C.A. Haussler, S.A. Slater, M.R. Haussler, C.C. Thompson, Physical and functional interaction between the vitamin D receptor and hairless corepressor, two proteins required for hair cycling. J. Biol. Chem. **278**, 38665–38674 (2003)
22. P.J. Malloy, R. Xu, A. Cattani, L. Reyes, D. Feldman, A unique insertion/substitution in helix H1 of the vitamin D receptor ligand binding domain in a patient with hereditary 1,25-dihydroxyvitamin D-resistant rickets. J. Bone Miner. Res. **19**, 1018–1024 (2004)
23. P.J. Malloy, R. Xu, L. Peng, S. Peleg, A. Al-Ashwal, D. Feldman, Hereditary 1,25-dihydroxyvitamin D resistant rickets due to a mutation causing multiple defects in vitamin D receptor function. Endocrinology **145**, 5106–5114 (2004)
24. G.K. Whitfield, S.H. Selznick, C.A. Haussler, J.C. Hsieh, M.A. Galligan, P.W. Jurutka, P.D. Thompson, S.M. Lee, J.E. Zerwekh, M.R. Haussler, Vitamin D receptors from patients with resistance to 1,25-dihydroxyvitamin D3: point mutations confer reduced transactivation in response to ligand and impaired interaction with the retinoid X receptor heterodimeric partner. Mol. Endocrinol. **10**, 1617–1631 (1996)
25. P.J. Malloy, R. Xu, L. Peng, P.A. Clark, D. Feldman, A novel mutation in helix 12 of the vitamin D receptor impairs coactivator interaction and causes hereditary 1,25-dihydroxyvitamin D-resistant rickets without alopecia. Mol. Endocrinol. **16**, 2538–2546 (2002)
26. N.S. Ma, P.J. Malloy, P. Pitukcheewanont, D. Dreimane, M.E. Geffner, D. Feldman, Hereditary vitamin D resistant rickets: identification of a novel splice site mutation in the vitamin D receptor gene and successful treatment with oral calcium therapy. Bone **45**, 743–746 (2009)
27. P.J. Malloy, D. Feldman, Genetic disorders and defects in vitamin D action. Endocrinol. Metab. Clin. North Am. (2010) **39**, 333–346
28. P.J. Malloy, J. Wang, T. Srivastava, D. Feldman, Hereditary 1,25-dihydroxyvitamin D-resistant rickets with alopecia resulting from a novel missense mutation in the DNA-binding domain of the vitamin D receptor. Mol. Genet. Metab. **99**, 72–79 (2010)
29. Y. Weisman, I. Bab, D. Gazit, Z. Spirer, M. Jaffe, Z. Hochberg, Long-term intracaval calcium infusion therapy in end-organ resistance to 1,25-dihydroxyvitamin D. Am. J. Med. **83**, 984–990 (1987)