

Allelic loss at the vitamin D receptor (VDR) locus in parathyroid tissue from one patient affected by refractory uremic hyperparathyroidism

Alberto Falchetti¹
Alessia Gozzini¹
Laura Masi¹
Valentina Martinetti¹
Sandro Bandini²
Maria Luisa Brandi¹

¹ Department of Internal Medicine, University of Florence, ² Unit of Dialysis, AOUC, Florence, Florence, Italy.

Address for correspondence:
Maria Luisa Brandi, M.D., Ph.D.
Department of Internal Medicine,
University of Florence
Viale G. Pieraccini 6, 50139 Florence, Italy
Ph. +39 055 4296585
Fax +39 055 4296586
E-mail: m.brandi@dmi.unifi.it

Summary

It has been established that secondary hyperparathyroidism (SHPT) represents a long-term complication of chronic renal failure (CRF) associated to diffuse or nodular hyperplasia of parathyroid glands. The molecular mechanisms underlying both hyperactivity and proliferation rate of parathyroid cells in uremic patients have not yet been elucidated. A decreased sensitivity to vitamin D feedback is considered one of the causes accounting for parathyroid hyperplasia in CRF. Thus, we investigated the possible role that VDR gene may play in the development of parathyroid tumors in three female patients exhibiting refractory SHPT. We detected in one parathyroid nodular lesion from one patient a loss of heterozygosity within the VDR locus with acquisition of the haplotype previously described to segregate with lower bone mass values and lower intestinal calcium absorption efficiency in Italian postmenopausal women. These findings suggest that qualitative/quantitative deficiency of VDR physiological activity may have a role in the pathogenesis of secondary hyperparathyroidism in uremic patients.

KEY WORDS: parathyroid tumorigenesis, vitamin D receptor, genetic polymorphisms.

Introduction

As in other human complex disorders, genetic association studies may provide data that could be helpful in precocious detection of subjects at risk to develop parathyroid hormone deregulation in presence, or not, of specific environmental factors. Many recent reports are focusing on the existence of a possible segregation between specific VDR gene polymorphisms and parathyroid proliferative disorders outcome in different ethnical groups (1-3). Such polymorphic sites can be useful also to investigate the role that the VDR gene may play in the pathogenesis of parathyroid outgrowth at tumoral level, unraveling the association between a particular VDR gene haplotype and calci-

um homeostasis disorder. VDR expression has been shown to be negatively correlated with parathyroid gland potential in hemodialysis patients affected by secondary hyperparathyroidism (SHPT). A previous immunohistochemical study in parathyroid glands from uremic patients demonstrated a lower VDR density in parathyroid cells in nodular hyperplasia when compared to the diffuse lesions, suggesting a direct involvement of VDR in the pathogenesis of refractory SHPT (4). More recently, Yano et al. demonstrated a decreased expression of both calcium sensing receptor (CaSR) and VDR in parathyroid glands from patients affected by uremic SHPT (5) suggesting an important role for both the receptor proteins in the increased proliferative activity of parathyroid cells in these individuals. Moreover, association studies investigating intron 8 (*B/b* and *A/a* alleles) and exon 9 (*T/t* alleles) of VDR gene polymorphisms, detected by BsmI, ApaI and TaqI endonucleases respectively, showed a higher prevalence of *baT* haplotype in Swedish patients affected by primary hyperparathyroidism (PHPT) (1-2). These results were not confirmed in a Japanese population study (3), even if an association study on a Spanish postmenopausal women population with PHPT failed to demonstrate a role of BsmI VDR polymorphism in the pathogenesis of parathyroid adenomas (6). However, genetic differences among ethnical groups could account for such discrepancies. The *baT* haplotype could be related to an altered expression of VDR gene with reduction of specific mRNA levels (7). The possible influence of VDR polymorphisms on quantitative/qualitative defects of its own mRNA has not been defined. Several clonality studies demonstrated the existence of monoclonal lesions in parathyroid from uremic patients both by X-chromosome inactivation pattern and loss of heterozygosity (LOH) at several different autosomal loci (8-11), indicating the existence of a high degree of genetic heterogeneity. A report on LOH analysis in parathyroid glands from patients with refractory SHPT seemed to confirm these data with some parathyroid nodules exhibiting LOH at different loci, including loci at 12q12-14, the region harboring the VDR gene (12). Considering all these data we decided to investigate the possible role of the VDR gene in the pathogenesis of refractory SHPT in uremic patients.

Patients and methods

After obtainment of a signed informed consent, approved by the Local Ethical Board, from each participating patient, DNA samples from seven hyperplastic parathyroid glands (2 diffuse and 3 nodular forms) from three female patients with refractory SHPT (3 from patient I, 2 from patient II and 2 from patient III) (age range 53- 56 years) and from matched peripheral blood were analyzed. Selective PCR amplification of a 740 bp and a 265 bp DNA fragments, using a forward primer in intron 8 and a reverse primer in exon 9 for the 740 bp fragment (11-12) and primers on both sides flanking exon 1 of VDR gene (13) for the second fragment, followed by restriction fragment length polymorphism (RFLP) analysis with Apa I, Taq I (intron 8/exon 9) and FokI (exon 1) were performed (14) (Figure 1). The RFLPs were coded as *Aa* (ApaI), *Tt* (TaqI) and *Ff* (FokI), upper case letter indicates the absence and lowercase the presence or the

restriction site. The patients resulted to be heterozygous both in constitutive and parathyroid tissue DNA for all RFLPs analysis (*Aa*, *Tt* and *Ff*), but patient I homozygous for *Apal* site (*AA*). In order to define the size of the lost region we performed also the VDR poly-A microsatellite analysis at 3'UTR (15) (Figure 1). All patients resulted to be informative (data not shown).

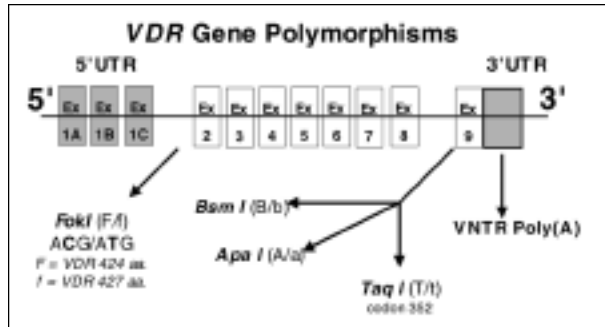


Figure 1 - Genomic organization and described polymorphisms at the VDR gene. Grey boxes represent untranslated regions (UTR) and white boxes (from Ex 2 to Ex 9) represent the coding regions. The specific restriction endonucleases and corresponding detected alleles (within brackets) are given.

Results

Only one parathyroid gland with a nodular pattern of growth from patient III, constitutively genotyped as *Aa/Tt*, exhibited the loss of "T" and "a" allele (Figure 2A and 2B), while both the corresponding FokI (Figure 3) and VDR poly-A polymorphisms

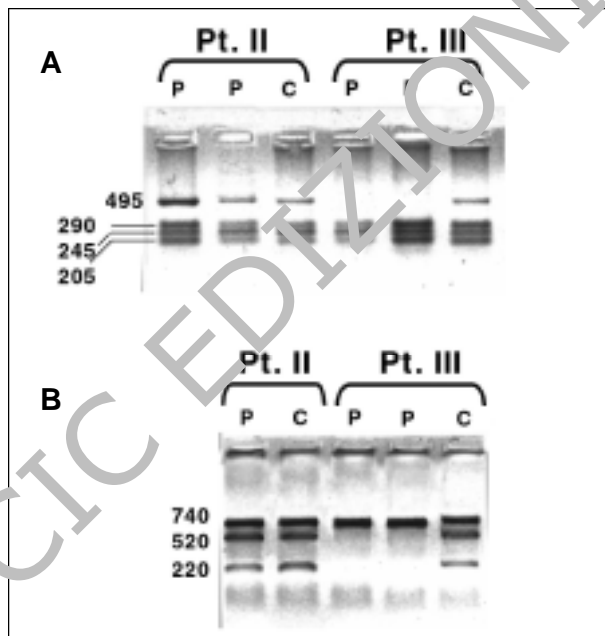


Figure 2 - A) TaqI and B) ApaI restriction analysis of the 740 bp PCR product of VDR gene in parathyroid glands (P) of patient II and III and the matched constitutive DNA (C). Two separate nodular lesions from patient II and one nodular lesion from patient III (duplicated) are here described. The size of detected alleles (bp) is reported on the left of the agarose gel picture. Parathyroid from patient III exhibited loss of "T" allele (495 bp) in A and of "a" allele (520+220 bp) in B.

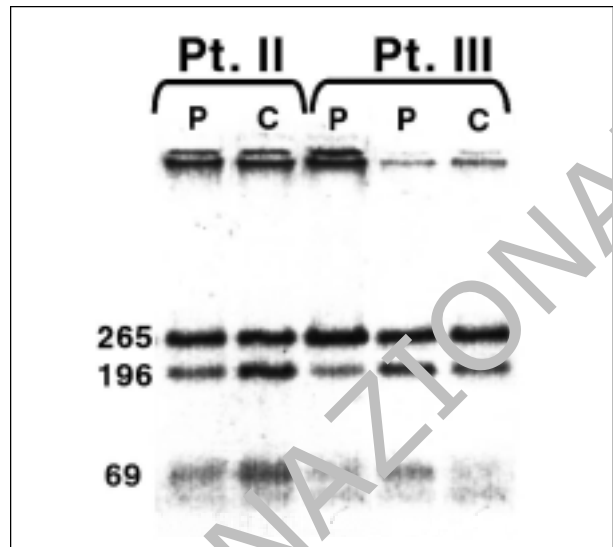


Figure 3 - FokI restriction analysis of the 265 bp PCR product of the same samples described in Figure 2 of patients II and III. All the parathyroid glands retained the DNA.

were informative and the DNA retained. Thus, this gland acquired the "hemizygous" haplotype *Af* with an intragenic deletion included between the FokI site on exon 2 and the VDR poly-A at the 3'UTR.

Discussion

Present result supports the theory of genetic heterogeneity at the molecular basis of SHPT, representing the first evidence of "intra-genic" chromosomal deletion at the VDR locus. A previous report excluded tumor-specific deletion, insertions or point mutations in the VDR gene in 37 parathyroid tumors from uremic patients by multiple independent methods (16). We consider our result to be an occasional, if not unique, molecular event that probably do not represent a frequent promoting mechanism in the tumorigenesis of such parathyroid lesions. In fact, a similar nodular pattern was also exhibited by the glands without VDR intragenic deletion. Thus, we confirm that it is unlikely that the VDR locus may play as a tumor suppressor gene in severe secondary or tertiary hyperparathyroidism tumorigenesis. In a previous association study on an Italian postmenopausal women population, it has been reported the existence of a strong correlation between the homozygous genotype *AA/tt*, lower bone mass values and lower intestinal calcium absorption efficiency, suggesting the existence of a qualitative/quantitative deficiency of VDR physiological activity (17). The acquisition of such defective molecular mechanism in uremic parathyroid glands could also account for the observed refractory behavior, may be due to a decreased sensitivity of parathyroid cells to vitamin D regulation with different pharmacological response to vitamin D treatment and consequent development of a parathyroid clonal expansion. Efforts should be addressed to assess the VDR function in a larger number of uremic parathyroid glands in order to confirm our data.

Acknowledgements

This report was supported by grants from Associazione Italiana per la Ricerca sul Cancro (A.I.R.C. to M.L.B.), Consiglio Nazionale

delle Ricerche (CNR 95.003116.PF.39 and 96.00520.PF.39), and M.U.R.S.T. 40% to M.L.B.

References

1. Carling T, Kindmark A, Hellman P et al. Vitamin D receptor genotypes in primary hyperparathyroidism. *Nat Med.* 1995;1:1309-1311.
2. Carling T, Kindmark A, Hellman P, Holmberg L, Åkerström G, Rastad J. Vitamin D receptor alleles b, a and T: risk factors for sporadic primary hyperparathyroidism (HPT) but not HPT of uremia or MEN 1. *Biochem Biophys Res Commun.* 1997;231:329-332.
3. Nagasaka S, Ishikawa S, Matoba H, Kubota K, Murakami T, Saito T. Vitamin D receptors and hyperparathyroidism. *Nat Med.* 1996;2:834.
4. Fukuda N, Tanaka H, Tominaga Y, Fukagawa K, Seino Y. Decreased 1,25-dihydroxyvitamin D3 receptor density is associated with a more severe form of parathyroid hyperplasia in chronic uremic patients. *J Clin Invest.* 1993;92:1436-1443.
5. Yano S, Sugimoto T, Tsukamoto T, Chihara K, Kobayashi A, Kitazawa S, Maeda S, Kitazawa R. Decrease in vitamin D receptor and calcium-sensing receptor in highly proliferative parathyroid adenomas. *Eur J Endocrinol.* 2003;148(4):403-11.
6. Pacheco D, Menarguez J, Cristobal E, Arribas B, Alcazar JA, Carrion R, Polo JR. BsmI vitamin D receptor polymorphism and pathogenesis of parathyroid adenoma. *Med Sci Monit.* 2000;6(4):658-60.
7. Carling T, Rastad J, Åkerström G, Westin G. Vitamin D receptor (VDR) and parathyroid hormone messenger ribonucleic acid levels correspond to polymorphic VDR alleles in human parathyroid tumors. *J Clin Endocrinol Metab.* 1998;83(7):2255-2259.
8. Arnold A, Brown MF, Urena P et al. Monoclonality of parathyroid tumors in chronic renal failure and in primary parathyroid hyperplasia. *J Clin Invest.* 1995;95:2047-2053.
9. Falchetti A, Bale AE, Amorosi A et al. Progression of uremic hyperparathyroidism involves allelic loss on chromosome 11. *J Clin Endocrinol Metab.* 1993;76:139-144.
10. Tominaga Y, Kohara S, Namii Y et al. Clonal analysis of nodular parathyroid hyperplasia in renal hyperparathyroidism. *World J Surg.* 1996;20:744-750.
11. Farnebo F, Teh BN, Dotzenrath C et al. Differential loss of heterozygosity in familial, sporadic, and uremic hyperparathyroidism. *Hum Genet.* 1997;99:342-349.
12. Chudek J, Ritz E, Kovacs G. Genetic abnormalities of parathyroid nodules of uremic patients. *Clin Cancer Res.* 1997;4:211-214.
13. Morrison NA, Cheng JQI, Akifumi T et al. Prediction of bone density from vitamin D receptor alleles. *Nature* 1994;371:284-287.
14. Gross C, Eccleshall TR, Malloy P et al. The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. *J Bone Miner Res.* 1996; 11:1850-1855.
15. Morrison NA, Tokita A, Cheng JQI et al. Allelic variation in the vitamin D receptor gene: major haplotypes in caucasians. *Bone* 1995;16(S1):105. Abstract #81.
16. Brown SB, Wesley Mike J, Palamisamy N et al. Vitamin D receptor as a candidate tumor suppressor gene in severe hyperparathyroidism of uremia. *Bone* 1998;23(5) Supplement Abstract #T231.
17. Grimaldi L, Beccherini L, Masi L et al. Vitamin D receptor genotypes and intestinal calcium absorption in postmenopausal women. *Calcif Tissue Int.* 1997;61:460-463.