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ORIGINAL PAPER

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Polymorphism of the vitamin D_3 receptor in patients with psoriasis

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Abstract 1,25-Dihydroxyvitamin D is the biologically active form of vitamin D for the treatment of skin eruptions in patients with psoriasis. 1,25-$(OH)_2D_3$ elicits its action on skin eruptions through the vitamin D receptor (VDR). Allelic frequencies of VDR were studied in 86 normal subjects and 50 patients with psoriasis. Genomic DNA was extracted from peripheral blood leukocytes and the VDR gene was amplified using a heminested polymerase chain reaction (PCR). The products were digested with respective restriction enzymes *Apa*I, *Taq*I and *Bsm*I. The restriction fragment length polymorphisms (RFLP) were coded as Aa, Tt or Bb. The frequencies of *Apa*I, *Bsm*I and *Taq*I RFLP genotypes in psoriasis patients showed no significant differences compared with normal controls. The frequency of the AA genotype was significantly higher in pustulosis palmaris et plantaris patients than in psoriasis vulgaris patients ($P<0.05$), and in psoriasis vulgaris patients than in psoriasis pustulosa patients ($P<0.01$). In patients with psoriasis, the levels of serum alanine 2-oxoglutarate aminotransferase (ALT) were significantly higher in patients with the AA genotype (54.0±22.0 IU/l, $n=4$) than in those with the aa genotype (24.0±15.9 IU/l, $n=27$; $P<0.02$). The distribution of *Apa*I, *Bsm*I, *Taq*I RFLP VDR genotypes showed no significant relationship to the PASI score, serum aspartate 2-oxoglutarate aminotransferase or triglyceride levels, or age at onset. These results show that the VDR genotype contributes to the liver dysfunction in patients with psoriasis, although no correlation was found between VDR genotype and the skin eruptions of psoriasis.

Keywords Psoriasis · Vitamin D_3 receptor · Polymorphism

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Introduction

Psoriasis is a common inflammatory and hyperproliferative skin disease of unknown etiology, affecting up to 2% of the population [1]. Vitamin D_3 is an effective drug for the treatment of skin eruptions in patients with psoriasis [2]. However, the skin eruptions show considerable variation in their response to 1,25-$(OH)_2D_3$ [3]. 1,25-$(OH)_2D_3$ elicits its action on the target tissues through the vitamin D receptor (VDR). It has been reported that the VDR gene shows considerable polymorphism. In the present study, we investigated the possible contribution of VDR gene polymorphism to the occurrence of skin eruptions in patients with psoriasis. Possible associations between VDR gene polymorphism and clinicolaboratory findings was also studied. We also investigated the relationship between VDR genotypes and hyperlipidemia and liver dysfunction in patients with psoriasis, because high values of cholesterol, triglycerides (TG) and depressed HDL-cholesterol concentrations [4] and liver cirrhosis [5] have been observed in patients with psoriasis.

Materials and methods

Patients

The patient group comprised 50 psoriasis patients and the control group 86 normal individuals. All the subjects enrolled into this study were Japanese. The psoriasis patients (35 men and 15 women, aged 14–87 years, mean age 51.1 years) comprised 38 with psoriasis vulgaris, 6 with psoriasis pustulosa, 1 with psoriasis arthropathica, 4 with pustulosis palmaris et plantaris (PPP) and 1 with acrodermatitis continua. The psoriasis patients included 24 (49.0%) with early-onset disease (onset not later than 40 years of age). The patients were clinically evaluated using the psoriasis area and severity index (PASI) [6]. The 86 normal controls (36 men and 50 women, aged 8–92 years, mean age 51.0 years) were randomly recruited. Serum aspartate 2-oxoglutarate aminotransferase (AST), serum alanine 2-oxoglutarate aminotransferase (ALT) and serum TG levels were determined in all subjects.

Vitamin D receptor genotyping

Genomic DNA (QIAamp DNA mini kit; Qiagen, Hilden, Germany) was extracted from peripheral blood leukocytes using standard methods [7]. The VDR gene was amplified using a heminested polymerase chain reaction (PCR). For detection of *Apa*I and *Taq*I sites, primer 1 (5′-CAGAGCATGG-ACAGGGAG-CAAG-3′) in intron 8, and primer 2 (5′-GCAACTCCTCATG-GCTGAGGTCTCA-3′) and primer 3 (5′-AGGGG-TTAGGTTG-GACAG-GAGAGAG-3′) in exon 9 were used. For detection of the *Bsm*I site, primer 1 (5′-CAAC-CAAGACTACAAGTACC-GCGTCAGTGA-3′) in exon 7, primer 2 (5′-TGGCGGCAGCG-GATGTA-CGTCTGC-3′) in exon 9, and primer 3 (5′-AAC-CAGCGGGAAGAGGTCAAGGG-3′) in intron 8 were used [8]. The first PCR product was obtained using primers 1 and 2 with genomic DNA. The second PCR product was obtained using primer 1 and 3 with the first PCR product. Each sample was subjected to 35 cycles in a DNA thermal cycler (Perkin Elmer Cetus, Ct.). After organic extraction of the second PCR product, the products were digested with the respective restriction enzymes (New England Biolabs, Beverly, Mass.). The digested samples were size-fractionated by electrophoresis in a 1% agarose gel. Visualization after ethidium bromide staining was performed by means of ultraviolet fluorescence. Gels were photographed using a Funaroid camera FP-6000 (FAC) and analyzed for restriction fragment length polymorphism (RFLP). The RFLP were coded as Aa (*Apa*I), Tt (*Taq*I), or Bb (*Bsm*I), where an uppercase letter signifies the absence of the site and a lowercase letter signifies the presence of the site. DNA analysis was performed blind to the clinical data.

Statistical analysis

Differences in values between groups shown as means±SD were evaluated using Mann-Whitney's *U*-test. The differences in values between groups shown as occurrence rates were evaluated by chi-squared analysis.

Results

The distribution of *Apa*I, *Taq*I and *Bsm*I VDR genotypes in normal controls and patients with psoriasis

The first PCR products of 740 bp and 1850 bp were obtained using primers 1 and 2 for the *Apa*I and *Taq*I sites, and the *Bsm*I site, respectively. Then, 689 bp and 825 bp second PCR products were obtained using primers 1 and 3, respectively. Figure 1 shows the results of restriction enzyme digestion. Lanes 2–4 show the results of digestion with restriction enzyme *Apa*I. Lane 2 represents the 689 bp fragment, lane 3 represents the 689 bp, 478 bp and 211 bp fragments, and lane 4 represents the 478 bp and 211 bp fragments bands, respectively. Lanes 2–4 represent AA, Aa and aa genotypes, respectively. Lanes 5–7 show the results of digestion with restriction enzyme *Bsm*I. Lanes 5–7 represent BB, Bb and bb genotypes, respectively. Lanes 8 and 9 show the results of digestion with restriction enzyme *Taq*I. Lanes 8 and 9 represent TT and Tt genotypes, respectively.

The distributions of VDR genotypes after restriction enzyme digestion of the second PCR product were studied in normal controls and psoriasis patients (Table 1). In the normal controls, the frequencies of the AA, BB and TT genotypes were 10.5%, 4.7% and 83.7%, respectively. In the psoriasis patients, the frequencies of the AA, BB and TT genotypes were 8.0%, 6.0% and 78.0%, respectively. The distribution of *Apa*I, *Bsm*I and *Taq*I RFLP genotypes in the psoriasis patients showed no significant differences compared with the normal controls.

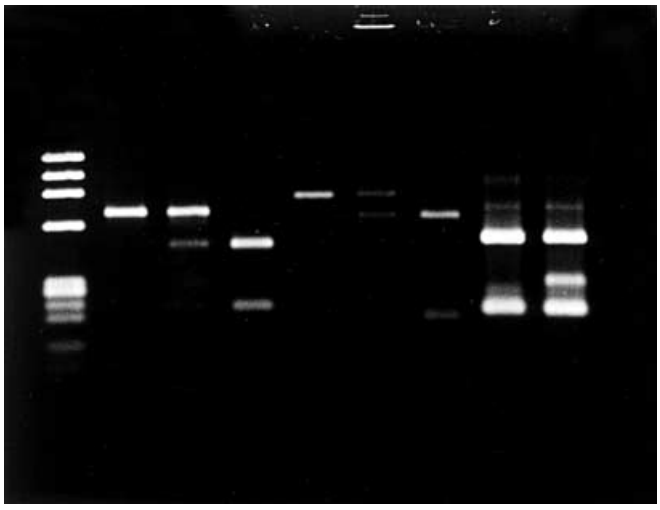

Fig. 1 Results of restriction enzyme digestion. *Lane 1* molecular weight size marker. *Lanes 2–4* restriction *Apa*I enzyme digestion: *lane 2* 689 bp fragment; *lane 3* 689 bp, 478 bp and 211 bp fragments; *lane 4* 478 bp and 211 bp fragments, respectively. *Lanes 2–4* represent AA, Aa and aa genotypes, respectively. *Lane 5–7* restriction enzyme *Bsm*I digestion. *Lanes 8 and 9* restriction enzyme *Taq*I digestion

Table 1 Distribution *Apa*I, *Bsm*I and *Taq*I RFLP VDR genotypes. There were no significant differences between the normal controls and psoriasis patients

Genotype	Normal controls (*n*=86)	Psoriasis patients (*n*=50)
AA	9 (10.5%)	4 (8.0%)
Aa	41 (47.7%)	19 (38.0%)
aa	36 (41.9%)	27 (54.0%)
BB	4 (4.7%)	3 (6.0%)
Bb	12 (14.0%)	7 (14.0%)
bb	70 (81.4%)	40 (80.0%)
TT	72 (83.7%)	39 (78.0%)
Tt	14 (16.3%)	11 (22.0%)
tt	0.0 (0%)	0.0 (0%)

The relationship between *Apa*I VDR genotypes and clinicolaboratory findings in psoriasis patients

The distributions of VDR genotypes were studied in relation to gender, and serum AST, ALT and TG levels in normal controls and psoriasis patients (Table 2). No significant differences were found in the distributions between normal controls and psoriasis patients.

In psoriasis patients, the degree of correlation between the distribution of VDR genotypes and the PASI score, and serum AST, ALT and TG levels, and age at onset was

Table 2 Laboratory findings in normal controls and psoriasis patients (means±SD). There were no significant differences between the normal controls and psoriasis patients

	Aspartate 2-oxo-glutarate amino-transferase (IU/l)	Alanine 2-oxo-glutarate amino-transferase (IU/l)	Triglyceride (mg/dl)
Normal controls	27.5±30.7	28.0±37.4	134.4±73.2
Psoriasis patients	28.6±27.1	28.3±24.1	155.1±94.0

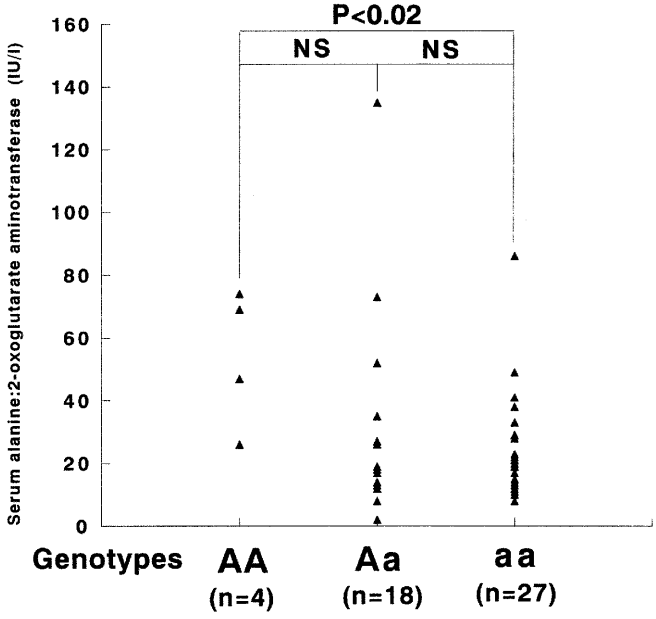

Fig. 2 Serum ALT levels associated with the AA, Aa and aa genotypes. The mean±SD levels were 54.0±22.0 IU/l (n=4), 29.1±31.3 IU/l (n=18) and 24.0±15.9 IU/l (n=27), respectively. The ALT levels associated with the AA genotype were significantly higher than those associated with the aa genotype (P<0.02)

determined. The serum ALT levels associated with the AA, Aa, and aa genotypes were 54.0±22.0 IU/l (n=4), 29.1±31.3 IU/l (n=18) and 24.0±15.9 IU/l (n=27), respectively. The serum ALT levels associated with the AA genotype were significantly elevated compared with those associated with the aa genotype (P<0.02, Fig. 2). No correlation was found between the distribution of the *Apa*I VDR genotypes and the PASI score, serum AST and TG levels or age at onset. No significant differences were found among the *Bsm*I or *Taq*I RFLP VDR genotypes in relation to the PASI score, serum AST and TG levels or age at onset in psoriasis patients. In normal controls, the degree of correlation between the distribution of the VDR genotypes and serum AST, ALT and TG levels was determined. No significant differences were found among the *Apa*I, *Bsm*I, *Taq*I RFLP VDR genotypes in relation to serum AST, ALT or TG levels in normal controls.

Table 3 Correlation between *Apa*I RFLP VDR genotypes and type of psoriasis

Type of psoriasis	*Apa*I VDR genotype		
	AA	Aa	aa
Psoriasis vulgaris	2	16	20
Psoriasis pustulosa	0**	3	3
Pustulosis palmaris et plantaris	2*	0	2
Psoriasis arthropathica	0	0	1
Acrodermatitis continua	0	0	1

*P<0.05, pustulosis palmaris et plantaris vs psoriasis vulgaris; **P<0.01, psoriasis pustulosa vs psoriasis vulgaris

Correlation between *Apa*I, *Bsm*I, *Taq*I RFLP VDR genotypes and types of psoriasis

The frequency of the AA genotype in PPP patients was significantly higher than in psoriasis vulgaris patients (P<0.05). The frequency of the AA genotype in psoriasis vulgaris patients was significantly higher than in psoriasis pustulosa patients (P<0.01, Table 3). No significant differences were found among the others.

Discussion

ALT levels in psoriasis patients with the AA genotype were significantly elevated compared with the levels in those with the aa genotype, and the frequency of the AA genotype was significantly higher in PPP patients than in psoriasis vulgaris patients, and significantly higher in psoriasis vulgaris patients than in psoriasis pustulosa patients. However, we found no significant differences between psoriasis patients and normal controls in terms of the frequency of *Apa*I, *Bsm*I, *Taq*I RFLP VDR genotypes, or serum AST, ALT and TG levels. We found no relationships between *Apa*I, *Bsm*I, *Taq*I RFLP VDR genotypes and age at onset, PASI score, or serum AST and TG levels.

Park et al. have reported a significantly higher frequency of the A allele by *Apa*I RFLP in psoriasis patients than in control subjects, and the tendency is more accentuated in early-onset psoriasis [9]. They also reported a significant association between VDR genotype and mean age at onset. However, Kontula et al. found no significant difference in VDR allele frequency between calcipotriol responders and nonresponders [10]. Whitfield et al. studied *Fok*I restriction site (F/f) in exon II and a singlet repeat in exon IX (L/S) [11]. They found that the activity of 1,25-$(OH)_2D_3$ in the FF, LL VDR genotypes. Our results showed no VDR genotype differences between normal controls and psoriasis patients. These results may indicate that abnormalities in exon IX are rare in Japanese psoriasis patients.

The observation that both vitamin D_3 and 1,25-$(OH)_2D_3$ significantly promote normal liver recovery after partial HX illustrates the role of the vitamin D endocrine system

in normal cell physiology in vivo [12]. Hepatocytes from vitamin D-deficient animals have been reported to contain primarily lipid [12]. These studies indicate that elevated ALT levels in patients with VDR AA allele psoriasis are caused by inhibition of hepatocyte regeneration, and lipids are increased in the hepatocytes because vitamin D_3 dose not act in the regeneration of hepatocytes.

Psoriasis is a disease characterized by inflammation and an increased turnover of the epidermis [1]. 1,25-$(OH)_2D_3$ suppresses the proliferation of keratinocytes in culture [13]. VDR has been shown to be upregulated by 1,25-$(OH)_2D_3$ in human keratinocytes [14]. At picomolar concentrations, 1,25-$(OH)_2D_3$ inhibits the growth-promoting lymphokine interleukin-2, which has been shown to be produced by human T lymphocytes activated in vitro by the mitogen phytohemagglutinin [15]. Correlations between VDR polymorphisms and osteoporosis [6], lupus nephritis [16], insulin-dependent diabetes mellitus (IDDM) [17], Crohn's disease [18] and malignancies have been reported. The proliferation of mitogen-activated lymphocytes is also inhibited by 1,25-$(OH)_2D_3$. These studies indicate that abnormalities in the VDR contribute to the suppression of inhibition of keratinocyte proliferation and inflammation. The results of the present study showed no relationship between psoriasis and the *Apa*I, *Bsm*I, *Taq*I RFLP VDR genotypes, although other abnormalities of the VDR genotypes cannot be dismissed. The abnormalities may contribute to the occurrence and progression of psoriasis.

References

1. Krueger GG, Bergstresser PR, Lowe NJ, Voorhees JJ, Weinstein GD (1984) Psoriasis. J Am Acad Dermatol 11:937–947
2. van de Kerkhof PCM (1995) Biological activity of vitamin D analogues in the skin, with special reference to antipsoriatic mechanisms. Br J Dermatol 132:675–682
3. Kragballe K, Gjertsen BT, De Hoop D, Karlsmark T, van de Kerkhof PC, Larko O, Nieboer C, Roed-Petersen J, Strand A, Tikjob G (1991) Double-blind, right/left comparison of calcipotriol and betamethasone valerate in treatment of psoriasis vulgaris. Lancet 337:193–196
4. Ena P, Madeddu P, Glorioso N, Cerimele D, Rappelli A (1985) High prevalence of cardiovascular diseases and enhanced activity of the renin-angiotensin system in psoriatic patients. Acta Cardiol 40:199–205
5. Stern RS, Lange R, and Members of the Photochemotherapy Follow-Up Study (1988) Cardiovascular disease, cancer, and cause of death in patients with psoriasis: 10 years prospective experience in a cohort of 1,380 patients. J Invest Dermatol 91: 197–201
6. Fredriksson T, Pettersson U (1978) Severe psoriasis – oral therapy with a new retinoid. Dermatologica 157:238–244
7. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York
8. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA (1994) Prediction of bone density from vitamin D receptor alleles. Nature 367:284–287
9. Park B-S, Park J-S, Lee D-Y, Youn J-I, Kim I-G (1999) Vitamin D receptor polymorphism is associated with psoriasis. J Invest Dermatol 112:113–116
10. Kontula K, Valimaki S, Kainulainen K, Viitanen A-M, Keski-Oja J (1997) Vitamin D receptor polymorphism and treatment of psoriasis with calcipotriol. Br J Dermatol 136:977–978
11. Whitfield GK, Remus LS, Jurutka PW, Zitzer H, Oza AK, Dang HT, Haussler CA, Galligan MA, Thatcher ML, Dominguez CE, Haussler MR (2001) Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Mol Cell Endocrinol 177:145–159
12. Ethier C, Kestekian R, Beaulieu C, Dube C, Havrankova J, Gascon-Barre M (1990) Vitamin D depletion retards the normal regeneration process after partial hepatectomy in the rat. Endocrinology 126:2947–2959
13. Holick MF, Chen ML, Kong XF, Sanan DK (1996) Clinical uses for calciotropic hormones 1,25-dihydroxyvitamin D_3 and parathyroid hormone-related peptide in dermatology: a new perspective. J Invest Dermatol 1:1–9
14. Reichrath J, Muller SM, Kerber A, Baum H-P, Bahmer FA (1997) Biologic effects of topical calcipotriol (MC 903) treatment in psoriatic skin. J Am Acad Dermatol 36:19–28
15. Tsoukas CD, Provvedini DM, Manolagas SC (1984) 1,25-Dihydroxy vitamin D_3: a novel immunoregulatory hormone. Science 224:1438–1440
16. Ozaki Y, Nomura S, Nagahama M, Yoshimura C, Kagawa H, Fukuhara S (2000) Vitamin-D receptor genotype and renal disorder in Japanese patients with systemic lupus erythematosus. Nephron 85:86–91
17. McDermott MF, Ramachandran A, Ogunkolade BW, Aganna E, Curtis D, Boucher BJ, Snehalatha C, Hitman GA (1997) Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. Diabetologia 40:971–975
18. Simmons JD, Mullighan C, Welsh KI, Jewell DP (2000) Vitamin D receptor gene polymorphism: association with Crohn's disease susceptibility. Gut 47:211–214