Vitamin D receptor Polymorphism is Associated with Psoriasis

Byung-Soon Park, Jeong-Soo Park,* Dong-Youn Lee, Jai-Il Youn, and In-Gyu Kim*

Departments of Dermatology and *Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul, Korea

Vitamin D receptor is a trans-acting transcriptional factor that mediates 1α,25-dihydroxyvitamin D3 action in the regulation of target gene expression. Recent studies have shown that clinical response of psoriasis to 1α,25-dihydroxyvitamin D3 is correlated with the vitamin D receptor mRNA expression level, which may be influenced by the genotype of the vitamin D receptor. In this study, we have explored a possible association between psoriasis and the polymorphism in the gene encoding the vitamin D receptor. We examined the allelic frequencies of the vitamin D receptor in psoriasis patients (n = 104) and in healthy controls (n = 104) by analyzing the restriction pattern of the polymerase chain reaction products. A significant increase in the frequency of the A allele (absence of the restriction site at intron 8) by Apal restriction fragment length polymorphism was observed in psoriasis patients compared with that of the control group, and the tendency was more accentuated in early onset psoriasis. Odds ratios (95% confidence interval) for psoriasis of AA and Aa genotypes were 5.0 (1.3–19.1) and 2.4 (1.3–4.3), and odds ratios for early onset of AA and Aa genotypes were 6.4 (1.6–25.0) and 3.1 (1.7–5.9), respectively. Allele frequencies for A and a alleles were 0.317 and 0.683 in the psoriasis group and 0.168 and 0.832 in the control group (p = 0.001). A significant association between vitamin D receptor genotypes and the mean age at onset was observed (p < 0.05). Our findings suggest that allelic variance in the vitamin D receptor gene itself or other genes in linkage disequilibrium with this gene, could predispose to the development of psoriasis. J Invest Dermatol 112:113–116, 1999

Psoriasis is a common and persistent papulosquamous disease of unknown etiology, which affects up to 2% of the population (Krueger et al., 1984). It is characterized by hyperproliferation of the keratinocytes and inflammation. The therapeutic efficacy of 1α,25-dihydroxyvitamin D3 [1,25(OH)2D3] and its analogs has been tested and proved to be effective for the treatment of psoriasis (Kraghalle et al., 1991). 1,25(OH)2D3 is the endogenously produced, hormonally active form of vitamin D3. In addition to the known effect of 1,25(OH)2D3 on controlling calcium and bone metabolism (Reichel et al., 1989), it inhibits proliferation and induces terminal differentiation of cultured human keratinocytes (Smith et al., 1986), and can also modulate the immune system in a variety of ways. 1,25(OH)2D3 elicits its action on target tissues through the vitamin D receptor (VDR). The VDR is a member of the steroid/thyroid hormone receptor superfamily, which is a group of ligand-dependent transcription factors. The receptor-hormone complex binds to hormone response elements in regulatory regions of target genes, and modulates the gene transcription. It has been noted, however, that cultured fibroblasts and keratinocytes from some psoriatic patients have partial resistance to 1,25(OH)2D3 mediated anti-proliferative activity (MacLaughlin et al., 1985; Smith et al., 1988). Furthermore, clinical response to 1,25(OH)2D3 treatment is variable in patients with psoriasis. It has been shown that the intracellular level of VDR protein correlates with the cellular response to 1,25(OH)2D3 in cultured human colon cancer cell line (Zhao and Feldman, 1993), and altered induction of VDR mRNA in the treated psoriatic plaques is a marker for clinical responsiveness to 1,25(OH)2D3 treatment (Chen et al., 1996).

Recently, it has been reported that allelic variations of the VDR gene are associated with the risk of developing prostate cancer in men and osteoporosis in post-menopausal women (Morrison et al., 1994; Taylor et al., 1996). The polymorphism of the VDR gene can predict the differences in bone density, accounting for up to 75% of the total genetic effect on bone density in healthy individuals (Morrison et al., 1994). Although it was suggested that cDNA differences in the 3’ untranslated region of each VDR genotype may alter VDR mRNA levels (Morrison et al., 1994), the molecular mechanisms by which bone density is regulated by the VDR gene are not fully understood. The 3’ untranslated region polymorphisms are in strong linkage disequilibrium with restriction fragment length polymorphisms (RFLP) located in intron 8 (BsmI and Apal) and exon 9 (TaqI). Conflicting results have ensued concerning the association of the BsmI RFLP in the VDR gene and bone density (Eisman, 1995). Despite the controversy, the physiologic parameters that are regulated by 1,25(OH)2D3, such as serum osteocalcin level and calcium absorption, are found to be correlated with VDR genotype (Morrison et al., 1992; Dawson-Hughes et al., 1995). These results suggest that genetic polymorphism of the VDR gene may influence 1,25(OH)2D3 mediated normal physiologic response of keratinocytes and can explain the variable responsiveness.

Although an association between VDR genotype and clinical response to 1,25(OH)2D3 or its analogs has been tested and proved to be effective for the treatment of psoriasis (Kraghalle et al., 1991). 1,25(OH)2D3 is the endogenously produced, hormonally active form of vitamin D3. In addition to the known effect of 1,25(OH)2D3 on controlling calcium and bone metabolism (Reichel et al., 1989), it inhibits proliferation and induces terminal differentiation of cultured human keratinocytes (Smith et al., 1986), and can also modulate the immune system in a variety of ways. 1,25(OH)2D3 elicits its action on target tissues through the vitamin D receptor (VDR). The VDR is a member of the steroid/thyroid hormone receptor superfamily, which is a group of ligand-dependent transcription factors. The receptor-hormone complex binds to hormone response elements in regulatory regions of target genes, and modulates the gene transcription. It has been noted, however, that cultured fibroblasts and keratinocytes from some psoriatic patients have partial resistance to 1,25(OH)2D3 mediated anti-proliferative activity (MacLaughlin et al., 1985; Smith et al., 1988). Furthermore, clinical response to 1,25(OH)2D3 treatment is variable in patients with psoriasis. It has been shown that the intracellular level of VDR protein correlates with the cellular response to 1,25(OH)2D3 in cultured human colon cancer cell line (Zhao and Feldman, 1993), and altered induction of VDR mRNA in the treated psoriatic plaques is a marker for clinical responsiveness to 1,25(OH)2D3 treatment (Chen et al., 1996).

Recently, it has been reported that allelic variations of the VDR gene are associated with the risk of developing prostate cancer in men and osteoporosis in post-menopausal women (Morrison et al., 1994; Taylor et al., 1996). The polymorphism of the VDR gene can predict the differences in bone density, accounting for up to 75% of the total genetic effect on bone density in healthy individuals (Morrison et al., 1994). Although it was suggested that cDNA differences in the 3’ untranslated region of each VDR genotype may alter VDR mRNA levels (Morrison et al., 1994), the molecular mechanisms by which bone density is regulated by the VDR gene are not fully understood. The 3’ untranslated region polymorphisms are in strong linkage disequilibrium with restriction fragment length polymorphisms (RFLP) located in intron 8 (BsmI and Apal) and exon 9 (TaqI). Conflicting results have ensued concerning the association of the BsmI RFLP in the VDR gene and bone density (Eisman, 1995). Despite the controversy, the physiologic parameters that are regulated by 1,25(OH)2D3, such as serum osteocalcin level and calcium absorption, are found to be correlated with VDR genotype (Morrison et al., 1992; Dawson-Hughes et al., 1995). These results suggest that genetic polymorphism of the VDR gene may influence 1,25(OH)2D3 mediated normal physiologic response of keratinocytes and can explain the variable responsiveness.

Although an association between VDR genotype and clinical response to 1,25(OH)2D3 or its analogs has been tested and proved to be effective for the treatment of psoriasis (Kraghalle et al., 1991). 1,25(OH)2D3 is the endogenously produced, hormonally active form of vitamin D3. In addition to the known effect of 1,25(OH)2D3 on controlling calcium and bone metabolism (Reichel et al., 1989), it inhibits proliferation and induces terminal differentiation of cultured human keratinocytes (Smith et al., 1986), and can also modulate the immune system in a variety of ways. 1,25(OH)2D3 elicits its action on target tissues through the vitamin D receptor (VDR). The VDR is a member of the steroid/thyroid hormone receptor superfamily, which is a group of ligand-dependent transcription factors. The receptor-hormone complex binds to hormone response elements in regulatory regions of target genes, and modulates the gene transcription. It has been noted, however, that cultured fibroblasts and keratinocytes from some psoriatic patients have partial resistance to 1,25(OH)2D3 mediated anti-proliferative activity (MacLaughlin et al., 1985; Smith et al., 1988). Furthermore, clinical response to 1,25(OH)2D3 treatment is variable in patients with psoriasis. It has been shown that the intracellular level of VDR protein correlates with the cellular response to 1,25(OH)2D3 in cultured human colon cancer cell line (Zhao and Feldman, 1993), and altered induction of VDR mRNA in the treated psoriatic plaques is a marker for clinical responsiveness to 1,25(OH)2D3 treatment (Chen et al., 1996).

Recently, it has been reported that allelic variations of the VDR gene are associated with the risk of developing prostate cancer in men and osteoporosis in post-menopausal women (Morrison et al., 1994; Taylor et al., 1996). The polymorphism of the VDR gene can predict the differences in bone density, accounting for up to 75% of the total genetic effect on bone density in healthy individuals (Morrison et al., 1994). Although it was suggested that cDNA differences in the 3’ untranslated region of each VDR genotype may alter VDR mRNA levels (Morrison et al., 1994), the molecular mechanisms by which bone density is regulated by the VDR gene are not fully understood. The 3’ untranslated region polymorphisms are in strong linkage disequilibrium with restriction fragment length polymorphisms (RFLP) located in intron 8 (BsmI and Apal) and exon 9 (TaqI). Conflicting results have ensued concerning the association of the BsmI RFLP in the VDR gene and bone density (Eisman, 1995). Despite the controversy, the physiologic parameters that are regulated by 1,25(OH)2D3, such as serum osteocalcin level and calcium absorption, are found to be correlated with VDR genotype (Morrison et al., 1992; Dawson-Hughes et al., 1995). These results suggest that genetic polymorphism of the VDR gene may influence 1,25(OH)2D3 mediated normal physiologic response of keratinocytes and can explain the variable responsiveness.
et al, 1996; Kontula et al, 1997), the variable inducibility or stability of VDR mRNA by 1,25(OH)2D3 may reflect the heterogeneity in VDR genotype. Thus, the complexity of VDR genotype and variable responsiveness to 1,25(OH)2D3 treatment provide a basis to test whether VDR gene may be one of the susceptibility genes for psoriasis. Recent studies have found a strong genetic background for psoriasis (Elder et al, 1994a). Although genome-wide searches for linked genes in affected families have come up with several chromosome regions cosegregating with the disease, the strongest association has been established for genes of the major histocompatibility complex or other related genes (Hoehler et al, 1997). Psoriasis shows a mendelian or multifactorial pattern of inheritance, the latter term distinguished by the additional involvement of environmental factors. The high heritability of the disease and studies indicating that susceptibility alleles can be inherited from parents with no personal or family history of psoriasis, lends support to the contention that one or more additional genes, not necessarily linked to the HLA locus, is a determinant of psoriasis susceptibility (Elder et al, 1994a; Henseler, 1997).

In this study, we report that the VDR polymorphism is associated with psoriasis by comparing the allele frequencies of VDR genotypes in psoriasis patients with those of normal healthy controls.

MATERIALS AND METHODS

Patients One hundred and four unrelated psoriasis patients, 52 men and 52 women aged 8–73 y (mean age, 37.1 ± 15.3 y), were recruited randomly from the Department of Dermatology, Seoul National University Hospital, for this study. All patients had psoriasis vulgaris with a duration of 0.1–41 y (mean duration, 12.2 ± 9.7 y). The age at onset of psoriasis ranged from 5 to 71 y old (mean age at onset, 24.9 ± 14.6 y). The patient group included 86 patients with early onset (onset not later than at the age of 40 y). All the patients were clinically evaluated concerning their family history of psoriasis, nail involvement, psoriatic arthropathy, and psoriasis area and severity index score. The normal control population consisted of 104 unrelated, healthy persons. All control subjects and psoriasis patients enrolled in this study had ethnic Korean background.

VDR genotyping The genomic DNA was extracted from leukocytes using standard methods (Sambrook et al, 1989). The VDR gene was amplified by using hemi-nested polymerase chain reaction (PCR). For detection of Apal and TaqI sites, primer 1 (5'-CAGAGGACTGGA-\textsubscript{CATGGGAGAC}-3') in intron 8, and primer 2 (5'-GCAACTCCTCATGCGGTAGGTCCTCA-3') and primer 3 (5'-AAGGGTTAGGTTGGAACGGAGAGAGGACG-3') in exon 9 were used. For detection of BsmI site, primer 1 (5'-CACCAAGACTCAAGTACCGCGTCAGTGA-3') and primer 2 (5'-CAACCAAGACTCAAGTACCGCGTCAGTGA-3') in exon 9 were used. In addition, this tendency was more accentuated in the psoriasis patients with early onset. Chi square test was performed in the analysis of associations between other clinical variables and VDR genotypes, in the analysis of sexual difference in VDR genotypes. The p value less than 0.05 was regarded as statistically significant.

RESULTS

Psoriasis patients showed significantly different Apal RFLP VDR genotypes and allele frequencies 740 bp and 1850 bp first PCR product were obtained using primers 1 and 2 for Apal and TaqI sites, and for the BsmI site, respectively. Then, 689 bp and 825 bp second PCR products were obtained using primers 1 and 3, respectively. In each subject, the VDR genotypes were identified after restriction enzyme digestion of the second PCR product. In the psoriasis group, the frequencies for the BB, Bb, and bb genotypes were 1.8%, 5.5%, and 92.7%, respectively, and the frequencies for the TT, Tt, and tt genotypes were 94.5%, 5.5%, and 0.0%, respectively. Compared with the control group, no significant differences were observed for BsmI and TaqI RFLP genotype frequencies.

In contrast, the significant difference in frequencies for Apal RFLP genotype was observed between the psoriasis group and the control group. Digestion of the PCR segment with Apal resulted in three genotypes: in the AA type only the 689 bp band was present; the Aa type included 689, 478, and 211 bp bands; and the aa type included 478 and 211 bp bands (Fig 1). Frequencies for AA, Aa, and aa genotypes were 10 (9.6%), 46 (44.2%), and 48 (46.2%) in the psoriasis group, and three (2.9%), 29 (27.9%), and 72 (69.2%) in the control group, respectively. We found a significant increase in subjects carrying AA and Aa genotypes in psoriasis patients (Table I). In addition, this tendency was more accentuated in the psoriasis patients with early onset. Odds ratios (95% confidence interval) were calculated to test whether VDR genotypes were associated with the outcome of psoriasis with early onset as compared with those of normal healthy controls. The p value less than 0.05 was regarded as statistically significant.

### Table I. Distribution of the VDR Apal genotypes among psoriasis patients and controls, and odds ratios for psoriasis

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (n = 104)</th>
<th>All psoriasis (n = 104)</th>
<th>Early onset (n = 86)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>3 (2.9%)</td>
<td>10 (9.6%)</td>
<td>9 (10.5%)</td>
</tr>
<tr>
<td>Aa</td>
<td>26 (27.9%)</td>
<td>46 (44.2%)</td>
<td>43 (50.0%)</td>
</tr>
<tr>
<td>aa</td>
<td>72 (69.2%)</td>
<td>48 (46.2%)</td>
<td>34 (39.5%)</td>
</tr>
<tr>
<td>Odds ratio [95% CI]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA/aa</td>
<td>1.0</td>
<td>5.0 [1.3–19.1]</td>
<td>6.4 [1.6–25.0]</td>
</tr>
<tr>
<td>Aa/aa</td>
<td>1.0</td>
<td>2.4 [1.3–4.3]</td>
<td>3.1 [1.7–5.9]</td>
</tr>
</tbody>
</table>

a, number of investigated subjects. cb, confidence interval.

Onset not later than at the age of 40 y.
controls (n = 5).

Early onset (n = 104) 0.354 0.832 1.0

Apa I RFLP of the VDR gene. The distributions of age at onset in individuals with aa (n = 48), Aa (n = 46), and AA (n = 10) genotypes in psoriasis patients. Solid vertical bars indicate mean ± SD for each group (\( p = 0.05 \), \( **p < 0.01 \)).

Table II. Allele frequencies in controls and psoriasis patients

<table>
<thead>
<tr>
<th>Allele frequency</th>
<th>Odds ratio</th>
<th>[95% CI]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All psoriasis (n = 104)(^a)</td>
<td>0.317</td>
<td>0.683</td>
<td>2.3 [1.4–3.7]</td>
</tr>
<tr>
<td>Early onset (n = 86)(^b)</td>
<td>0.354</td>
<td>0.645</td>
<td>2.7 [1.7–4.4] &lt; 0.001</td>
</tr>
<tr>
<td>Controls (n = 104)</td>
<td>0.168</td>
<td>0.832</td>
<td>1.0</td>
</tr>
</tbody>
</table>

\(^a\) Number of investigated subjects.

\(^b\) CI, confidence interval.

No sexual difference in VDR genotype We have evaluated whether there is any sexual difference in the VDR genotypes. The frequencies for AA, Aa, and aa genotypes were seven (13.5%), 21 (40.4%), and 24 (46.1%) in the 52 male psoriasis patients, and three (5.8%), 25 (48.1%), and 24 (46.1%) in the 52 female psoriasis patients, respectively. There was no significant difference in VDR genotypes between male and female psoriasis patients.

Significant correlation between age at onset and VDR genotypes Means of age at onset, defined by the VDR polymorphism in the psoriasis patients, were compared among the three groups. Mean age at onset was 29.3 ± 17.2 (n = 48), 21.5 ± 11.1 (n = 46), and 19.1 ± 10.2 (n = 10) years old for homozygotes aa, heterozygotes Aa, and homozygotes AA, respectively, showing a significant relationship between VDR polymorphism and age at onset of psoriasis (p < 0.05) (Fig 2); however, other clinical variables such as family history of psoriasis, nail involvement, psoriatic arthropathy, and psoriasis area and severity index score, did not show any relationship with the VDR polymorphism (data not shown).

**DISCUSSION**

This study shows that there is a significant difference in allele frequencies and VDR genotypes between normal controls and psoriasis patients, especially with early onset. This suggests that allelic variance in VDR or genes in linkage disequilibrium with the VDR gene, may be a risk factor for development of psoriasis. Epidemiologic and clinical studies have shown that the peak age of onset for psoriasis is bimodally distributed. Early onset psoriasis is associated with a more severe and recurrent course, and increased inheritability in Caucasians (Henseler and Christophers, 1985). This tendency also applies to Koreans (manuscript in preparation). In this study, the more increased odds ratio of having AA or Aa alleles in the early onset group, and the significant difference in the mean age at onset among the three VDR genotype groups, underlines the significance of VDR polymorphism. Dominant genetic effect of carrying allele A was suggested by the excess of heterozygotes (Thomson and Bodmer, 1977). Kontula et al. (1997) have previously shown that there is no difference in allelic variation of the Bsm I site in intron 8 of the VDR gene between psoriasis patients and controls. The apparent discrepancy between their results and ours may be explained by the fact that the size of the population was smaller, and that vitamin D responsiveness, rather than presence or absence of disease, was investigated in that study. Also, a different restriction site, e.g., Bsm I, was examined in that study. Allelic variances in interleukin-1 receptor antagonist gene and tumor necrosis factor-\( \alpha \) gene, as well as numerous HLA loci, were reported to be associated with psoriasis (Hoehler et al., 1997; Tarlow et al., 1997). Based upon the current clinical and genetic knowledge, psoriasis patients may show heterogeneous genetic make up (Elder et al., 1994; Ortonne, 1996). Lack of absolute difference in VDR genotypes between psoriasis and normal controls further supports the heterogeneity of cause. There may be several other factors contributing to these relative differences: e.g., interaction of the VDR gene alleles with the environment or with other genes. Linkage disequilibrium to a nearby gene could also explain the lack of absolute difference between cases and controls.

It is unknown whether psoriasis is related with an intrinsic abnormality of the vitamin D \( _3 \) signaling pathway, and vitamin D \( _3 \) analogs improve psoriasis by overcoming an intrinsic abnormality of the vitamin D \( _3 \) signaling pathway in psoriatic skin (Kragballe, 1997). As the VDR mRNA levels may be influenced by the allelic variance of VDR (Morrison et al., 1994), the allelic differences between psoriasis and normal controls in this study may suggest the differences in the VDR mRNA levels; however, the VDR mRNA and protein levels were found to be similar between normal skin and involved and uninvolved psoriatic skin (Sflysten et al., 1996). These results indicate that the quantity of the VDR mRNA or protein may not be the only explanation for variable responsiveness to 1,25(OH)\(_2\)D\(_3\) or the role of the VDR gene in the pathogenesis of psoriasis. The other possible explanations could be obtained from the studies of functional difference of VDR in relation to VDR genotype. These include interaction with other receptors and affinity for the target gene(s) that especially modulate the immune function.

The observed associations, which are not quite as strong, however, suggest another possible interpretation. Because association studies test the correlated occurrence of disease and an allele in a population, the population characteristics, such as population admixture in an ancient Korean population, could result in positive association of VDR polymorphism with psoriasis (Lander and Schork, 1994). This spurious association could arise if psoriasis-causing genes as well as allele A of the VDR gene happen to be more common in the tested Korean population. Therefore, further studies on this association, such as a transmission disequilibrium test, will be required to exclude this possibility (Spielman et al., 1993).
In conclusion, we report an association of a polymorphism in the VDR gene with psoriasis. This suggests that the VDR gene can be one of some candidate genes implicated in the pathogenesis of psoriasis in the Korean population. We cannot completely rule out the possibility that the association could represent population stratification, because a transmission disequilibrium test was not performed. It has already been revealed that there is a marked ethnic difference in the VDR genotypes (Tokita et al., 1996). It remains to be elucidated whether this polymorphism in the pathogenesis of psoriasis is also applicable to other ethnic groups. Further explanation of the molecular biologic or physiologic mechanism upon the basis of the VDR genotypes should be investigated.

This work was supported by grant (1995) from Seoul National University Hospital Research Fund.

REFERENCES


Lander ES, Schork NJ: Genetic dissection of complex traits. Science 265:2037–2048, 1994


THE JOURNAL OF INVESTIGATIVE DERMATOLOGY