CLINICAL STUDY

Vitamin D receptor genotype is associated with Addison's disease

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Abstract

Objective: Autoimmune Addison's disease is a rare disorder which results from the T cell-mediated destruction of adrenocortical cells. A number of genetic susceptibility markers are shared by Addison's disease, type 1 diabetes, Graves' disease and Hashimoto's thyroiditis. The vitamin D endocrine system has been shown to influence immune regulation. Variants of the nuclear vitamin D receptor (VDR) gene were found to be associated with type 1 diabetes and thyroid autoimmunity amongst others. We therefore investigated the role of VDR polymorphisms in Addison's disease.
Design and methods: Patients ($n = 95$) and controls ($n = 220$) were genotyped for VDR polymorphisms *Fok*I, *Bsm*I, *Apa*I and *Taq*I.
Results: The '*ff*' (13.7% vs 5.5%; $P = 0.0243$; odds ratio = 2.75) and the '*tt*' (28.4% vs 14.1%; $P = 0.0043$; odds ratio = 2.42) genotypes were significantly more frequent in patients than in controls. Furthermore, the *Bsm*I genotype distribution differed significantly between patients and controls ($\chi^2 = 6.5016$ (2 d.f.) $P = 0.0387$).
Conclusions: These data suggest that the VDR genotype is associated with Addison's disease. The mechanisms by which distinct receptor variants might confer disease susceptibility remain to be elucidated.

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Introduction

Autoimmune Addison's disease is a rare disorder causing primary adrenal failure. Its prevalence is estimated to be 40–110 cases per 1 million inhabitants (1, 2). About 50–60% of patients with Addison's disease develop additional autoimmune endocrinopathies during their life as manifestations of the polyglandular syndrome type 1 or 2 (3–5). Genetic susceptibility conferred by the human leukocyte antigen (HLA)-DQ is a feature shared by type 1 diabetes, Graves' disease, Hashimoto's thyroiditis and Addison's disease (6). Compared with other endocrine autoimmune disorders, little is known about the mechanisms involved in the pathogenesis of Addison's disease (reviewed by Betterle & Volpato (7) and Peterson and colleagues (8)). Addison's disease is thought to be mainly T cell mediated (9, 10), and it is strongly associated with the major histocompatibility complex on chromosome 6. Both HLA class II haplotypes, DR4-DQ8 and DR3-DQ2 (6), as well as polymorphisms within the cytotoxic T lymphocyte antigen 4 (CTLA4) gene (11, 12), confer disease susceptibility. Autoantibodies to adrenal cortex and 21-hydroxylase can be found in about 80% of

autoimmune Addison's patients (13) and autoantibody levels have been correlated with the degree of adrenal dysfunction in individuals without overt disease (14).

Besides its effect on bone metabolism, the vitamin D endocrine system has important immunomodulatory properties (15–17). Administration of the most active natural vitamin D metabolite, 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$), ameliorates experimental autoimmune diseases such as type 1 diabetes (18), thyroiditis (19) and encephalitis (20). On a molecular level, $1,25(OH)_2D_3$ leads to reduced expression of HLA class II molecules on endocrine cells (21, 22) and inhibits T cell proliferation and the secretion of cytokines (23, 24). An effect of vitamin D on thymic education (referred to as 'hormonal imprinting') has been reported in animals (25, 26) and lends further support for its immunomodulatory properties. The actions of $1,25(OH)_2D_3$ are mediated via the nuclear vitamin D receptor (VDR). Its gene, located on chromosome 12q12–14 harbors several polymorphisms and was found to be associated with type 1 diabetes (27–29) and thyroid autoimmunity (30, authors, unpublished data).

Given the shared genetic susceptibility among endocrine autoimmune disorders and the association of

VDR variants with type 1 diabetes and thyroid auto-immunity, we investigated the distribution of the VDR polymorphisms *Fok*I (exon 2), *Bsm*I, *Apa*I (both intron 8) and *Taq*I (exon 9) in patients with Addison's disease compared with healthy controls.

Subjects and methods

Subjects

Patients with Addison's disease ($n = 95$) were seen regularly at the endocrine outpatient clinic of the University Hospital Frankfurt am Main, Germany. Seventy-one patients were females and twenty-four were males. Addison's disease was diagnosed by primary adreno-cortical insufficiency without evidence of tuberculosis or adrenoleukodystrophy. Adrenal autoantibodies were detected with indirect immunofluorescence on cryostat sections; these were confirmed to be directed against 21-hydroxylase in a subgroup of patients by radioimmunoassay. 21-Hydroxylase antibodies were found in 80% of patients (31). Thirty-four patients (35.8%) suffered either from thyroid autoimmune disease or were thyroid autoantibody positive as part of a polyglandular syndrome type 2. None of the patients had type 1 diabetes. The age of onset varied from 15 to 42 years and no neurological deficits could be detected. Two hundred and twenty healthy sex-matched controls (139 females and 81 males), who were collected at random from the population of Frankfurt am Main, Germany, had no family history of type 1 diabetes, Graves' disease, Hashimoto's thyroiditis or Addison's disease. Although the adrenal function of the controls was not formally assessed, all individuals had normal thyroid function and were thyroid autoantibody negative. All patients and controls were of Caucasian origin and informed consent was obtained from all individuals.

Genotype analysis

DNA was isolated from whole blood according to standard protocols and subjected to PCR amplification using Taq polymerase (Promega, Madison, WI, USA) on a Multicycler PTC 200 (MJ Research, Las Vegas, NE, USA) as previously described (28). Amplified DNA was digested for 3 h with restriction enzymes – purchased from New England Biolabs (Beverly, MA, USA) and AGS (Heidelberg, Germany) – according to the manufacturer's instructions. Digested PCR fragments were separated on a 2% agarose gel and stained with ethidium bromide (Roth, Karlsruhe, Germany).

The *Fok*I and the *Bsm*I polymorphisms were examined as previously described (28). The *Taq*I and *Apa*I polymorphisms (annealing temperature: 60 °C) were examined in a 740 bp fragment of intron 8/exon 9 using the primers Taq/Apa-forward (5′-CAG AGC ATG GAC AGG GAG CAA-3′) and Taq/Apa-reverse (5′-GCA

ACT CCT CAT GGC TGA GGT CTC-3′). Digestion with *Apa*I revealed two fragments of 210 and 530 bp when the restriction site was present. Digestion with *Taq*I resulted in three fragments of 290, 245 and 205 bp in the presence of the polymorphic site and two fragments of 245 and 495 bp in its absence, due to an additional monomorphic *Taq*I site. In samples heterozygous for the respective restriction site, both digested and undigested DNA fragments were visible. Genotypes were assessed blindly and were designated by a lower case letter for the presence of a restriction site and by an upper case letter for its absence. Each batch included a control DNA homozygously cut by the respective restriction enzyme to rule out partial digestions. Approximately 10% of samples were genotyped in duplicate. HLA-DQ haplotypes of patients and controls had previously been assessed using standard allele-specific primers (6). Fifty-one patients (53.7%) and 41 controls (18.6%) carried a high-risk HLA-DQ2 (DQA1*0501–DQB1*0201) and/or an HLA-DQ8 (DQA1*0301–DQB1*0302) haplotype.

Statistical analysis

Observed and expected genotype frequencies were compared based on the Hardy–Weinberg equation using the BiAS statistical package version 7.01 (Epsilon, Weinheim, Germany). Patients and controls were compared using allele-wise and genotype-wise χ^2 testing. All probabilities (P) were corrected according to the respective degrees of freedom (d.f.) but not for the number of polymorphisms tested, and regarded as significant if $P < 0.05$. The strength of association was estimated by the odds ratio (OR) given with the respective 95% confidence interval (95% CI) using the BiAS software.

Results

The observed *Fok*I, *Bsm*I, *Apa*I and *Taq*I genotype frequencies were in accordance with the Hardy–Weinberg equilibrium in both patients and controls (data not shown).

Analysis of the *Fok*I polymorphism (see Table 1) revealed the '*ff*' genotype to be significantly more frequent in patients than in controls (13.7% vs 5.5%; genotype-wise $\chi^2 = 6.7000$ (2 d.f.) $P = 0.0351$; OR (95% CI) = 2.75 (1.23–6.12)). However, the allele frequency of '*f*' did not differ significantly (31.1% of patients vs 28.0% of controls; $P = 0.3390$). Significantly fewer patients than controls were homo- or heterozygous carriers of '*F*' (86.3% vs 94.5%; $\chi^2 = 5.0754$ (1 d.f.) $P = 0.0243$; OR (95% CI) = 0.39 (0.16–0.81)).

Similar results were found comparing the distribution of the *Taq*I polymorphism in patients with Addison's disease and healthy controls (see Table 2).

Table 1 Distribution of the *Fok*I polymorphism in patients with Addison's disease and controls.

	Patients (*n* (%))	Controls (*n* (%))	OR (95% CI)
Genotype-wise comparison[a]			
FF	47 (49.5)	109 (49.5)	1.00 (0.62−1.61)
Ff	35 (36.8)	99 (45.0)	0.71 (0.44−1.17)
ff	13 (13.7)	12 (5.5)	2.75 (1.23−6.12)
Allele-wise comparison[b]			
F	129 (67.9)	317 (72.0)	0.82 (0.57−1.19)
f	61 (32.1)	123 (28.0)	1.22 (0.84−1.76)
Combined genotype-wise comparison[c]			
FF and *ff*	82 (86.3)	208 (94.5)	0.39 (0.16−0.81)
ff	13 (13.7)	12 (5.5)	2.75 (1.23−6.12)

[a] $\chi^2 = 6.7000$ (2 d.f.) $P = 0.0351$; [b] $\chi^2 = 0.9141$ (1 d.f.) $P = 0.3390$; [c] $\chi^2 = 5.0754$ (1 d.f.) $P = 0.0243$.

Table 3 Distribution of the *Bsm*I polymorphism in patients with Addison's disease and controls.

	Patients (*n* (%))	Controls (*n* (%))	OR (95% CI)
Genotype-wise comparison[a]			
BB	21 (22.1)	39 (17.7)	1.32 (0.73−2.39)
Bb	43 (45.3)	133 (60.5)	0.54 (0.33−0.88)
bb	31 (32.6)	48 (21.8)	1.74 (1.02−2.96)
Allele-wise comparison[b]			
B	85 (44.7)	211 (48.0)	0.88 (0.62−1.24)
b	105 (55.3)	229 (52.0)	1.14 (0.81−1.60)
Combined genotype-wise comparison[c]			
BB and *Bb*	69 (72.6)	172 (78.2)	0.74 (0.43−1.29)
bb	26 (27.4)	48 (21.8)	1.35 (0.78−2.35)

[a] $\chi^2 = 6.5016$ (2 d.f.) $P = 0.0387$; [b] $\chi^2 = 0.4300$ (1 d.f.) $P = 0.5120$; [c] $\chi^2 = 0.8493$ (1 d.f.) $P = 0.3567$.

Significantly more patients than expected carried the '*tt*' genotype (28.4% of patients vs 14.1% of controls; genotype-wise $\chi^2 = 10.0608$ (2 d.f.) $P = 0.0065$; OR (95% CI) = 2.42 (1.36−4.31)). Also, the '*t*' allele was more frequent among patients showing a trend (44.7% vs 36.8%; allele-wise $\chi^2 = 3.1667$ (1 d.f.) $P = 0.0752$). '*T*' positivity was significantly less frequent among patients compared with controls (71.6% vs 85.9%; $P = 0.0043$).

The analysis of *Bsm*I revealed that genotype frequencies differed between patients and controls (genotype-wise $\chi^2 = 6.5016$ (2 d.f.) $P = 0.0387$; see Table 3). However, the '*b*' allele was not more frequent in patients with Addison's disease (55.3% vs 52.0%; $P = 0.5120$). No significant differences were observed for the *Apa*I polymorphism in either genotype- ($P = 0.4058$) or allele-wise ($P = 0.9161$) analysis. Unequivocal construction of *Bsm*I/*Apa*I/*Taq*I haplotypes was possible in only 47 patients and 87 controls because the remaining individuals were heterozygous for more than one polymorphism. Frequencies of the *Bsm*I/*Apa*I/*Taq*I haplotypes differed significantly in this subset ($P = 0.0011$) with the 'BAT' allele being significantly less (OR (95% CI) = 0.23 (0.08−0.63)) and the

'bAt' allele being significantly more frequent (OR (95% CI) = 4.49 (1.77−11.43)) in patients than in controls.

VDR genotype frequencies differed significantly between HLA-DQ2- and HLA-DQ8-negative patients and controls (TaqI: $P < 0.001$; BsmI: $P = 0.0199$; FokI: $P = 0.0061$). Whereas the distribution of the TaqI genotype also differed significantly between HLA-DQ2/DQ8-positive patients and controls ($P = 0.0126$), the distribution of BsmI and FokI in this subset did not differ. Whereas the '*tt*' genotype conferred an OR of 0.54 (0.22−1.35) in HLA-DQ2/DQ8-positive individuals, it conferred a significantly greater OR of 4.96 (2.33−10.54) in DQ2/DQ8-negative subjects. Analysis of the other VDR polymorphisms revealed no such difference.

Discussion

In the present study, we investigated the role of the VDR polymorphisms *Fok*I, *Bsm*I, *Apa*I and *Taq*I in Addison's disease. The genotypes '*ff*' and '*tt*' appear to be associated with susceptibility to Addison's disease. Also, the genotype distribution of *Bsm*I differed significantly between patients and controls, but neither the '*b*' allele, nor the '*bb*' genotype nor the *Apa*I polymorphism showed an association with Addison's disease. These findings correspond to observations that VDR polymorphisms are associated with type 1 diabetes (27−29) and thyroid autoimmunity (30, authors, unpublished data). Taken together, VDR variants are associated with susceptibility to several endocrine autoimmune diseases. In a previous study, we found that VDR haplotypes, but not individual polymorphisms, predicted susceptibility to type 1 diabetes when studying families (28). Thereby, VDR haplotypes '*baT*' and '*BAt*' predispose to and haplotypes '*BAT*' and '*bat*' are protective against type 1 diabetes mellitus. In contrast, our case-control study reveals genotypes '*ff*' and '*tt*' to be associated with Addison's disease. Interestingly, the

Table 2 Distribution of the *Taq*I polymorphism in patients with Addison's disease and controls.

	Patients (*n* (%))	Controls (*n* (%))	OR (95% CI)
Genotype-wise comparison[a]			
TT	37 (38.9)	89 (40.5)	0.94 (0.57−1.54)
Tt	31 (32.6)	100 (45.4)	0.58 (0.35−0.96)
tt	27 (28.4)	31 (14.1)	2.42 (1.36−4.31)
Allele-wise comparison[b]			
T	105 (55.3)	278 (63.2)	0.72 (0.51−1.02)
t	85 (44.7)	162 (36.8)	1.39 (0.98−1.96)
Combined genotype-wise comparison[c]			
TT and *Tt*	68 (71.6)	189 (85.9)	0.41 (0.23−0.74)
tt	27 (28.4)	31 (14.1)	2.42 (1.36−4.31)

[a] $\chi^2 = 10.0608$ (2 d.f.) $P = 0.0065$; [b] $\chi^2 = 3.1667$ (1 d.f.) $P = 0.0752$; [c] $\chi^2 = 8.1667$ (1 d.f.) $P = 0.0043$.

distribution of *Bsm*I/*Apa*I/*Taq*I haplotypes differed significantly between the subset of patients and controls in which direct assignment of haplotypes was possible. Although this difference might not be representative for those subjects for whom no haplotypes could be assigned, the finding that 'bAt' confers a significantly increased risk for Addison's disease whereas the 'BAT' haplotype is protective is in accordance with our previous observations in type 1 diabetes (28). Therefore the investigated polymorphisms may only be markers linked to a functionally relevant VDR variation conferring susceptibility to Addison's disease.

VDR genotypes differed significantly between Addison's patients and controls carrying at least one high-risk HLA-DQ haplotype (DQ2 or DQ8). Although the distribution of TaqI genotypes also differed among the subsets of HLA-DQ2/DQ8-negative patients and controls, the '*tt*' genotype appeared to confer a significantly greater risk in the latter subset. This finding might indicate a significant interaction between HLA-DQ2/DQ8 and the VDR genotype '*tt*' in Addison's disease but it needs to be confirmed in a larger study.

Two other studies reported higher '*ff*' genotype frequencies in Caucasians than in our control population (32, 33). As a larger number of individuals would be required to determine the true *Fok*I frequencies in Caucasians and *Fok*I allele frequencies exhibit a considerable inter-study variability in Asians (34, 35), we do not consider the differences between other study populations and our control population as significant. Furthermore, our controls have genotype distributions for all other VDR polymorphisms as well as for HLA-DQ and CTLA4 that are very similar to other reports.

Experimentally, $1,25(OH)_2D_3$ prevents autoimmune diabetes (18), thyroiditis (19), rheumatoid arthritis (36) and encephalitis (20). In humans, $1,25(OH)_2D_3$ serum levels were found to be significantly lower in autoimmune hyperthyroidism than in non-autoimmune hyperthyroidism (37), and administration of $1,25(OH)_2D_3$ to patients with untreated Graves' disease was reported to ameliorate hyperthyroidism (38). Blood levels of $1,25(OH)_2D_3$ in patients with type 1 diabetes are significantly reduced, even at disease onset (39). Vitamin D supplementation in early infancy was reported to result in a lower incidence of type 1 diabetes (40).

The '*f*' allele results in a VDR protein 3 amino acids longer than the '*F*' variant (41). *Fok*I alleles differ functionally (42, 43) due to altered VDR affinity and transactivation (44). An *in vitro* study demonstrated an increased transcription rate (1.7-fold) of the VDR gene in cells with the '*FF*' genotype (45). However, another study failed to show any relevant alteration in either the affinity of binding of the VDR protein or in the steady-state transcription rate of the VDR gene (46).

The *Taq*I polymorphism in exon 9 is due to a silent base exchange and therefore does not alter the amino acid sequence of VDR. Interestingly, VDR mRNA levels were reported to be associated with the *Taq*I polymorphism (47). The *Apa*I site is located within intron 8 of the VDR gene. Alterations in intronic sequences may influence protein expression, but the VDR intron 8 polymorphisms were not found to influence VDR mRNA levels (48, 49). The *Bsm*I polymorphism was found to correlate with $1,25(OH)_2D_3$ serum levels in one study (50), although other reports failed to detect such a correlation (5, 51). In summary, VDR variants appear to differ functionally. The identification of a single locus responsible for an altered VDR expression or affinity for either its ligand, DNA-binding sites or the retinoid receptor – with which it forms heterodimers – could explain how an impairment in vitamin D action might contribute to an immune dysregulation eventually leading to Addison's disease.

Transmission analyses of extended VDR haplotypes will help to further identify those alleles most strongly associated with Addison's disease. How these VDR variants exert their distinct actions in various tissues, including lymphocytes and adrenocortical cells, needs to be addressed in functional studies. In conclusion, this study provides evidence that the VDR represents a genetic marker for susceptibility to Addison's disease. The issue of a potential interaction between HLA-DQ haplotype and the VDR *Taq*I polymorphisms needs to be further pursued.

References

1 Kong MF & Jeffcoate W. Eighty-six cases of Addison's disease. *Clinical Endocrinology* 1994 **41** 757–761.

2 Laureti S, Vecchi L, Santeusanio F & Falorni A. Is the prevalence of Addison's disease underestimated? *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 1762.

3 Ahonen P, Myllarniemi S, Sipila I & Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *New England Journal of Medicine* 1990 **322** 1829–1836.

4 Betterle C, Volpato M, Greggio AN & Presotto F. Type 2 polyglandular autoimmune disease (Schmidt's syndrome). *Journal of Pediatric Endocrinology and Metabolism* 1996 **9** 113–123.

5 Bell NH, Morrison NA, Nguyen TV, Eisman J & Hollis BW. ApaI polymorphisms of the vitamin D receptor predict bone density of the lumbar spine and not racial difference in bone density in young men. *Journal of Laboratory and Clinical Medicine* 2001 **137** 133–140.

6 Badenhoop K, Walfish PG, Rau H, Fischer S, Nicolay A, Bogner U et al. Susceptibility and resistance alleles of human leukocyte antigen (HLA) DQA1 and HLA DQB1 are shared in endocrine autoimmune diseases. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 2112–2117.

7 Betterle C & Volpato M. Adrenal and ovarian autoimmunity. *European Journal of Endocrinology* 1998 **138** 16–25.

8 Peterson P, Uibo R & Krohn KJ. Adrenal autoimmunity: results and developments. *Trends in Endocrinology and Metabolism* 2000 **11** 285–290.

9 Nerup J & Bendixen G. Anti-adrenal cellular hypersensitivity in Addison's disease. II. Correlation with clinical and serological findings. *Clinical and Experimental Immunology* 1969 **5** 341–353.

10 Fairchild RS, Schimke RN & Abdou NI. Immunoregulation abnormalities in familial Addison's disease. *Journal of Clinical Endocrinology and Metabolism* 1980 **51** 1074–1077.

11 Donner H, Braun J, Seidl C, Rau H, Finke R, Ventz M *et al.* Codon 17 polymorphism of the cytotoxic T lymphocyte antigen 4 gene in Hashimoto's thyroiditis and Addison's disease. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 4130–4132.

12 Kemp EH, Ajjan RA, Husebye ES, Peterson P, Uibo R, Imrie H *et al.* Cytotoxic T lymphocyte antigen-4 (CTLA-4) gene polymorphism is associated with autoimmune Addison's disease in English patients. *Clinical Endocrinology* 1998 **49** 609–613.

13 Betterle C, Volpato M, Pedini B, Chen S, Smith BR & Furmaniak J. Adrenal-cortex autoantibodies and steroid-producing cell auto-antibodies in patients with Addison's disease: comparison of immunofluorescence and immunoprecipitation assays. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 618–622.

14 Laureti S, De Bellis A, Muccitelli VI, Calcinaro F, Bizzarro A, Rossi R *et al.* Levels of adrenocortical autoantibodies correlate with the degree of adrenal dysfunction in subjects with preclinical Addison's disease. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 3507–3511.

15 Thomasset M. Vitamin D and the immune system. *Pathologie Biologie* 1994 **42** 163–172.

16 Lemire J. 1,25-Dihydroxyvitamin D3 – a hormone with immuno-modulatory properties. *Zeitschrift für Rheumatologie* 2000 **59** 24–27.

17 Cantorna MT. Vitamin D and autoimmunity: is vitamin D status an environmental factor affecting autoimmune disease preva-lence? *Proceedings of the Society for Experimental Biology and Medicine* 2000 **223** 230–233.

18 Mathieu C, Waer M, Laureys J, Rutgeerts O & Bouillon R. Preven-tion of autoimmune diabetes in NOD mice by 1,25 dihydroxyvita-min D3. *Diabetologia* 1994 **37** 552–558.

19 Fournier C, Gepner P, Sadouk M & Charreire J. *In vivo* beneficial effects of cyclosporin A and 1,25-dihydroxyvitamin D3 on the induction of experimental autoimmune thyroiditis. *Clinical Immunology and Immunopathology* 1990 **54** 53–63.

20 Lemire JM & Archer DC. 1,25-Dihydroxyvitamin D3 prevents the *in vivo* induction of murine experimental autoimmune encephalo-myelitis. *Journal of Clinical Investigation* 1991 **87** 1103–1107.

21 Tokuda N, Mano T & Levy RB. 1,25-Dihydroxyvitamin D3 antagonizes interferon-gamma-induced expression of class II major histocompatibility antigens on thyroid follicular and testicular Leydig cells. *Endocrinology* 1990 **127** 1419–1427.

22 Hahn HJ, Kuttler B, Mathieu C & Bouillon R. 1,25-Dihydroxy-vitamin D3 reduces MHC antigen expression on pancreatic beta-cells *in vitro*. *Transplantation Proceedings* 1997 **29** 2156–2157.

23 Muller K & Bendtzen K. Inhibition of human T lymphocyte pro-liferation and cytokine production by 1,25-dihydroxyvitamin D3. Differential effects on CD45RA+ and CD45R0+ cells. *Autoimmunity* 1992 **14** 37–43.

24 Willheim M, Thien R, Schrattbauer K, Bajna E, Holub M, Gruber R *et al.* Regulatory effects of 1α,25-dihydroxyvitamin D3 on the cytokine production of human peripheral blood lymphocytes. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 3739–3744.

25 Ravid A, Koren R, Novogrodsky A & Liberman UA. 1,25-Di-hydroxyvitamin D3 inhibits selectively the mitogenic stimulation of mouse medullary thymocytes. *Biochemical and Biophysical Research Communications* 1984 **123** 163–169.

26 Provvedini DM, Sakagami Y & Manolagas SC. Distinct target cells and effects of 1α,25-dihydroxyvitamin D3 and glucocorticoids in the rat thymus gland. *Endocrinology* 1989 **124** 1532–1538.

27 McDermott MF, Ramachandran A, Ogunkolade BW, Aganna E, Curtis D, Boucher BJ *et al.* Allelic variation in the vitamin D recep-tor influences susceptibility to IDDM in Indian Asians. *Diabetologia* 1997 **40** 971–975.

28 Pani MA, Knapp M, Donner H, Braun J, Baur MP, Usadel KH *et al.* Vitamin D receptor allele combinations influence genetic susceptibility to type I diabetes in Germans. *Diabetes* 2000 **49** 504–507.

29 Chang TJ, Lei HH, Yeh JI, Chiu KC, Lee KC, Chen MC *et al.* Vitamin D receptor gene polymorphisms influence susceptibility to type 1 diabetes mellitus in the Taiwanese population. *Clinical Endocrin-ology* 2000 **52** 575–580.

30 Ban Y, Ban Y, Taniyama M & Katagiri T. Vitamin D receptor initiation codon polymorphism in Japanese patients with Graves' disease. *Thyroid* 2000 **10** 375–380.

31 Yassin N, Seissler J, Gluck M, Boehm BO, Heinze E, Pfeiffer EF *et al.* Insulin autoantibodies as determined by competitive radiobinding assay are positively correlated with impaired beta-cell function – the Ulm–Frankfurt Population Study. *Klinische Wochenschrift* 1991 **69** 736–741.

32 Lucotte G, Mercier G & Burckel A. Fok I polymorphism at the human vitamin D receptor gene locus in Europeans and Africans. *Clinical Genetics* 1999 **55** 281–282.

33 Langdahl BL, Gravholt CH, Brixen K & Eriksen EF. Polymorphisms in the vitamin D receptor gene and bone mass, bone turnover and osteoporotic fractures. *European Journal of Clinical Investigation* 2000 **30** 608–617.

34 Ban Y & Taniyama M. Vitamin D receptor gene polymorphism is associated with Graves' disease in the Japanese population. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 4639–4643.

35 Chen WC, Chen HY, Lu HF, Hsu CD & Tsai FJ. Association of the vitamin D receptor gene start codon Fok I polymorphism with calcium oxalate stone disease. *British Journal of Urology Inter-national* 2001 **87** 168–171.

36 Cantorna MT, Hayes CE & DeLuca HF. 1,25-Dihydroxycholecalci-ferol inhibits the progression of arthritis in murine models of human arthritis. *Journal of Nutrition* 1998 **128** 68–72.

37 Czernobilsky H, Scharla S, Schmidt-Gayk H & Ziegler R. Enhanced suppression of 1,25(OH)2D3 and intact parathyroid hormone in Graves' disease as compared to toxic nodular goiter. *Calcified Tissue International* 1988 **42** 5–12.

38 Kawakami-Tani T, Fukawa E, Tanaka H, Abe Y & Makino I. Effect of 1α-hydroxyvitamin D3 on serum levels of thyroid hormones in hyperthyroid patients with untreated Graves' disease. *Metabolism* 1997 **46** 1184–1188.

39 Baumgartl HJ, Standl E, Schmidt-Gayk H, Kolb HJ, Janka HU & Ziegler AG. Changes of vitamin D3 serum concentrations at the onset of immune-mediated type 1 (insulin-dependent) diabetes mellitus. *Diabetes Research* 1991 **16** 145–148.

40 EURODIAB Substudy 2 Study Group, Vitamin D supplement in early childhood and risk for type I (insulin-dependent) diabetes mellitus. *Diabetologia* 1999 **42** 51–54.

41 Gross C, Eccleshall TR, Malloy PJ, Villa ML, Marcus R & Feldman D. 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. *Journal of Bone and Mineral Research* 1996 **11** 1850–1855.

42 Colin EM, Weel AE, Uitterlinden AG, Buurman CJ, Birkenhager JC, Pols HA *et al.* Consequences of vitamin D receptor gene poly-morphisms for growth inhibition of cultured human peripheral blood mononuclear cells by 1,25-dihydroxyvitamin D3. *Clinical Endocrinology* 2000 **52** 211–216.

43 Jurutka PW, Remus LS, Whitfield GK, Thompson PD, Hsieh JC, Zitzer H *et al.* The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulat-ing interaction with transcription factor IIB. *Molecular Endocrin-ology* 2000 **14** 401–420.

44 Miyamoto K, Takentani Y, Tohna E & Nishisho T. A novel poly-morphism in the vitamin D receptor and bone mineral density: a study of vitamin D receptor expression and function in COS-7 cells. *Journal of Bone and Mineral Research* 1996 **11** 116S.

45 Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K *et al.* A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. *Journal of Bone and Mineral Research* 1997 **12** 915–921.

46 Gross C, Krishnan AV, Malloy PJ, Eccleshall TR, Zhao XY & Feldman D. The vitamin D receptor gene start codon polymorphism: a functional analysis of FokI variants. *Journal of Bone and Mineral Research* 1998 **13** 1691–1699.

47 Yamagata M, Nakajima S, Tokita A, Sakai N, Yanagihara I, Yabuta K *et al.* Analysis of the stable levels of messenger RNA derived from different polymorphic alleles in the vitamin D receptor gene. *Journal of Bone and Mineral Metabolism* 1999 **17** 164–170.

48 Mocharla H, Butch AW, Pappas AA, Flick JT, Weinstein RS, De Togni P *et al.* Quantification of vitamin D receptor mRNA by competitive polymerase chain reaction in PBMC: lack of correspondence with common allelic variants. *Journal of Bone and Mineral Research* 1997 **12** 726–733.

49 Gross C, Musiol IM, Eccleshall TR, Malloy PJ & Feldman D. Vitamin D receptor gene polymorphisms: analysis of ligand binding and hormone responsiveness in cultured skin fibroblasts.

Biochemical and Biophysical Research Communications 1998 **242** 467–473.

50 Tokita A, Matsumoto H, Morrison NA, Tawa T, Miura Y, Fukamauchi K *et al.* Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women. *Journal of Bone and Mineral Research* 1996 **11** 1003–1009.

51 Suarez F, Zeghoud F, Rossignol C, Walrant O & Garabedian M. Association between vitamin D receptor gene polymorphism and sex-dependent growth during the first two years of life. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 2966–2970.