International Journal of Infectious Diseases (2009) **13**, e437—e443

http://intl.elsevierhealth.com/journals/ijid

Staphylococcus aureus nasal carriage might be associated with vitamin D receptor polymorphisms in type 1 diabetes

Charalambos Panierakis [a], George Goulielmos [b], Dimitris Mamoulakis [a], Sofia Maraki [c], Efstathios Papavasiliou [d], Emmanouil Galanakis [a,*]

[a] *Department of Pediatrics, University of Crete, POB 2208, Heraklion 71003, Greece*
[b] *Laboratory of Internal Medicine, University of Crete, Heraklion, Greece*
[c] *Laboratory of Microbiology, University Hospital, Heraklion, Greece*
[d] *Department of Endocrinology, University Hospital, Heraklion, Greece*

Received 21 November 2008; received in revised form 16 February 2009; accepted 18 February 2009
Corresponding Editor: Sunit K. Singh, Hyderabad, India

KEYWORDS
Vitamin D receptor polymorphisms;
Type 1 diabetes;
Staphylococcus aureus colonization;
Staphylococcus aureus carriage;
Susceptibility to infection

Summary
Background: Polymorphisms in the vitamin D receptor (VDR) gene have been associated with susceptibility to several diseases, including type 1 diabetes (T1D) and infections. In this study we investigated whether VDR gene polymorphisms influence nasal carriage of *Staphylococcus aureus* in individuals with T1D.
Methods: In 93 T1D patients, VDR polymorphisms on *Fok*I *F*>*f*, *Bsm*I *B*>*b*, *Apa*I *A*>*a*, and *Taq*I *T*>*t* were determined in DNA extracted from peripheral blood leukocytes, and a nasal swab was obtained to detect colonization by *S. aureus*. A repeat swab was obtained in 76/93 subjects for the estimation of persistent *S. aureus* carriage.
Results: The prevalence of *S. aureus* nasal colonization was 31.2% and the prevalence of persistent carriage was 25%. The presence of *Taq*I *T* allele was related to higher rates of *S. aureus* colonization, and *Taq*I *TT* homozygotes were more colonized (48.5% vs. 21.7%; *p* 0.007; OR 3.40, 95% CI 1.36—8.52) and more persistent carriers (37.9% vs. 17.0%; *p* 0.039; OR 2.98, 95% CI 1.02—8.67). The presence of *Apa*I *A* allele was related to lower rates of *S. aureus* colonization, and *Apa*I *AA* homozygotes were less colonized (17.6% vs. 39.0%; *p* 0.026; OR 0.34, 95% CI 0.12—0.94) and less persistent carriers (11.5% vs. 32%; *p* 0.043; OR 0.28, 95% CI 0.07—1.06). No differences were observed for *Bsm*I and *Fok*I genotypes.
Conclusions: Our findings suggest that VDR polymorphisms may be associated with nasal carriage of *S. aureus* in individuals with T1D, and further contribute to the better understanding of the immunomodulatory role of vitamin D in the human host's response and susceptibility to infection.

* Corresponding author.
E-mail address: emmgalan@med.uoc.gr (E. Galanakis).

1201-9712/$36.00
doi:10.1016/j.ijid.2009.02.012

Introduction

The vitamin D endocrine system has pleiotropic effects on various processes including calcium absorption and bone biology, cell growth and differentiation, and modulation of the immune system.[1,2] Vitamin D mediates its actions by binding to the vitamin D receptor (VDR), which is a soluble protein present in various tissues and in T and B lymphocytes, monocytes, and macrophages.[1–4] Polymorphisms in the human VDR gene have been associated with several diseases including type 1 diabetes (T1D),[2,5–7] and with susceptibility to several bacterial and viral infections.[3,4,8–12]

Staphylococcus aureus is a major pathogen for humans with increasing resistance to antibiotics. The anterior nares are the primary ecological reservoir of *S. aureus*, and nasal carriage has been related to progress to staphylococcal disease.[13–20] The host's response seems to be crucial in *S. aureus* nasal colonization and carriage, but little is known in this field.[20] Vitamin D metabolites are known to affect macrophage activation, and neutrophils have been demonstrated to express functional VDR,[10,21] hence VDR gene polymorphisms may affect colonization and infection by microorganisms such as *S. aureus* that might behave as an intracellular pathogen.

Individuals with T1D are considered to be susceptible to infections in terms of frequency, severity, and complications, including infections by *S. aureus*.[13,15,16,22] Although VDR polymorphisms are related both with T1D and susceptibility to infection, and T1D is further associated to *S. aureus* infection, to date no study has investigated potential links between VDR polymorphisms on the one hand and T1D and *S. aureus* disease or carriage on the other. In this study we investigated whether the four most commonly studied polymorphisms in the VDR gene, i.e., *Fok*I *F*>*f*, *Bsm*I *B*>*b*, *Apa*I *A*>*a*, and *Taq*I *T*>*t* are associated with colonization by and persistent carriage of *S. aureus* in individuals with T1D.

Patients and methods

Patient recruitment

The study group consisted of a cohort of 93 T1D patients, followed up at the outpatient diabetic clinic of the Department of Pediatrics, University Hospital of Heraklion, Crete, Greece. This T1D outpatient clinic is the only one on the island of Crete. Diagnosis of T1D follows the American Diabetes Association guidelines.[23] The study protocol was approved by the institutional committees of the University Hospital of Heraklion and the Medical School, University of Crete.

S. aureus carriage

Nasal specimens were obtained by rotating a sterile fiber-tipped swab four times in both anterior nares of each enrolled individual. The swabs were immediately placed in Amies transport medium (BioMerieux, Marcy L'Etoile, France) and kept at 4 °C before being inoculated onto mannitol salt agar and Columbia agar with 5% sheep blood. The culture plates were incubated at 36 °C for 48 hours. *S. aureus* was identified on the basis of colony morphology, Gram stain, catalase and coagulase test, and the API 20 Staph system (BioMerieux). A second nasal swab culture was obtained after a minimum interval of 3 months. Subjects were classified as persistent carriers if both cultures were positive, as non-carriers if both cultures were negative, and as intermittent carriers in cases where one culture was positive and the other was negative.[13,15,17–20,24] Individuals with a single nasal swab available were excluded from further analysis of persistent carriage.

DNA analysis

Whole blood was collected in EDTA-containing tubes, and genomic DNA was isolated from peripheral blood leukocytes using the Puregene kit (Gentra Systems, Minnesota, USA). The extracted DNA was stored at −20 °C until analysis. DNA was amplified with standard PCR techniques. The cDNA of the human VDR (vitamin D 1,25-dihydroxyvitamin D_3 receptor) sequence (GenBank accession number **J03258**) was used to design primers for PCR amplification of the fragments, as shown in Table 1. Both undigested and digested PCR products were visualized on 2.5% agarose gels stained with ethidium bromide. Genotypes were determined according to the presence or absence of an appropriate restriction site, and alleles were designated respective to actual base change according to the dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/). Genotypes were scored blindly and analysis of all ambiguous samples was repeated. Moreover, 10% of the samples were amplified twice to check the accuracy of results. The selection of the four enzyme sites followed previous protocols, and the established nomenclature for restriction fragment length polymorphism alleles was used in this study.[2,7,10–12,18] The lowercase allele represents the presence of the restriction site (*f*: *Fok*I, *b*: *Bsm*I, *a*: *Apa*I, or *t*: *Taq*I) and the uppercase allele represents the absence of the restriction site (*F*, *B*, *A*, or *T*, respectively).[25] Allele types, SNP reference numbers, and PCR conditions for all the analyzed polymorphisms are shown in Table 2.

Confirmation of polymorphisms by direct sequencing

Selected PCR amplified fragments from the four VDR examined polymorphisms, corresponding to heterozygous or mutant homozygous genotypes, were completely sequenced, both strands, in a LiCor 4200L sequencer at the laboratory of microchemistry (IMBB-FORTH, Crete, Greece) in order to confirm that the amplified products represent genuine VDR regions.

Statistical analysis

Statistical analysis was based on contingency tables, including calculations of odds ratio (OR) and of the lower and upper limits of the 95% confidence interval (95% CI). The one-tailed Fisher's exact test was applied to determine whether distributions of categorized variables between the groups were significant. The Hardy–Weinberg equilibrium was tested by comparing expected and observed genotype frequencies by

Table 1 PCR primers designed to amplify fragments harboring the vitamin D receptor single nucleotide polymorphisms (SNPs)

SNP	PCR primer	Fragment size
*Apa*I	Forward (5′—3′) CAG AGC ATG GAC AGG GAG CAA	740 bp uncleaved
	Reverse (3′—5′) GCA ACT CCT CAT GGC TGA GGT C CTC	530 bp, 210 bp
*Bsm*I	Forward (5′—3′) CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA	825 bp uncleaved
	Reverse (3′—5′) AAC CAG CGG GAA GAG GTC AAG GG	650 bp, 175 bp
*Fok*I	Forward (5′—3′) AGC TGG CCC TGG CAC TGA CTC TGC TCT	265 bp uncleaved
	Reverse (3′—5′) ATG GAA ACA CCT TGC TTC TTC TCC CTC	196 bp, 69 bp
*Taq*I	Forward (5′—3′) CAG AGC ATG GAC AGG GAG CAA	740 bp uncleaved
	Reverse (3′—5′) GCA ACT CCT CAT GGC TGA GGT C CTC	495 bp, 245 bp or 290 bp, 245 bp and 205 bp

Chi-square test. The conventional level of $p < 0.05$ was considered to be significant.

Results

During the 21-year period, 1986—2006, 147 individuals were diagnosed with T1D and followed-up at the outpatient clinic. Of these, 93 (63.3%) were enrolled in this study. The 93 individuals (43 female and 50 male) were aged 3.3 to 25.3 (mean 12.3, median 12.4) years, and the duration of T1D from diagnosis ranged from 0.1 to 17.0 (mean 4.4, median 2.8) years.

VDR gene *Fok*I, *Bsm*I, *Apa*I, and *Taq*I genotypes and allele frequencies are shown in Table 3. In the initial nasal swab sampling, 29/93 individuals (31.2%) were found to be colonized by *S. aureus*. Among the 76 individuals from whom a second culture was obtained, 19 (25%) were defined as persistent *S. aureus* carriers, 21 (27.6%) as intermittent carriers, and 36 (47.4%) as non-carriers for *S. aureus*.

VDR polymorphisms and initial *S. aureus* carriage

As shown in Table 3, *S. aureus* nasal colonization was more common in individuals with *Taq*I *T* than *t* allele (37.0% vs. 20.9%; *p* 0.016; OR 2.22, 95% CI 1.11—4.46), and *Taq*I *TT* homozygotes were more colonized by *S. aureus* than genotypes *Taq*I *tt* and *Tt* combined (48.5% vs. 21.7%; *p* 0.007; OR 3.40, 95% CI 1.36—8.52). *S. aureus* nasal colonization was less common in individuals with *Apa*I *A* rather than *a* allele, although not at a statistically significant level (27.3% vs. 38.5%; *p* 0.081; OR 0.60, 95% CI 0.31—1.14); furthermore *Apa*I *AA* homozygotes were less colonized than *Apa*I *aa* and *Aa* genotypes combined (17.6% vs. 39.0%; *p* 0.026; OR 0.34, 95% CI 0.12—0.94). No significant differences were observed for *Bsm*I and *Fok*I genotypes.

VDR polymorphisms and persistent *S. aureus* carriage

As shown in Table 4, *S. aureus* persistent nasal colonization was more common in individuals with *Taq*I *T* than *t* allele (29.3% vs. 17.0%), although at a marginally significant level (*p* 0.068; OR 2.03, 95% CI 0.88—4.68); furthermore *Taq*I *TT* homozygotes were persistently colonized by *S. aureus* more than genotypes *Taq*I *tt* and *Tt* combined (37.9% vs. 17.0%; *p* 0.039; OR 2.98, 95% CI 1.02—8.67). *S. aureus* nasal colonization was more common in individuals with *Apa*I *a* than *A* allele, although not at a significant level (21.9% vs. 30.4%; *p* 0.17; OR 0.64, 95% CI 0.30—1.36); furthermore, *Apa*I *AA* homozygotes were less persistent carriers than *Apa*I *aa* and *Aa* genotypes combined (11.5% vs. 32%; *p* 0.043; OR 0.28, 95% CI 0.07—1.06). No significant differences were observed for *Bsm*I and *Fok*I genotypes.

Table 2 Allele types, single nucleotide polymorphism (SNP) reference numbers, and PCR conditions

SNP	Allele type	Ref. number	PCR conditions
*Apa*I	*a* allele: T→G transition in intron 8	rs7975232	Denaturation at 94 °C for 5 min, followed by addition of the polymerase, and then samples were exposed to 35 cycles of denaturing (at 94 °C for 30 s), annealing (at 70 °C for 30 s), and chain extension (at 72 °C for 1 min), followed by a final extension step at 72 °C for 7 min
*Bsm*I	*b* allele: G→A transition	rs1544410	Initial heating at 94 °C for 5 min, followed by addition of the polymerase and then 35 cycles of denaturing (at 94 °C for 15 s), annealing (at 60 °C for 30 s), and chain extension (at 72 °C for 30 s), followed by a final extension step at 72 °C for 7 min
*Fok*I	*f* allele: C→T transition at the junction of intron 1 and exon 2	rs10735810	Initial heating at 94 °C for 5 min, followed by 35 cycles of denaturing (at 94 °C for 15 s), annealing (at 60 °C for 30 s), and chain extension (at 72 °C for 30 s), followed by a final extension step at 72 °C for 7 min
*Taq*I	*t* allele: silent T→C transition in exon 9	rs731236	Denaturation at 94 °C for 5 min, followed by addition of the polymerase, and then samples were exposed to 35 cycles of denaturing (at 94 °C for 30 s), annealing (at 70 °C for 30 s), and chain extension (at 72 °C for 1 min), followed by a final extension step at 72 °C for 7 min

Table 3 Distribution of VDR genotype and haplotype frequencies in 93 individuals with type 1 diabetes as related to *S. aureus* nasal colonization

VDR gene polymorphism	HWE[a] *p*-value	*S. aureus* colonized (%)	*S. aureus* non-colonized (%)	Total	*p*-Value; OR (95% CI)[b]
FokI					
Genotype	0.67 (0.91)				NS
ff		0 (0)	7 (100)	7	
Ff		16 (40)	24 (60)	40	
FF		13 (28.3)	33 (71.7)	46	
Allele					NS
f		16 (29.6)	38 (70.4)	54	
F		42 (31.8)	90 (68.2)	132	
BsmI					
Genotype	0.12 (0.30)				NS
bb		6 (31.6)	13 (68.4)	19	
Bb		19 (35.2)	35 (64.8)	54	
BB		4 (20)	16 (80)	20	
Allele					NS
b		31 (33.7)	61 (66.3)	92	
B		27 (28.7)	67 (71.3)	94	
ApaI					
Genotype	0.01 (0.05)				0.026; 0.34 (0.12—0.94)[c]
aa		2 (33.3)	4 (66.7)	6	
Aa		21 (39.6)	32 (60.4)	53	
AA		6 (17.6)	28 (82.4)	34	
Allele					0.081; 0.60 (0.31—1.14)[d]
a		25 (38.5)	40 (61.5)	65	
A		33 (27.3)	88 (72.7)	121	
TaqI					
Genotype	0.02 (0.07)				0.007; 3.40 (1.36—8.52)[e]
tt		1 (14.3)	6 (85.7)	7	
Tt		12 (22.6)	41 (77.4)	53	
TT		16 (48.5)	17 (51.5)	33	
Allele					0.016; 2.22 (1.11—4.46)[f]
t		14 (20.9)	53 (79.1)	67	
T		44 (37.0)	75 (63.0)	119	
Total patients		29 (31.2)	64 (68.8)	93	
Total alleles		58 (31.2)	128 (68.8)	186	

VDR, vitamin D receptor; NS, not significant; OR, odds ratio; CI, confidence interval.
[a] Hardy—Weinberg equilibrium (calculations with 1 degree of freedom; in parentheses values after calculation with 2 degrees of freedom).
[b] *p*-Values and odds ratios calculated for colonized vs. non-colonized T1D individuals.
[c] (*ApaI aa* + *ApaI Aa*) vs. *ApaI AA*.
[d] *A* allele vs. *a* allele.
[e] *TaqI TT* vs. (*TaqI tt* + *TaqI Tt*).
[f] *T* allele vs. *t* allele.

Discussion

Our findings suggest that two VDR polymorphisms, both located in the 3′ region of the VDR gene, are related to colonization by and persistent nasal carriage of *S. aureus* in individuals with T1D. In this study we focused on both initial colonization and persistent carriage, as persistent carriers have higher *S. aureus* loads and a higher risk of acquiring *S. aureus* infection.[13] The rates of colonization and persistent *S. aureus* carriage in the study population (31% and 25%, respectively) are consistent with the ranges reported elsewhere.[15—19,26]

The ability of *S. aureus* to colonize the human host and progress to infection depends both on virulence properties of the invading strain and on the individual host's response. Not much is known on the intrinsic properties of *S. aureus* strains that facilitate colonization and long-term carriage.[18—20] By contrast, evidence is emerging and pointing to host genetic factors affecting *S. aureus* colonization and carriage.[18,20,24,26] Staphylococcal carriage has long been associated with the presence of HLA-DR3.[27] In individuals with T1D, dendritic cells have been shown to produce less interferon-α,[28] and polymorphonuclear cells have demonstrated impaired killing function against *S. aureus*.[16] Our findings provide evidence for

Table 4 Distribution of VDR genotype and haplotype frequencies in 76 individuals with type 1 diabetes as related to *S. aureus* persistent nasal carriage

VDR gene polymorphism	HWE[a] *p*-value	Persistent carriers (%)	Intermittent carriers (%)	Non-carriers (%)	Total	*p*-Value; OR (95% CI)[b]
FokI						
Genotype	0.98 (1.00)					NS
ff		0 (0.0)	1 (14.3)	6 (85.7)	7	
Ff		11 (34.4)	9 (28.1)	12 (37.5)	32	
FF		8 (21.6)	11 (29.7)	18 (48.7)	37	
Allele						NS
f		11 (23.9)	11 (23.9)	24 (52.2)	46	
F		27 (25.5)	31 (29.2)	48 (45.3)	106	
BsmI						
Genotype	0.07 (0.19)					NS
bb		4 (26.7)	3 (20.0)	8 (53.3)	15	
Bb		13 (28.3)	13 (28.3)	20 (44.4)	46	
BB		2 (13.3)	5 (33.3)	8 (53.4)	15	
Allele						NS
b		21 (27.6)	19 (25.0)	36 (47.4)	76	
B		17 (22.4)	23 (30.3)	36 (47.3)	76	
ApaI						
Genotype	0.03 (0.10)					0.043; 0.28 (0.07—1.06)[c]
aa		1 (16.7)	3 (50.0)	2 (33.3)	6	
Aa		15 (34.1)	8 (18.2)	21 (47.7)	44	
AA		3 (11.5)	10 (38.5)	13 (50.0)	26	
Allele						0.17; 0.64 (0.30—1.36)[d]
a		17 (30.4)	14 (25.0)	25 (44.6)	56	
A		21 (21.9)	28 (29.2)	47 (48.9)	96	
TaqI						
Genotype	0.10 (0.26)					0.039; 2.98 (1.02—8.67)[e]
tt		1 (16.7)	2 (33.3)	3 (50.0)	6	
Tt		7 (17.1)	14 (34.1)	20 (48.8)	41	
TT		11 (37.9)	5 (17.2)	13 (44.8)	29	
Allele						0.068; 2.03 (0.88—4.68)[f]
t		9 (17.0)	18 (34.0)	26 (49.0)	53	
T		29 (29.3)	24 (24.2)	46 (46,5)	99	
Total patients		19 (25.0)	21 (27.6)	36 (47.4)	76	
Total alleles		38 (25.0)	42 (27.6)	72 (47.4)	152	

VDR, vitamin D receptor; NS, not significant; OR, odds ratio; CI, confidence interval.
[a] Hardy—Weinberg equilibrium (calculations with 1 degree of freedom; in parentheses values after calculation with 2 degrees of freedom).
[b] *p*-Values and odds ratios calculated for persistent vs. intermittent plus non-carriage state.
[c] (*ApaI aa* + *ApaI Aa*) vs. *ApaI AA*.
[d] *A* allele vs. *a* allele.
[e] *TaqI TT* vs. (*TaqI tt* + *TaqI Tt*).
[f] *T* allele vs. *t* allele.

involvement of the vitamin D immunomodulatory system. Interestingly, no significant association between VDR gene polymorphism and *S. aureus* nasal carriage status was demonstrated in a study with healthy volunteers.[17] Hence, we assume that it is in conditions with impaired immunity, such as in T1D, where VDR polymorphisms may affect susceptibility to *S. aureus* colonization and carriage. VDR gene polymorphisms may be involved in colonization by *S. aureus*, since vitamin D metabolites have been shown to play a role in macrophage activation and differentiation[10,11,21] and neutrophils have been demonstrated to play an important role in the innate immunity against *S. aureus*. Cole et al.[18] demonstrated that persistent *S. aureus* nasal carriage is accompanied by the release of epithelial- and neutrophil-derived host defense peptides into nasal secretions, providing a possible explanation for the failure of *S. aureus* clearance in individuals with macrophage dysfunction.

The *Apa*I genotype has been shown to modulate susceptibility to *Mycobacterium malmoense*[3] and hepatitis B virus,[4] and the *Taq*I genotype susceptibility to mycobacteria,[3,9] lower respiratory tract infections,[10] and periodontitis.[11,12] Vitamin D metabolites are known to suppress

tumor necrosis factor-α, interleukin (IL)-1, and IL-2, to stimulate transforming growth factor-β1 and IL-4 production, to inhibit T cell proliferation and inflammatory activity,[1] and thus to modulate Th1/Th2 balance,[3] to modulate expression of neutrophil genes, and restrict association and invasion of macrophages by pathogens including *S. aureus*.[20,29] *S. aureus* carriage is associated with opsonization,[13,14] and persistent *S. aureus* nasal carriage has been shown to be accompanied by the release of epithelial- and neutrophil-derived peptides into nasal secretions.[18] The *Taq*I polymorphism has been proposed to affect VDR-mediated production of IL-1α and IL-6 and production of anti-proteinase TIMP-1, which is a natural inhibitor of metalloproteinase-9.[10] On the other hand, VDR gene polymorphisms *Fok*I, *Bsm*I, *Apa*I, and *Taq*I are well known to influence the occurrence of T1D, although protective or predisposing effects vary greatly among studies and populations worldwide.[5–7]

Our findings are based on a relatively small population and need to be confirmed in larger cohorts. However, as we enrolled the majority of T1D individuals in the island of Crete, our study provides the benefits of a well-defined area and of a population with one of the lowest T1D incidence rates in Europe.[30] Violation of Hardy–Weinberg equilibrium in *Apa*I and *Taq*I polymorphisms in our study should be attributed to the small cohort size, and to inbreeding, a minimized migration rate, and closed gene pool of our island population. In addition, following suggestions by Chen and Chatterjee,[31] we used a 2-degrees-of-freedom Chi-square 'model-free' test of association that has robust power under unknown modes of genetic effects, and confirmed that Hardy–Weinberg equilibrium was not violated any more (Tables 3 and 4). Violation of Hardy–Weinberg equilibrium has been observed in several other studies investigating VDR polymorphisms in T1D and has been attributed to disease-related gene, non-random mating, and common genetic background of study populations.[32–34]

As polymorphisms *Apa*I and *Taq*I are probably non-functional and do not modify the VDR polypeptide sequence, linkage disequilibrium with one or more truly functional polymorphisms elsewhere in the VDR gene is assumed to explain the impact on VDR functionality. Research for additional polymorphisms across the VDR gene is therefore required to verify this hypothesis. In a sequence analysis performed in our laboratory using Genomatix (Genomatix Software GmbH 1998–2008), the polymorphic site was not found to disrupt any transcription factor binding site and, therefore, it does not seem plausible that the SNPs under study affect the expression levels of VDR. Our study was only of a descriptive nature, and future research should include functional tests on immune cells and focus on underlying mechanisms and on genetic and environmental factors potentially interfering in the relationship between VDR polymorphisms and *S. aureus* carriage and disease. Furthermore, future research might use peripheral blood mononuclear cells instead of whole blood.

Our findings suggest that genetic variation in the vitamin D receptor gene affects nasal colonization by *S. aureus* in individuals with type 1 diabetes, and further contribute to the understanding of the immunoregulatory role of vitamin D in the host's response and susceptibility to infection.

Acknowledgements

We thank Professor Dimitrios T. Boumpas, Laboratory of Internal Medicine, University of Crete, for his contribution to this study.

Conflict of interest: No conflict of interest to declare.

References

1. Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. *FASEB J* 2001;**15**:2579–85.
2. Uitterlinden AG, Fang Y, van Meurs JB, van Leeuwen H, Pols HA. Vitamin D receptor gene polymorphisms in relation to vitamin D related disease states. *J Steroid Biochem Mol Biol* 2004;**89–90**:187–93.
3. Gelder CM, Hart KW, Williams OM, Lyons E, Welsh KI, Campbell IA, et al. Vitamin D receptor gene polymorphisms and susceptibility to *Mycobacterium malmoense* pulmonary disease. *J Infect Dis* 2000;**181**:2099–102.
4. Suneetha PV, Sarin SK, Goyal A, Kumar GT, Shukla DK, Hissar S. Association between vitamin D receptor, CCR5, TNF-alpha and TNF-beta gene polymorphisms and HBV infection and severity of liver disease. *J Hepatol* 2006;**44**:856–63.
5. Marti Audi L, Marti G, Esteban C, Oyarzabal M, Chueca M, Gussinye M, et al. VDR gene polymorphism at exon 2 start codon (*Fok*I) may have influenced type 1 diabetes mellitus susceptibility in two Spanish populations. *Diabet Med* 2004;**21**:393–4.
6. Guja C, Marshall S, Welsh K, Merriman M, Smith A, Todd JA, et al. The study of CTLA-4 and vitamin D receptor polymorphisms in the Romanian type 1 diabetes population. *J Cell Mol Med* 2002;**6**:75–81.
7. Pani MA, Knapp M, Donner H, Braun J, Baur MP, Usadel KH, et al. Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. *Diabetes* 2000;**49**:504–7.
8. Selvaraj P, Vidyarani M, Alagarasu K, Prabhu Anand S, Narayanan PR. Regulatory role of promoter and 3′ UTR variants of vitamin D receptor gene on cytokine response in pulmonary tuberculosis. *J Clin Immunol* 2008;**28**:306–13.
9. Goulart LR, Ferreira FR, Goulart IM. Interaction of *Taq*I polymorphism at exon 9 of the vitamin D receptor gene with the negative lepromin response may favor the occurrence of leprosy. *FEMS Immunol Med Microbiol* 2006;**48**:91–8.
10. Roth DE, Jones AB, Prosser C, Robinson JL, Vohra S. Vitamin D receptor polymorphisms and the risk of acute lower respiratory tract infection in early childhood. *J Infect Dis* 2008;**197**:676–80.
11. Tachi Y, Shimpuku H, Nosaka Y, Kawamura T, Shinohara M, Ueda M, et al. Vitamin D receptor gene polymorphism is associated with chronic periodontitis. *Life Sci* 2003;**73**:3313–21.
12. de Brito Junior RB, Scarel-Caminaga RM, Trevilatto PC, de Souza AP, Barros SP. Polymorphisms in the vitamin D receptor gene are associated with periodontal disease. *J Periodontol* 2004;**75**:1090–5.
13. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis* 2005;**5**:751–62.
14. van den Akker EL, Nouwen JL, Melles DC, van Rossum EF, Koper JW, Uitterlinden AG, et al. *Staphylococcus aureus* nasal carriage is associated with glucocorticoid receptor gene polymorphisms. *J Infect Dis* 2006;**194**:814–8.
15. Canario DG, Idris MH, Cunha BA. Methicillin-resistant and -sensitive *Staphylococcus aureus* nasal colonization of insulin dependent children with juvenile onset diabetes mellitus. *Am J Infect Control* 2004;**32**:371–2.
16. Ahluwalia A, Sood A, Sood A, Lakshmy R, Kapil A, Pandey RM. Nasal colonization with *Staphylococcus aureus* in patients with diabetes mellitus. *Diabet Med* 2000;**17**:487–91.

17. Claassen M, Nouwen J, Fang Y, Ott A, Verbrugh H, Hofman A, et al. *Staphylococcus aureus* nasal carriage is not associated with known polymorphism in the vitamin D receptor gene. *FEMS Immunol Med Microbiol* 2005;**43**:173—6.
18. Cole AM, Tahk S, Oren A, Yosjioka D, Kim YH, Park A, et al. Determinants of *Staphylococcus aureus* nasal carriage. *Clin Diagn Lab Immunol* 2001;**8**:1064—9.
19. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* 1997;**10**:505—20.
20. Peacock SJ, de Silva I, Lowy FD. What determines nasal carriage of Staphylococcus aureus? *Trends Microbiol* 2001;**9**: 605—10.
21. Takahashi K, Nakayama Y, Horiuchi H, Ohta T, Komoriya K, Ohmori H, et al. Human neutrophils express messenger RNA of vitamin D receptor and respond to 1alpha,25-dihydroxyvitamin D_3. *Immunopharmacol Immunotoxicol* 2002;**24**: 335—47.
22. Breen J, Karchmer AW. *Staphylococcus aureus* infections in diabetic patients. *Infect Dis Clin North Am* 1995;**9**:11—24.
23. American Diabetes Association. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 2003;**26**(Suppl 1):S5—20.
24. Nouwen JL, Ott A, Kluytmans-Vandenbergh MF, Boelens HA, Hofman A, van Belkum A, et al. Predicting the *Staphylococcus aureus* nasal carrier state: derivation and validation of a 'culture rule'. *Clin Infect Dis* 2004;**39**:806—11.
25. Reis AF, Hauache OM, Velho G. Vitamin D endocrine system and the genetic susceptibility to diabetes, obesity and vascular disease: a review of evidence. *Diabetes Metab* 2005;**31**:318—25.
26. Nouwen J, Boelens H, van Belkum A, Verbrugh H. Human factor in *Staphylococcus aureus* nasal carriage. *Infect Immun* 2004;**72**: 6685—8.
27. Kinsman OS, McKenna R, Noble WC. Association between histocompatibility antigens (HLA) and nasal carriage of *Staphylococcus aureus*. *J Med Microbiol* 1983;**16**:215—20.
28. Summers KL, Marleau AM, Mahon JL, McManus R, Hramiak I, Singh B. Reduced IFN-alpha secretion by blood dendritic cells in human diabetes. *Clin Immunol* 2006;**121**:81—9.
29. Anand PK, Kaul D, Sharma M. Synergistic action of vitamin D and retinoic acid restricts invasion of macrophages by pathogenic mycobacteria. *J Microbiol Immunol Infect* 2008;**41**:17—25.
30. Mamoulakis D, Galanakis E, Bicouvarakis S, Paraskakis E, Sbyrakis S. Epidemiology of childhood type I diabetes in Crete, 1990—2001. *Acta Paediatr* 2003;**92**:737—9.
31. Chen J, Chatterjee N. Exploiting Hardy—Weinberg equilibrium for efficient screening of single SNP associations from case—control studies. *Hum Hered* 2007;**63**:196—204.
32. Mimbacas A, Trujillo J, Gascue C, Javiel G, Cardoso H. Prevalence of vitamin D receptor gene polymorphism in a Uruguayan population and its relation to type 1 diabetes mellitus. *Genet Mol Res* 2007;**6**:534—42.
33. 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. *Clin Endocrinol (Oxf)* 2000;**52**:575—80.
34. Györffy B, Vásárhelyi B, Krikovszky D, Madácsy L, Tordai A, Tulassay T, et al. Gender-specific association of vitamin D receptor polymorphism combinations with type 1 diabetes mellitus. *Eur J Endocrinol* 2002;**147**:803—8.