Vitamin D Pathway Gene Polymorphisms and Keratinocyte Cancers: A Nested Case-Control Study and Meta-Analysis

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Abstract. *Background: The vitamin D endocrine system is implicated in skin carcinogenesis and polymorphisms in genes associated with the vitamin D receptor (VDR) gene may alter the risk of keratinocyte cancers (basal cell carcinoma (BCC) and squamous cell carcinoma (SCC)). Materials and Methods: In a nested case-control study of 1,124 adults, we investigated associations between polymorphisms in VDR-related pathways and incident keratinocyte cancers during 11 years of follow-up using adjusted multivariate regression analysis. We also performed a meta-analysis of rs2228570, rs7975232, rs1544410 and rs739837 polymorphisms. Results: A total of 286 BCCs and 161 SCCs were newly-diagnosed during follow-up. Participants with rs2228570 and rs927650 recessive genotypes had a decreased risk of SCC (odds ratio (OR)=0.34, 95% confidence interval (CI)=0.17-0.68; OR=0.48, CI=0.27-0.84, respectively). Meta-analysis showed a lower SCC risk in rs1544410 recessive genotypes (summary OR (SOR)=0.74, CI=0.53-0.94), while rs7975232 and rs739837 recessive genotypes were associated with a decreased BCC risk (SOR=0.74, CI=0.56-0.98; SOR=0.65, CI=0.43-0.88). Conclusion: Our meta-analysis indicated that vitamin D receptor polymorphisms may be associated with the risk of keratinocyte cancers.*

Skin cancers are the most common cancer type in most white-skinned populations and impose a substantial burden on health care systems (1). The most important environmental risk factor for keratinocyte cancers, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), is high exposure to ultraviolet radiation (UVR) (2). High UVR exposure causes DNA damage and potentiates malignant cell transformation (2). In the skin, UVR also induces the synthesis of the steroid hormone vitamin D (3). In its biologically active form, vitamin D is a potent ligand for the vitamin D receptor (VDR).

A number of mechanisms have been proposed through which the cutaneous vitamin D endocrine system, UVR and skin carcinogenesis may be associated. Firstly, UVR induces characteristic alterations in DNA, such as mutations in the tumour suppressor gene *TP53* [Online Mendelian Inheritance in Man (OMIM) database: 191170], which result in increased mutant p53 expression (4, 5). If left unrepaired, a decrease in functional p53 results in uncontrolled cell proliferation (6). Secondly, activation of the VDR and its target genes inhibits cell proliferation and promotes keratinocyte differentiation (5, 7). Thirdly, initiation of the vitamin D pathway suppresses the hedgehog signaling pathway, that is typically activated in all BCC and some SCCs (8, 9); mice lacking the *VDR* gene have increased hedgehog signalling, leading to increased keratinocyte skin cancer risk (6, 10).

Polymorphisms in genes associated with the vitamin D signalling pathway, in particular the *VDR* [OMIM: 601769] gene, are thought to increase susceptibility to malignant transformation of skin cells (11). The *VDR* gene is highly polymorphic with over 600 variants reported to date (12). The most widely studied VDR polymorphisms include rs2228570 (Fok1), rs7975232 (Apa1), rs1544410 (Bsm1) and rs739837 (Bgl1). rs7975232, rs1544410 and rs739837 are restriction fragment length polymorphisms, which correspond to G/T, A/G and G/T substitutions, respectively (13). They are considered silent single nucleotide polymorphisms (SNPs), that they may decrease the stability of the *VDR* mRNA or reduce the intracellular activity of the vitamin D receptor (14, 15). rs2228570 is located in exon 4 and corresponds to a C/T substitution causing a longer VDR

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protein that is less transcriptionally active (16). At present it is the only *VDR* polymorphism known to result in a functional change in the VDR protein.

Despite the plausible association between vitamin D pathway and development of keratinocyte skin cancers, very few studies have been able to address this issue in population-based investigations to date. We, therefore, used data from a well-established cohort study of skin cancer to analyse whether *VDR*-related polymorphisms are associated with development of BCC and SCC. We also carried out a meta-analysis, combining the results from our study with that of all other evidence, to summarise current knowledge on the role of the four most studied *VDR* polymorphisms to date in keratinocyte cancer development.

Materials and Methods

Nambour Skin Cancer Study. This 11-year prospective community-based study was conducted amongst Caucasian residents of the Australian township of Nambour (26˚S latitude) who participated in the Nambour Skin Cancer Prevention Trial (1992-1996) (17). Briefly, the Trial involved 2,095 community members aged 20 to 69 years, who were randomly selected from the state electoral roll (enrolment is compulsory in Australia) in 1986, and invited to take part in a 5-year field study to evaluate the preventive effects of daily sunscreen application and daily beta-carotene supplementation on skin cancer. This study had ethical approval from the Queensland Institute of Medical Research ethics committee and participants provided written consent. On completion of the trial in 1996, participants were asked to continue in a post-trial follow-up study.

Questionnaires in 1992 and 1996 ascertained demographic and personal factors, including skin colour and smoking history, as well as sun exposure history. Intensive skin cancer surveillance consisted of twice-yearly questionnaires about all treated skin cancers between 1992 and 2002 and yearly questionnaires from 2003 to 2007. In 1992, 1994, 1995, 2000 and 2007, all active participants received a full skin examination for skin cancer and related signs, such as solar elastosis of the neck, by a dermatologist or a dermatologically trained medical practitioner. Self-reported skin cancers and those diagnosed during clinical examinations were confirmed histologically and cross-checks with independent pathology services throughout Queensland revealed any further lesions diagnosed in study participants, ensuring virtually 100% ascertainment of all skin cancers diagnosed between 1992 and 2007.

Twenty-nine SNPs from the *VDR* gene and vitamin D signalling pathway-associated genes that had previously been investigated for an association with skin cancer (any type) or other cancer types were selected based on the published literature. This resulted in the following SNPs being selected: *VDR* gene: rs2228570, rs739837, rs1544410, rs4516035, rs11568820, rs11574143, rs12717991, rs2107301, rs2238135, rs2254210, rs2853564, rs3118523, rs4760648, rs4760658, rs757343, rs7975232. *CYP24A1* gene: rs927650, rs2585428, rs6013897, rs6097809. *CYP2R1* [OMIM: 608713] gene: rs10741657. GC (Vitamin D binding) [OMIM 139200] gene: rs1155563, rs12512631, rs16846876, rs1746825, rs7041. *NADSYN1* [OMIM: 608285] gene: rs12785878. *TMC8* [OMIM: 605829] gene: rs7208422. *RXRA* [OMIM: 180245] gene: rs7861779. DNA extraction from peripheral blood samples was performed using QiAMP blood kit (Qiagen, Inc., Chadstone Centre, VIC, Australia). NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Scoresby, VIC, Australia) quantified DNA samples, 5 μg of each gDNA was aliquoted into 384 well plates and dried at room temperature. Well positions of DNA samples were randomly assigned. Assay reproducibility was assured on each plate by including 36 randomly chosen repeat samples and four negative, template-free controls. Genotyping assays were designed using standard procedures and SNPs were typed using iPLEXTM chemistry on a MALDI-TOF Mass Spectrometer (Sequenom Inc., San Diego, CA, USA) (18). Primer sequences are available on request. Of the 1,191 subjects for which genotyping was attempted, 67 were excluded due to failed attempts or missing data for at least 5 SNPs. A chi-square test was performed to assess whether genotypes were in Hardy-Weinberg equilibrium.

Blood was drawn from study participants in August 1996 (when average seasonal UV index is moderate to high). Serum 25(OH)-vitamin D levels were measured by Liaison 25(OH)D assay (19). High intra-assay correlation was demonstrated by measuring twelve random samples in duplicate (20); a further 29 random samples were compared with high-performance liquid chromatography analysis. Median serum vitamin D values in the control population were used to categorise the variable into 'high' and 'low' levels.

Meta-analysis. The four most commonly researched *VDR* polymorphisms in relation to keratinocyte cancers were selected for meta-analysis; 'rs2228570', 'rs7975232', 'rs1544410' and 'rs739837'. The Pubmed, Web of Science, Embase and Scopus databases were searched to identify studies that had investigated the association between keratinocyte skin cancer risk and *VDR* polymorphisms and were published in English, with the last updated search being performed on January 10th, 2015. The following search terms were used: 'skin cancer' or 'SCC' or 'BCC' or 'non-melanoma skin cancer' or 'keratinocyte caner', as well as 'VDR' or 'vitamin D receptor' and/or 'polymorphism' 'rs2228570' 'rs7975232' 'rs1544410' 'rs739837'. Studies were required to meet the following inclusion criteria: (i) Evaluation of keratinocyte cancer risk and *VDR* polymorphisms, (ii) available genotype frequencies in cases and controls, (iii) Hardy-Weinberg equilibrium must apply to genotype frequency of control population. The following exclusion criteria were used: (a) Reviews and abstracts, (b) studies in which genotype frequencies were not reported, (c) studies that did not distinguish between SCC and BCC outcomes, (d) if Hardy-Weinberg equilibrium in control genotype frequencies was not met.

Two independent authors assessed and reached a consensus on all potentially relevant studies. A third author assessed the study if dispute arose. The following items were extracted from each article: first author, country of study and year of publication, cancer types, total number of cases and controls, as well as genotype distributions.

Statistical analysis. Outcomes were the occurrence of histologically-confirmed BCC or SCC at any time after completion of the trial and DNA collection in 1996 through to 2007. Logistic regression models were used to calculate crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) to assess BCC and SCC risk. Associations were tested using recessive *versus* dominant models (hh *vs*. HH + Hh). The multivariate analyses were adjusted for age, sex, previous treatment allocation (daily sunscreen use *versus* discretionary sunscreen use and beta-carotene supplement *versus* placebo), skin colour and elastosis of the neck (indicator of high cumulative sun

Table I. *Characteristics of the study population (n=1,124) by BCC and SCC occurrence between 1996-2007.*

	BCC			SCC		
	Yes (n=286)	No (n=838)	*p*-Value	Yes (n=163)	No (n=961)	*p*-Value
Age (1996)						
Mean age (years)	62	58	<0.0001	63	52	<0.0001
Gender						
Male	141 (49%)	361 (43%)	0.07	88 (54%)	414 (43%)	0.01
Female	145 (51%)	477 (57%)		75 (46%)	547 (57%)	
Skin colour (1996)						
Fair	179 (63%)	450 (54%)	0.005	109 (67%)	520 (54%)	0.001
Medium	95 (33%)	327 (39%)		49 (30%)	373 (39%)	
Olive/black	12 (4%)	60 (7%)		5 (3%)	67 (7%)	
Elastosis of the neck (1996)						
Mild	32 (11%)	208 (25%)	<0.0001	7 (4%)	233 (24%)	<0.0001
Moderate	138 (49%)	400 (48%)		63 (39%)	475 (49%)	
Severe	114 (40%)	229 (27%)		91 (57%)	252 (26%)	
Smoking history (life-time pack years through to 1996)						
Mean	10.8	13.8	0.1	14.7	8.4	0.0001
Serum vitamin D levels (1996)						
Mean (nmol/L)	66.3	65.4	0.5	63.1	66.0	0.1

BCC, Basal cell carcinoma; SCC, squamous cell carcinoma.

exposure). Where multiple SNPs within one gene were tested, Bonferroni corrections for multiple comparisons were performed. SNPs significantly associated with keratinocyte cancers in multivariate analyses (rs2228570 and rs927650) were assessed further to determine whether vitamin D levels caused confounding (included in multivariate regression) or effect modification (with likelihood ratio test). Multivariate analyses were conducted with both disease-free controls and controls that included participants affected by other skin cancer types. Since there were no significant differences in the respective effect sizes demonstrated, results of the latter approach are shown. SNPs included in the multivariate regression analysis were selected based on the following criteria: (i) SNPs investigated in previous keratinocyte cancer studies (rs2228570, rs7975232, rs1544410, rs739837, rs11568820, rs4516035), (ii) any of the 30 selected SNPs that had a chi-squared *p*-value of <0.10 for allelic comparison (SCC: rs12512631, rs927650 and BCC: rs2254210). Statistical analyses were performed using SAS statistical software version 9.3 (SAS Institute Inc., Cary, NC, USA).

In the meta-analysis, summary ORs with 95% CI were obtained for genotype comparisons for all four polymorphisms (rs2228570, rs7975232, rs1544410, rs739837). Heterozygote (Hh *vs*. HH) and recessive (hh *vs*. HH + Hh) models were used to assess the associations. A meta-analysis with two studies was deemed appropriate providing there was no significant heterogeneity. Heterogeneity testing, using the X^2 based Q-test, was considered significant at $p<0.10$ (21). Summary odds ratios (SOR) were obtained using a fixed-effects model, where $p>0.10$ (22). Otherwise, a random effects model was applied (23). When between-studies heterogeneity was absent, these methods provide similar summary odds ratios. Statistical significance of the pooled OR was determined with a Z-test and results were considered significant at $p<0.05$. Egger's tests and funnel plots were used to assess potential publication bias in analyses with at least three included studies ($p<0.05$ was considered significant). All statistical tests for the meta-analysis were performed using STATA 11.0 software (StataCorp LP, College Station, TX, USA).

Results

Nambour Skin Cancer Study. From 1996 to completion of the trial in 2007, 286 (25%) of the 1,124 study participants developed BCC and 163 (15%) developed SCC. Those affected by BCC were older, had more severe elastosis of the neck and fair skin more commonly than persons not affected. Participants with SCC were older, more likely to be male, to have a fair skin type, more severe elastosis of the neck and were more likely to be current smokers compared to persons not affected by SCC (Table I). Genotype distributions were consistent with Hardy-Weinberg equilibrium in the controls (all $p>0.01$).

In unadjusted analyses, rs7975232 GG + GT dominant genotypes were associated with a decreased occurrence of BCC when compared with TT genotype (OR=0.67, CI=0.46-0.96, $p=0.029$). Study participants with recessive genotypes for the rs739837 polymorphism also had decreased risk of BCC in a univariate model (GG + GT *vs*. TT: OR=0.65, CI=0.45-0.95, $p=0.024$). Although the effect sizes remained similar, the associations were no longer statistically significant in the adjusted model and after correction for multiple testing within each gene (three SNPs in the *VDR* gene after adjusting for linkage disequilibrium, $p=0.05/3=0.017$) (Table II).

Table II. *Odds ratios from nested case-control study for the association between VDR pathway polymorphisms and BCC.*

Gene	SNP	Comparison	OR[a] basic model	OR[b] multivariate
VDR	rs2254210	AA *vs*. GG + GA (Ref)	(286/113)	(271/1084)
			1.39 (0.97-2.00)	1.29 (0.88-1.90)
			p=0.07	*p*=0.19
VDR	rs2228570	TT *vs*. CC + CT (Ref)	(285/1118)	(270/1079)
	(Fok1)		0.92 (0.62-1.37)	0.89 (0.59-1.36)
			p=0.69	*p*=0.60
VDR	rs7975232	TT *vs*. GG + GT (Ref)	(286/1122)	(271/1083)
	(Apa1)		0.67 (0.46-0.96)	0.73 (0.50-1.09)
			p=0.029	*p*=0.12
VDR	rs1544410)	AA *vs*. GG + AG (Ref)	(286/1123)	(271/1084)
	(Bsm1		1.00 (0.69-1.45)	0.98 (0.66-1.45)
			p=0.98	*p*=0.90
VDR	rs739837	TT *vs*. GG + GT (Ref)	(286/1121)	(271/1082)
	(Bgl1)		0.65 (0.45-0.95)	0.72 (0.48-1.06)
			p=0.024	*p*=0.09
VDR	rs11568820	AA *vs*. GG + GA (Ref)	(272/1056)	(259/1019)
	(Cdx2)		0.77 (0.38 –1.57)	0.81 (0.39-1.71)
			p=0.47	*p*=0.58
VDR	rs4516035	CC *vs*. TT + TC (Ref)	(284/1119)	(270/1079)
	(EcoRV)		1.07 (0.76-1.51)	0.97 (0.67-1.39)
		p=0.69	*p*=0.85	

[a]Crude odds ratio and 95% CI. [b]Adjusted for age, gender, sunscreen treatment, beta-carotene supplementation, skin colour and elastosis of the neck.

SCC risk was decreased in participants with rs2228570 TT *vs*. CC + CT genotypes and this remained statistically significant after adjusting for confounders and correction for multiple testing (OR_{adj}=0.34, CI=0.17-0.68, *p*=0.002). Vitamin D levels did not cause any confounding in this model. The interaction model with vitamin D levels was not significant (*p*=0.14), indicating absence of effect modification. Persons who were homozygous for the recessive rs927650 allele were also at decreased risk of SCC in both crude and adjusted models (O_{Radj}=0.48, CI=0.27-0.84, *p*=0.011). Vitamin D was neither a confounder nor effect modifier in this model (*p*=0.15) (Table III).

Meta-analysis. In addition to the Nambour Skin Cancer Study, we identified 3 other studies that met the meta-analysis inclusion criteria. The characteristics of selected studies are displayed in Table IV. For BCC analysis, three studies had recruited a total of 713 cases and 1,264 controls for rs2228570 and rs1544410, three studies recruited 518 cases and 1,030 controls for rs7975232, while two studies had recruited 376 cases and 888 controls for rs739837. For SCC analysis, two studies had recruited 439 cases and 1,240 controls for rs2228570 and rs1544410, two studies recruited 261 cases and 1,013 controls for rs7975232, whereas two studies recruited 261 cases and 1,013 controls for rs739837. Included studies used diverse genotyping methods, including SEQUENOM, TaqMan and PCR-RFLP. Genotype distributions were consistent with the Hardy-Weinberg equilibrium in the controls for all studies (*p*<0.01).

A summary of the meta-analyses results and heterogeneity tests is shown in Table V. Heterogeneity between studies was significant for rs2228570 in CT *vs*. CC (BCC) and TT *vs*. CT + CC (BCC and SCC) analyses. For the rs1544410 polymorphism, the AA genotype was associated with decreased SCC risk (AA *vs*. GG + AG: SOR=0.74, CI=0.53-0.94). Persons with the rs7975232 homozygous variant for the minor allele (TT) had a lower risk of BCC compared to those who had GG or GT genotypes (SOR=0.74, CI=0.56-0.98). rs739837 TT genotype was associated with a decreased risk of BCC when compared with GG + GT genotypes (TT *vs*. GG + GT: SOR=0.65, CI=0.43-0.88). There were no associations between rs2228570 genotypes and BCC or SCC susceptibility.

Publication bias was assessed by funnel plots and Egger's test. Shape symmetry of the funnel plots was visually inspected for the various genotype models and an Egger's test performed in models that included at least three studies, to provide statistical evidence of funnel plot symmetry. The results indicated no significant evidence of publication bias.

Discussion

rs2228570, known as the *FokI* polymorphism, results in a longer VDR protein and exerts less transcriptional activity (16). The T allele has been reported to cause a >10-fold

Table III. *Odds ratios from nested case-control study for the association between VDR pathway polymorphisms and SCC.*

Gene	SNP	Genotype	OR[a] basic model	OR[b] mutivariable	OR[c] multivariable
GC	rs12512631	CC *vs*. TT + TC (Ref)	(161/1098)	(153/1061)	
			1.18 (0.71-1.94) *p*=0.53	1.24 (0.70-2.19) *p*=0.46	-
CYP24A1	rs927650	TT *vs*. CC + CT (Ref)	(161/1114)	(153/1075)	
			0.48 (0.29-0.80) *p*=0.0048	0.48 (0.27-0.84) *p*=0.011	0.49 (0.26-0.92) *p*=0.027
VDR	rs2228570	TT *vs*. CC + CT (Ref)	(162/1118)	(154/1079)	
	(Fok1)		0.37 (0.19-0.73) *p*=0.0037	0.34 (0.17-0.68) *p*=0.0022	0.38 (0.18-0.79) *p*=0.005
VDR	rs7975232	TT *vs*. GG + GT (Ref)	(116/1122)	(155/1083)	-
	(Apa1)		0.73 (0.47-1.16) *p*=0.19	0.90 (0.54-1.48) *p*=0.67	
VDR	rs1544410	AA *vs*. GG + AG (Ref)	(163/1123)	(155/1084)	
	(Bsm1)		1.19 (0.77-1.84) *p*=0.44	1.17 (0.71-1.91) *p*=0.54	-
VDR	rs739837	TT *vs*. GG + GT (Ref)	(162/1121)	(154/1082)	
	(Bgl1)		0.73 (0.47-1.16) *p*=0.18	0.87 (0.53-1.45) *p*=0.60	-
VDR	rs11568820	AA *vs*. GG + GA (Ref)	(157/1056)	(149/1019)	
	(Cdx2)		0.67 (0.26-1.73) *p*=0.41	0.50 (0.17-1.48) *p*=0.21	-
VDR	rs4516035	CC *vs*. TT + TC (Ref)	(162/1119)	(154/1080)	
	(EcoRV)		0.93 (0.60-1.44) p=0.74	0.92 (0.57-1.48) p=0.72	-

[a]Crude odds ratio. [b]Adjusted for age, gender, sunscreen treatment, beta-carotene supplementation, skin colour and elastosis of the neck. [c]Adjusted for age, gender, sunscreen treatment, beta-carotene supplementation, skin colour, elastosis of the neck and vitamin D.

Table IV. *Characteristics of studies included in the meta-analysis.*

First author	Year	SNP	Cancer	Cases	Controls	Ethnicity	Country
Han *et al*. (26)	2007	rs2228570 (Fok1)	BCC	285	285	Caucasian	USA
		rs1544410 (Bsm1)	SCC	278	278		
Lesiak *et al*. (16)	2011	rs2228570 (Fok1)	BCC	142	142	Caucasian	Poland
		rs1544410 (Bsm1)					
		rs7975232 (Apa1)					
Köstner *et al*. (29)	2012	rs7975232 (Apa1)	BCC	90	51	Caucasian	Germany
		rs739837 (Bgl1)	SCC	100	51		
von Schuckmann	2016	rs2228570 (Fok1)	BCC	286	837	Caucasian	Australia
et al. (this study)		rs7975232 (Apa1)					
		rs1544410 (Bsm1)					
		rs739837 (Bgl1)	SCC	161	962		

BCC, Basal cell carcinoma; SCC, squamous cell carcinoma.

increased risk for BCC (13, 16). Despite this previously reported strong association, in our study there was no association between rs2228570 and BCC, whereas SCC risk was lower in persons with the recessive genotype for rs2228570. Gnagnarella and colleagues reported a significant association with rs2228570: SOR=1.24 (95% CI=1.01-1.54) for TT *versus* CC, but notably this study did not differentiate between skin cancer types (24). Unsurprisingly, the meta-analysis demonstrated a high degree of variation in the direction of the effects between studies. A recent large study conducted by Jsorgenson and colleagues (25) (534 BCC cases; 2,296 controls) was excluded from our meta-analysis because genotype frequencies were not reported. This study showed no significant association between the rs2228570 polymorphism and BCC risk (OR=1.10, 95% CI=0.96-1.26). For sensitivity analysis, we repeated our meta-analysis for rs2228570 and BCC, including the estimated genotype frequencies from Jorgensen and colleagues' study, but results were not materially altered: TT *vs*. CC + CT (SOR=1.17, 95% CI=0.85-1.51).

Our own study results showed that persons with rs927650 recessive genotypes were less likely to develop SCC. The rs927650 genotype is located on the *CYP24A1* gene, which encodes catabolic 24-hydroxylase. This enzyme is responsible for inactivating vitamin D metabolites. *CYP24A1* polymorphisms may alter SCC risk by increasing or

Table V. *Summary odds ratios and heterogeneity estimates from meta-analysis for the association between VDR polymorphisms and skin cancer.*

SNP	Cancer	Comparisons	SOR and 95% CI	Q test
rs2228570 (Fok1)	BCC	CT *vs*. CC	1.12 (0.64-1.61) R	0.04
		TT *vs*. CC + CT	1.39 (0.65-2.14) R	0.03
	SCC	CT *vs*. CC	0.98 (0.78-1.19) F	0.74
		TT *vs*. CC + CT	0.88 (0.16-1.91) R	0.001
rs7975232 (Apa1)	BCC	GT *vs*. GG	1.09 (0.79-1.39) F	0.18
		TT *vs*. GG + GT	0.74 (0.56-0.98) F	0.61
	SCC	GT *vs*. GG	1.00 (0.64-1.36) F	0.78
		TT *vs*. GG + GT	0.74 (0.43-1.04) F	0.98
rs1544410 (Bsm1)	BCC	AG *vs*. GG	1.13 (0.86-1.39) F	0.45
		AA *vs*. GG + AG	1.01 (0.76-1.26) F	0.75
	SCC	AG *vs*. GG	0.96 (0.73-1.20) F	0.34
		AA *vs*. GG + AG	0.74 (0.53-0.94) F	0.41
rs739837 (Bgl1)	BCC	GT *vs*. GG	1.03 (0.72-1.34) F	0.55
		TT *vs*. GG + GT	0.65 (0.43 0.88) F	0.95
	SCC	GT *vs*. GG	0.99 (0.63-1.34) F	0.57
		TT *vs*. GG + GT	0.75 (0.44-1.06) F	0.80

SOR, Summary odds ratio; CI, confidence interval; Q-test, X^2 test for heterogeneity; R, random effects model; F, fixed effects model.

decreasing activity of the enzyme, hence interfering with vitamin D metabolism and predisposing to cancer development. We have previously demonstrated that SCC incidence tends to be lower in persons with high serum 25(OH)-vitamin D concentrations (20).

rs1544410 is a restriction fragment length polymorphism located in intron 8 at the 3' end of the *VDR* gene. As a silent SNP, it does not change the amino acid sequence of the encoded protein, yet it may affect gene expression through regulating mRNA stability (20). In our study there was no association between rs1544410 genotypes and BCC or SCC. Han and colleagues on the other hand previously reported a 1.5-time higher risk of SCC in persons with rs1544410 dominant GG genotypes and a greater than two-fold risk in women with such genotypes and high vitamin D levels (26). As a result, our meta-analysis, comprising the findings of Han and colleagues and our findings, supported an overall decreased SCC risk in persons carrying the rs1544410 recessive AA genotype compared with GG + GA individuals.

Like rs1544410, rs7975232 polymorphism is located at the 3' end of the *VDR* gene, in intron 8. Carless and colleagues investigated the relationship between rs7975232 and solar keratoses, which are established SCC precursors and biomarkers (27). When comparing individuals with fair skin to individuals with medium or olive skin, solar keratosis prevalence was five-fold higher in individuals with GT genotypes (27). In our meta-analysis, however, we found no association between rs7975232 and SCC and, thus, heterogeneity between studies or populations may exist. In our study BCC risk was decreased in persons with the recessive TT genotype compared with GG+GT genotypes, yet this association was no longer significant in the adjusted model and may indicate an association between rs7975232 and a covariate. Lesiak and colleagues reported that rs7975232 heterozygous variants contributed to BCC risk (GT *vs*. GG: OR=1.94, 95% CI=1.04-3.60) (16). Our meta-analysis showed a decreased BCC susceptibility for persons carrying recessive TT genotype, thus indicating that the totality of evidence to date suggests that the recessive rs7975232 TT genotype may be protective for BCC.

The rs739837 polymorphism is located 303 base-pairs downstream of the exon 9 stop codon. Fair skin colour and Fitzpatrick's phenotype I/II, which are risk factors for keratinocyte cancers, are marginally associated with rs739837 (28). The study by Köstner and colleagues is the only other study that reported on this genotype in relation to keratinocyte cancers and they found no significant association with BCC or SCC (29). In comparison, our study had higher statistical power and our results showed a decreased BCC risk in recessive genotypes in the unadjusted model. These associations remained significant when results from Köstner and colleagues were pooled with our findings, with the TT genotype showing an overall protective association with BCC across the two studies.

The final two *VDR* SNPs that we assessed in relation to BCC and SCC, rs11568820 and rs4516035, lie in strong linkage disequilibrium (D=0.97) with three haplotypes accounting for >95% of genotypes (30). Consistent with our results, Jorgensen and colleagues demonstrated that neither rs4516035, nor rs11568820 polymorphisms were associated with an increased risk of BCC (25). Thus, within the limited published data there is no evidence for rs11568820 or rs4516035 involvement in keratinocyte cancer risk.

Our data and samples were drawn from a well-established cohort study of skin cancer in which all skin cancers were histologically confirmed and high-quality data on confounding factors were available. Case or control status was ascertained over an 11-year period by closely monitoring skin cancer incidence, reducing the possibility of false negatives. The study population was a community-based sample and not subject to selection bias. Our results were robust to the choice of comparison group, with conclusions being the same whether controls were chosen as persons without any type of keratinocyte cancer or when controls included persons who may have had another skin cancer type than the one being analysed. Our baseline assessment of serum vitamin D levels was carried out at the same time (season) for all participants and by one laboratory with high validity and repeatability. Due to the limited number of observations we dichotomised vitamin D status to above and below the median; if a larger number of observations had been available, analyses of sub-groups

defined by clinically relevant cut-points would have been preferred. Previous analyses showed that serum vitamin D levels were associated with keratinocyte cancer occurrence in this study population (20), thus we stratified analyses by serum levels to ascertain evidence of effect modification.

In relation to our meta-analysis, a number of the summary odds ratios were obtained from only two studies. Despite the high quality data on confounding factors in our own study, we were unable to adjust the meta-analysis for potential confounders (in particular serum vitamin D levels, dietary vitamin D intake, sun exposure and skin phenotype) because of inconsistency or absence in some studies. The lack of reproducibility of our own and other studies' results in meta-analysis may indicate the importance of confounders, and replication of these analyses in larger datasets with high quality skin cancer and confounder assessment would help increase the level of evidence available to date for a role of *VDR* polymorphisms in development of keratinocyte cancers.

Conclusion

The collective evidence to date suggests that persons with the recessive genotype for rs1544410 (GG) may have a reduced risk of SCC, while those with the recessive rs7975232 (TT) and rs739837 (TT) genotypes may have decreased susceptibility to BCC development. We found no conclusive evidence of an effect of rs2228570 polymorphisms on keratinocyte cancer risk. Our results further suggest that persons with the rs927650 recessive genotype may have a decreased risk of SCC, possibly due to alteration in the activity of the catabolic 24-hydroxylase enzyme responsible for inactivating vitamin D metabolites. Further studies with large numbers of participants and high quality information about possible confounding factors and vitamin D status are needed to confirm these relationships.

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