The influence of vitamin D receptor gene polymorphism on basal cell carcinoma development in the Polish population

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Abstract

Introduction: Basal cell carcinomas (BCC) are the most common cancers in Caucasian populations. Their pathogenesis is not fully known, but genetic and environmental factors are relevant. Among genetic disturbances, genes encoding intracellular signalling pathways and regulatory proteins are considered the most important. In recent years associations between polymorphisms in the *VDR* gene and different internal organs and skin neoplasms have been found.
Aim: To assess the frequency of *FokI*, *BsmI*, *TaqI* and *ApaI* polymorphisms in the *VDR* gene in patients with BCC.
Material and methods: *VDR* polymorphisms were assessed in 142 BCC patients using PCR-RFLP.
Results: The presence of TT genotype in *FokI* polymorphism was linked to the highest risk for BCC development (OR = 10.145, $p < 0.001$). Other genotypes such as GT in *ApaI* and TT and TC in *TaqI* also statistically increased the risk for BCC, but these associations were weaker. No correlation between the presence of any analysed polymorphisms and constitutive features such as hair or eye colour or skin phototype was found.
Conclusions: The obtained results confirm the role of the polymorphisms in the *VDR* gene in the development of basal cell carcinomas in patients of Polish origin.

Key words: basal cell carcinoma, *VDR* gene polymorphism, cancerogenesis.

Introduction

Over the last years, increasing incidence of non-melanocytic skin cancers (including basal cell carcinoma [BCC] and squamous cell carcinoma [SCC]) has been observed. Because in most European countries, including Poland, BCC are not taken into account in the national cancer registry, it is hard to determine their incidence [1, 2].

Excessive UV exposure is one of the greatest risk factors of BCC development, especially exposure to UVB, which can generate DNA damage and synthesis of vitamin D by keratinocytes. Because of this observation, it was suspected that synthesis of vitamin D in the epidermis is one of the primary protective mechanisms of UV-induced DNA damaged [3-5].

Genetic factors which play a role in BCC development include mutations in genes encoding intracellular *sonic hedgehog* signal transduction and polymorphism of the vitamin D receptor (VDR). Recent data have shown correlations between the above-mentioned genetic disturbances and pathogenesis of BCC development and have provided a new point of view on the pathogenesis of non-melanocytic skin cancers [6].

The vitamin D receptor gene is localized on chromosome 12q12-q14. *FokI* (rs2228570), *BsmI* (rs1544410), *ApaI* (rs7975232) and *TaqI* (rs731236) are among the most

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described polymorphisms of this gene in the literature, which play a role in BCC development. Vitamin D receptor is responsible for the effect of vitamin D in cells and regulates intracellular transduction, whose disturbance leads to cancerogenesis [7-9]. It was proved that various *VDR* gene polymorphisms have an influence on vitamin D synthesis, metabolism and degradation in skin, liver and kidney. Vitamin D receptor demonstrates expression on keratinocytes and is a natural ligand for calcitriol, which has the ability to suppress proliferation and induce differentiation of human keratinocytes [10].

Although many studies on the correlation between VDR polymorphisms and development of internal and skin neoplasms exist, data from different populations are not consistent [11, 12].

Aim

The aim of this study was to assess the prevalence of *FokI*, *BsmI*, *TaqI* and *ApaI* polymorphisms in the *VDR* gene in Polish patients with BCC.

Material and methods

The study group consisted of 142 Caucasian patients with BCC (71 males, 71 females, age range 45-78 years), treated between 2006 and 2009 in the Department of Dermatology and Venereology in Lodz and in the Department of Dermatology, Venereology and Allergology in Gdansk. Basal cell carcinoma diagnosis was confirmed by clinical manifestation and histopathological examination. The control group consisted of 142 healthy volunteers with negative cancer history, matched by age and sex. All patients gave written consent to participate in the study. The study was approved by the Local Ethics Committee at the Medical University of Lodz. In all patients and volunteers eye and hair colour and skin type were assessed by the Fitzpatrick scale [13]. Clinical characteristics of both groups are presented in Table 1. From all BCC patients and volunteers DNA was isolated from peripheral blood to assess four polymorphisms of the *VDR* gene: *ApaI*, *TaqI*, *FokI* and *BsmI*.

Genotyping of the *ApaI* polymorphism in *VDR*

Polymerase chain reaction (PCR) amplification of the region containing the polymorphism rs7975232 (64978G/T) was performed using the forward primer in intron 10 (5`-GCAAAGATAGCAGAGCAGAGTTCC-3`) and the reverse primer in exon 11 (5`-AGGTTGGACAGGAGAGAGAATGG-3`). The PCR conditions employed were as follows: 95°C for 5 min, and 35 cycles using the following temperature profile: 94°C for 30 s, 56°C for 30 s, 72°C for 45 s, and final elongation at 72°C for 7 min. The PCR products were 781 bp long (TT) and were digested with FastDigest® ApaI (Fermentas, Ontario, Canada) at 37°C for 20 min, and finally subjected to electrophoresis in 2% agarose gels containing ethidium bromide. The lengths of the restriction fragments were 469 bp and 312 bp (GG) and 781 bp, 469 bp and 312 bp (GT).

Genotyping of the *FokI* polymorphism in *VDR*

A fragment of exon 4 containing the polymorphism rs10735810 (2T/C, Met1Lys) was PCR amplified from DNA using specific primers in intron 3: 5'-CACCCTGGAAGTAAAACA-3' and in intron 4: 5'-ACCTGAAGAAGCCTTTGC-3'). The cycle parameters were as follows: 1 cycle at 95°C for 5 min for an initial denaturation, followed by 35 cycles of denaturation for 30 s at 94°C, primer annealing for 30 s at 56°C, primer extension for 45 s at 72°C and a final extension for 7 min at 72°C. The PCR products were digested with 1 µl of FastDigest® FokI (Fermentas, Ontario, Canada) at 37°C for 20 min, and separated on 2% agarose gels. The TT genotype was identified by the presence of 142 and 344 bp, CC 486 bp and TC 142 bp, 344 bp and 486 digestion products.

Exon 4 containing the polymorphism rs10735810 (2T/C, Met1Lys) was amplified by the polymerase chain reaction, using specific primers in intron 3: 5'-CACCCTGGAAGTAAAACA-3' (*forward*) and in intron 4: 5'-ACCTGAAGAAGCCTTTGC-3' (*reverse*).

Genotyping of the *BsmI* polymorphism in *VDR*

Polymerase chain reaction amplification of the region containing the polymorphisms rs1544410 (63980G/A) was performed using the forward primer in intron 10

Tab. 1. Clinical characteristics of BCC patients and control group

	BCC patients	Control group
Number of patients	142	142
Age [years]	56 (45-78)	51 (39-76)
Sex	F (*n* = 71), M (*n* = 71)	F (*n* = 72), M (*n* = 70)
Hair colour	Bright (*n* = 85), dark (*n* = 57)	Bright (*n* = 89), dark (*n* = 53)
Eye colour	Bright (*n* = 79), dark (*n* = 63)	Bright (*n* = 73), dark (*n* = 69)
Skin phototype	I/II (*n* = 58), III (*n* = 64), IV (*n* = 20)	I/II (*n* = 54), III (*n* = 66), IV (*n* = 22)

F – female, M – male

(5'-GGGGAGTATGAAGGACAAAGAC-3') and the reverse primer in the same intron (5'-TTCTCACCTCTAACCAGCGG-3'). The PCR conditions employed were as follows: 94°C for 30 s, 56°C for 30 s, 72°C for 45 s, and final elongation at 72°C for 7 min. The PCR products were digested with FastDigest® HinP1I (Fermentas, Ontario, Canada) at 37°C for 20 min, and finally subjected to electrophoresis in 2% agarose gels containing ethidium bromide. The lengths of the restriction fragments were 429 bp (AA), 282 bp and 147 bp (GG) and 429 bp, 282 bp and 147 bp (AG).

Genotyping of the *TaqI* polymorphism in *VDR*

PCR-RFLP based assays were used to identify alleles containing polymorphism rs731236 (1056C/T, Ile352Ile) in exon 11. *TaqI* RFLPs were determined in a single polymerase chain reaction (PCR) fragment of 740 bp from genomic DNA using a forward primer in intron 10 (5'-CAGAGCATGGACAGGGAGCAAG-3') and a reverse primer in exon 11 (5'-GCAACTCCTCATGGCTGAGGTCTC-3'). The polymorphic *TaqI* restriction endonuclease site is a synonymous isoleucine codon change in the coding region of the *VDR* gene. Polymerase chain reaction products were then digested with FastDigest® *TaqI* 65°C for 20 min (Fermentas, Ontario, Canada), and the products were examined after electrophoresis in 2% agarose gels. Homozygous CC results in two fragments of 245 bp and 495 bp, while homozygous TT results in 740 bp. Heterozygotes exhibit all three bands of 245 bp, 495 bp and 740 bp.

Statistical analysis

To assess the relationship between the dependent or independent variables logistic regression was used. Differences of genotype in the studied population were analyzed for deviation from Hardy-Weinberg equilibrium and χ^2 tests. Risk assessment of individual genotypes coexisting with the disease and other characteristics was performed using the odds ratio (OR). Results were considered statistically significant at the significance level $p < 0.05$. Statistical analysis was performed using the software Statistica.

Results

The distribution of genotypes of the *VDR* polymorphisms in both groups was consistent with the Hardy-Weinberg equilibrium.

Distribution of genotypes in *ApaI*, *FokI* and *TaqI* polymorphisms was statistically significantly different between the control group and BCC group. There were no differences in *BsmI* polymorphism. Genotype GG of *ApaI* polymorphism occurred in 26.8% of the BCC group, genotype GT in 49.3% and TT in 23.9%. Genotype TT of *TaqI* polymorphism occurred in 27.5% of patients, CT – 47.9% and CC – 24.6% (Tab. 2). Multiple logistic regression analysis showed that genotype TT of *FokI* increases risk of BCC development more than 10 times (OR = 10.145, $p < 0.001$) and genotype CT – 5 times (OR = 5.111, $p < 0.001$). Moreover, occurrence of genotype TC or TT and *TaqI* polymorphism increases BCC development more than 3 times (OR = 3.088, $p < 0.001$; OR = 3.384, $p = 0.001$). When analysed genotype in *ApaI* polymorphism, it was shown that the presence of GT genotype significantly increases the risk of BCC development BCC (OR = 1.942, $p = 0.036$), however this association was not so relevant (Tab. 3). Additionally we found no correlation between analysed polymorphisms in the *VDR* gene and constitutional features such as eye and hair colour and skin phototype ($p > 0.05$ for all comparisons).

Discussion

The vitamin D receptor is expressed on various malignant cells including basal cell carcinoma and squamous cell carcinoma. The main targets of this receptor include

Tab. 2. Distribution of genotypes in *ApaI*, *FokI*, *TaqI* and *BsmI* polymorphisms in VDR gene. BCC patients and control group

		BCC patients		Control group	
		N	%	*N*	%
ApaI	GG	38*	26.8	57	40.1
	GT	70*	49.3	49	34.5
	TT	34	23.9	36	25.4
FokI	CC	28*	19.7	83	58.5
	CT	69*	48.6	42	29.6
	TT	45*	31.7	17	12.0
TaqI	TT	39*	27.5	69	48.6
	TC	68*	47.9	49	34.5
	CC	35	24.6	24	16.9
BsmI	AA	27	19.0	34	23.9
	AG	55	38.7	59	41.5
	GG	60	42.3	49	34.5

**p < 0.05 statistically significant difference*

Tab. 3. Distribution of genotypes of *VDR* polymorphisms

	OR	–95% CI	+95% CI	Value of *p*
***FokI* CT vs. CC**	5.111	2.792	9.358	< 0.001
***FokI* TT vs. CC**	10.145	4.706	21.871	< 0.001
***TaqI* TC vs. CC**	3.088	1.671	5.708	< 0.001
***TaqI* TT vs. CC**	3.384	1.603	7.144	0.001
***ApaI* GT vs. GG**	1.942	1.045	3.608	0.036
***ApaI* TT vs. GG**	1.495	0.737	3.032	0.265

mineral metabolism, but also regulation of many metabolic pathways, such as those involved in the immune response and cancer. The vitamin D receptor is a transacting transcriptional factor that mediates 1α, 25-dihydroxyvitamin D_3 action in the regulation of target gene expression [3].

In recent years, the relevance of *VDR* polymorphisms for various types of cancer has been investigated by a great number of studies. It has been hypothesized that *VDR* polymorphisms may influence both the risk of cancer occurrence and prognosis. However, studies investigating the associations between specific *VDR* polymorphisms and cancer often provide controversial results [10].

VDR polymorphisms have been reported to be associated with cancer of the breast (*FokI*, *BsmI*, *TaqI*, *ApaI*, poly (A)), prostate (*FokI*, *BsmI*, *TaqI*, poly (A)), colorectum (*FokI*, *BsmI*), ovary (*FokI*, *ApaI*), and bladder (*FokI*), as well as renal cell carcinoma (*TaqI*, *ApaI*). To date, the association of *VDR* polymorphisms and cancer risk are the strongest for breast cancer, prostate cancer and malignant melanoma [5, 10].

There are scarce data on the association between *VDR* polymorphisms and non-melanoma skin cancers (NMSC). Gandini *et al.* [14] performed a meta-analysis with a total of 6805 skin cancer cases in regard to two polymorphisms, *FokI* and *BsmI*, and vitamin D intake. They found a correlation between melanoma and NMSC and two of the analysed polymorphisms with the relative risk of approximately 1. The association with vitamin D intake is not fully understood and only, according to the authors' observations, it can be relevant for MM development.

The *FokI* polymorphism in exon 4 results in an altered translation start site. The TT genotype was associated with an increased risk of melanoma (OR 1.90) in a hospital-based case-control study [15]. Han *et al.* [5] also reported a positive correlation between the TT genotype and risk for SCC and BCC. For SCC risk, moreover, they showed that the association of TT genotype was stronger among women with high vitamin D intake. They also showed that the *BsmI* AA genotype was significantly associated with an increased risk for SCC, but not for melanoma or BCC. They observed an interaction between the *BsmI* polymorphism and total vitamin D intake on SCC risk, suggesting that women with the AA genotype and high vitamin D intake have the highest risk of SCC. Higher levels of $1,25(OH)_2D_3$ were noted more frequently among subjects with the AA genotype than in people with the AG or GG genotype [16]. Actinic keratosis, which currently is regarded as SCC *in situ*, was found to develop more frequently among subjects with homozygote genotypes in *TaqI* polymorphism [17]. It is also known that genetic polymorphism of the *VDR* gene may influence the $1,25(OH)_2D_3$ mediated normal physiological response of keratinocytes and can explain the variable responsiveness [18].

In another study Santonocito *et al.* [19] revealed a strong link between *BsmI* GG genotype and melanoma along with Breslow thickness, suggesting that *BsmI* G allele may be a possible risk marker for melanoma and its aggressiveness.

In our study the presence of TT genotype in *FokI* polymorphism caused over 10-fold increased risk for BCC development while other genotypes such as GT in *ApaI* and TT and TC in *TaqI* were also statistically linked with cancer, although not so significantly. These observations are in line with the results obtained by Han *et al.* [5]. Similarly to other authors we found no correlation between BCC and *BsmI* polymorphism [14]. The risk ratio for TT *FokI* polymorphism obtained in our statistical analysis was much higher than in the meta-analysis presented by Gandini *et al.* [14]. These results should be proven by a study on a larger population. However, currently we may suggest TT genotype as one of the risk factors for BCC in the Polish population.

In conclusion, published reports and our own data do not allow one to draw any definitive and unequivocal statements on the role of VDR polymorphism in BCC development. However, the obtained results testify to their importance in skin cancerogenesis and further complex multicentre studies on these pathways are strongly required.

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