Association of methylenetetrahydrofolate reductase gene polymorphisms with basal cell carcinoma development

Karolina Wódz-Naskiewicz[1], Joanna Narbutt[2], Michał Rogowski-Tylman[2], Rafał Pawliczak[1], Michał Sobjanek[3], Anna Sysa-Jędrzejowska[2], Adam Włodarkiewicz[3], Aleksandra Lesiak[2]

[1]Department of Immunopathology, Medical University of Lodz, Poland
Head: Prof. Rafał Pawliczak MD, PhD
[2]Department of Dermatology and Venereology, Medical University of Lodz, Poland
Head: Prof. Anna Sysa-Jędrzejowska MD, PhD
[3]Department of Dermatology, Venereology and Allergology, Medical University of Gdansk, Poland
Head: Prof. Jadwiga Roszkiewicz MD, PhD

Post Dermatol Alergol 2011; XXVIII, 1: 1–5

Abstract

Introduction: Basal cell carcinomas (BCCs) are the most common cancers occurring among Caucasians. The main risk factors for development of BCC is excessive exposure to ultraviolet radiation, especially UVB, which simultaneously leads to DNA damage in keratinocytes. The genetic background of BCC includes multiple genes including the gene encoding methylenetetrahydrofolate reductase (MTHFR), which is a critical enzyme in folate metabolism and DNA repair.

Aim: To assess the frequency of 665C/T (rs1801133) and 1286A/C (rs1801131) polymorphisms in the *MTHFR* gene in patients with BCC.

Material and methods: The study comprised 142 patients with BCC and 142 healthy volunteers matched for age and sex as a control group. Presence of the two studied polymorphisms was assessed by PCR-RFLP.

Results: CT genotype in *MTHFR* 665C/T polymorphism and CC genotype in 1286A/C significantly increases the risk of BCC (OR = 3.39, p = 0.00008 and OR = 4.240, p = 0.032; respectively). Moreover, the presence of T allele in 665C/T MTHFR polymorphism was also associated with significantly increased risk of the development of BCC (OR = 2.094, p = 0.00068).

Conclusions: Based on the results it was found that the presence of CT genotype in the polymorphism 665C/T and CC genotype in the polymorphism 1286A/C in the *MTHFR* gene is an additional risk factor for basal cell skin cancers in the Polish population.

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

Introduction

Non-melanocytic skin cancers, including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are the most common malignancies in the Caucasian race. Basal cell carcinoma accounts for about 80% of all skin cancers. Although it is characterized by slow growth and local invasiveness, it may lead to the destruction of vast areas of the skin, posing a therapeutic problem [1, 2]. In the pathogenesis of BCC one should take into account the interaction between genetic and environmental factors, especially exposure to solar radiation.

Ultraviolet radiation (UVR), through DNA damage, generating photoproducts, and development of mutations in genes which regulate the cell cycle, contributes to the initiation of carcinogenesis [3]. Ultraviolet radiation can also cause a local state of immunosuppression, including by reducing the activity of Langerhans cells, which also promote this phenomenon [4, 5]. The gene encoding methylenetetrahydrofolate reductase (*MTHFR*) is located on chromosome 1 (1p36.3), consists of 11 exons and has 9 alternative transcripts. The *MTHFR* gene encodes an NADPH reductase, which catalyses the conversion of 5,10-methyl-

Address for correspondence: Aleksandra Lesiak MD, PhD, Department of Dermatology and Venereology, Medical University of Lodz, Krzemienicka 5, 94-017 Lodz, Poland, tel. +48 42 686 79 81, e-mail: lesiak_ola@interia.pl

enetetrahydrofolate to 5-methylenetetrahydrofolate [6], which plays a crucial role in DNA synthesis and repair.

Repair of DNA damage is a key phenomenon that protects the organism against development of cancers. This was a prerequisite to search for association of polymorphisms of genes involved in DNA repair with risk of cancer [7, 8]. Literature data also point to the participation of *MTHFR* gene polymorphisms in oncogenesis [7]. The most important functional polymorphism of the *MTHFR* gene is transition C/T at nucleotide 665 and A/C at nucleotide 1286 from the transcription start site [9-11].

Polymorphism 665C/T (exon 4) causes an alanine to valine substitution at codon 222 and polymorphism 1286A/C (exon 7) causes conversion of glutamic acid to alanine at codon 429 [7, 12], which leads to generation of a thermolabile form of MTHFR and a decrease of its enzymatic activity.

To date, there have been demonstrated relationships between selected polymorphisms of the *MTHFR* gene and increased risk of developing breast [13], colorectal [14] and gastric cancer [15], and squamous cell carcinoma [7, 12, 16, 17].

Because of the relatively sparse literature data concerning the role of *MTHFR* polymorphisms in the development of basal cell carcinoma [7, 12], the aim of our study was to determine the relationship between polymorphisms 665C/T and 1286A/C in the *MTHFR* gene and risk of BCC in the Polish population.

Material and methods

The study group consisted of 142 patients with histologically confirmed BCC and 142 healthy individuals, as a control group, divided according to sex and age. Clinical characteristics are shown in table 1.

Tab. 1. Clinical characteristics of patients with BCC and healthy volunteers in the control group

	Patients with BCC	Control group
Number of patients	142	142
Age [median]	56 (45-78)	51 (39-76)
Gender [female/male]	71/71	72/70
Hair colour, *n*		
fair	85	89
dark	57	53
Eye colour, *n*		
fair	79	73
dark	63	69
Skin phototype, *n*		
I/II	58	54
III	64	66

All patients gave written consent to participate in the study. The volunteers were generally healthy with a negative medical history of patient and family history for occurrence of skin cancer. The study was approved by the Local Ethics Committee at the Medical University of Lodz (No. RNN/171/06/KE).

Genotyping of the 665C/T *MTHFR* gene variant

Genomic DNA containing the polymorphism 665C/T was amplified by polymerase chain reaction, using 665CT1 primer in intron 5: 5´-AGGACTCTCTCTGCCCAG-3` (forward) and 665CT2 in intron 6: 5´-TCA CAA AGC GGA AGA ATG-3 (reverse) using the AccuPrime™ Taq polymerase system (Invitrogen, California, USA) and 50 ng of genomic DNA. This PCR reaction resulted in the synthesis of a 227 bp fragment (665CC). The *MTHFR* gene contains a C to T substitution at nucleotide 665; the alteration created a HinfI restriction site. Obtained PCR products were then digested with the restriction enzyme FastDigest® HinfI (Fermentas, Ontario, Canada), at 37°C for 30 min. FastDigest® HinfI did not digest the fragment derived from the C allele, whereas it digested the fragment of the same length from the T allele into 169 bp and 58 bp fragments. These fragments were then electrophoresed using a 2% agarose gel with ethidium bromide and visualized under UV light. Homozygous CC results in one fragment of 227 bp, while homozygous TT results in two fragments of 169 bp and 58 bp. Heterozygotes (CT) show all three bands of 227 bp, 169 bp and 58 bp (fig. 1).

Genotyping of the 1286A/C *MTHFR* gene variant

Genomic DNA containing the polymorphism 1286A/C was amplified by PCR, using the forward primer 1286AC1 in intron 8 (5´-TGA AGA GCA AGT CCC CCA AG-3`) and the reverse primer 1286AC2 in intron 9 (5´-CAA CAA AGA CCC AGC CTG TC-3`), using the same polymerase system. This reaction resulted in the synthesis of a 325 bp fragment (1286CC). In exon 8 of the *MTHFR* gene, an A to C substitution leads to an amino acid change from glutaminic acid to alanine at codon 429 of the protein. This Glu429Ala polymorphism is detectable with a restriction enzyme, FastDigest® MboII. Obtained fragments were then electrophoresed by using a 2% agarose gel with ethidium bromide and visualized under UV light. The *MTHFR* CC genotype was represented by a single fragment of 325 bp, AA genotype by two fragments of 253 bp and 72 bp, while heterozygotes (AC) showed all three bands of 325 bp, 253 bp and 72 bp (fig. 2).

Additionally, we analysed the association of these polymorphisms with phenotypic features, such as eye colour, hair and skin phototype according to Fitzpatrick [18].

Statistical analysis

To assess the relationships between dependent or independent variables, logistic regression was used. Fre-

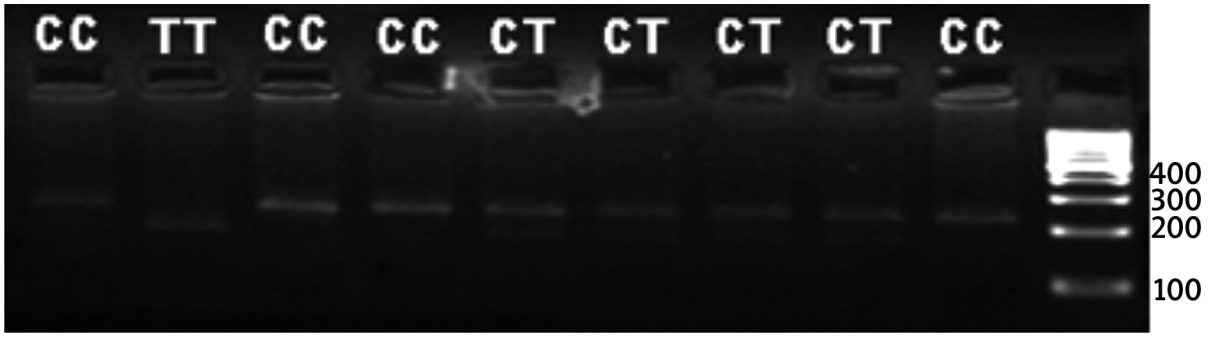

Fig. 1. Results of the RFLP analysis. The length of the PCR product containing the polymorphism 665C/T was 227 bp (CC); after digestion with FastDigest® HinfI enzyme, fragments of 179 bp and 48 bp (genotype TT), and fragments of 227 bp, 179 bp and 48 bp (CT) were produced

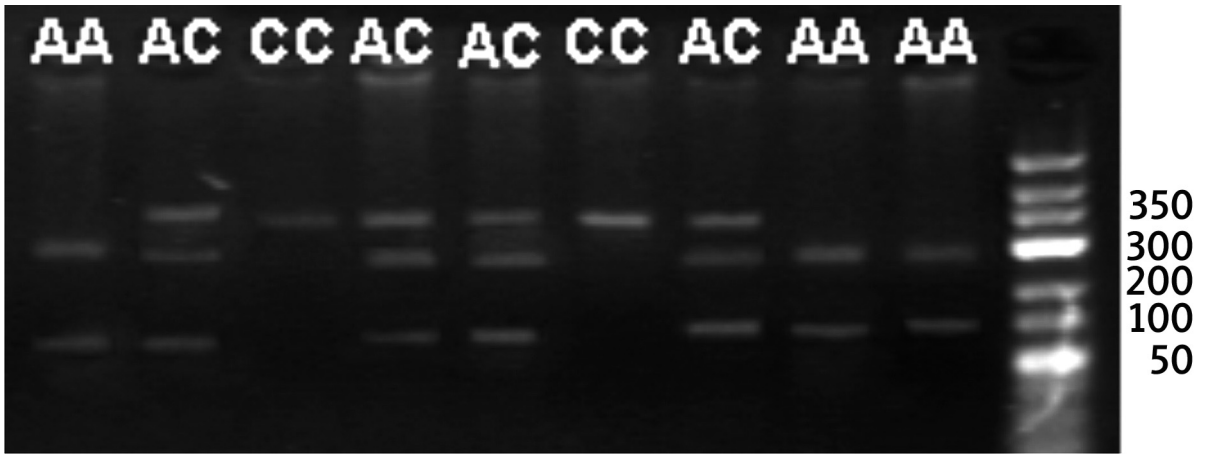

Fig. 2. Results of the RFLP analysis. The length of the PCR product containing the polymorphism 1286A/C was 325 bp (CC); after digestion with FastDigest® MboII enzyme, fragments of 253 bp and 72 bp (genotype AA) and fragments of 325 bp, 253 bp and 72 bp (AC) were produced

quencies of genotypes and alleles in the studied population were analysed for deviation from Hardy-Weinberg equilibrium and tests for association were performed (Institute of Human Genetics, Technical University Munich, Germany, http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). 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 STATISTICA software.

Results

The distribution of genotypes of the polymorphisms 665C/T and 1286 A/C in the *MTHFR* gene in both groups was consistent with Hardy-Weinberg equilibrium.

Analysing the distribution of genotypes for polymorphism 665C/ T in the control group showed that the CC genotype occurs with frequency of 80.3%, CT – 12.0%, TT – 7.7%.

In patients with BCC, compared with the control group, the heterozygous genotype CT occurred statistically significantly more often (30.3% vs. 11.97%, OR = 3.392, $p = 0.00008$). Moreover, in patients with BCC (39.6%), allele T (genotype CT and TT) is more frequent compared with the control group (19.7%). In the case of carriers of this allele, there was observed increased risk of developing BCC (OR = 2.094, $p = 0.00068$), compared with the control group (tab. 2, fig. 3).

Analysing the distribution of genotypes 1286A/C *MTHFR* in the control group showed that genotype AA occurs with frequency 52.1%, AC – 45.8% , CC – 2.11%. In patients with BCC the presence of CC genotype was statistically significantly more frequent (7.7% *vs.* 2.1%, OR = 4.24, $p = 0.032$). Moreover, in patients with BCC, compared with the control group, allele C was more frequently present (genotype AC and CC, 54.9% *vs.* 47.9%, respectively), but this association showed no statistical significance ($p > 0.05$) (tab. 2, fig. 4).

There were no relationships between investigated polymorphisms and eye colour, hair and skin phototype ($p < 0.05$ for all comparisons).

Tab. 2. Distribution of genotypes of the polymorphisms 665C/T and 1286 A/C in the *MTHFR* gene in patients with BCC and healthy individuals

MTHFR polymorphism		Patients with BCC *n* (%)	Control group *n* (%)
665	CC	85 (59.9)*	114 (80.3)
	CT	243 (30.3)*	17 (12)
	TT	14 (9.9)	11 (7.7)
1286	AA	64 (45.1)	74 (52.1)
	AC	67 (47.2)	65 (45.8)
	CC	11 (7.7)	3 (2.1)

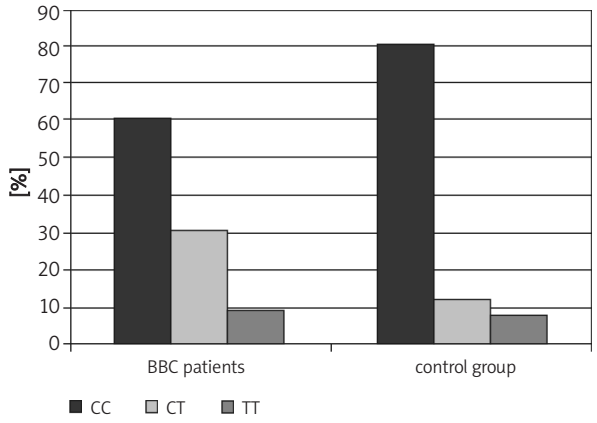

Fig. 3. Frequencies of genotypes of 677C/T MTHFR gene in patients with BCC and control group

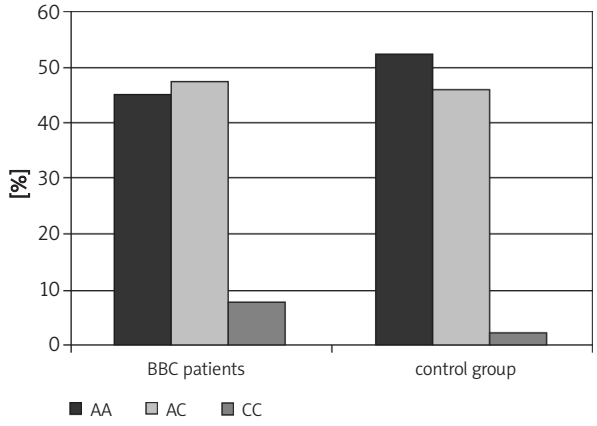

Fig. 4. Frequencies of genotypes of 1286A/C *MTHFR* gene in patients with BCC and control group

Discussion

Normal serum folate level is essential, in physiological conditions, for repair of DNA damaged by ultraviolet radiation. Hence, folates, and their metabolites, are necessary for normal cell proliferation and DNA repair in rapidly dividing cells, such as keratinocytes. In the metabolism of folates, a crucial role is played by methylenetetrahydrofolate reductase, which is involved in the folate pathway and catalyses a reduction reaction from 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, a substrate for the remethylation of homocysteine to methionine [19-23].

The most common genetic phenomenon in the process of carcinogenesis is incorrect DNA methylation [24, 25]. Polymorphisms in the *MTHFR* gene give rise to reductase with decreased activity and thus affect the folic acid metabolic pathway, which leads to disorders in methylation and initiation of carcinogenesis. In spite of numerous studies, the relationship between two common polymorphisms of the *MTHFR* gene with diminished DNA methylation in the development of BCC has not been analysed [17]. Festa *et al.* [7] in studies of patients with BCC, among the Scandinavian population, have shown that the presence of a combination of genotypes of TT/AA, both polymorphisms 665 C/T and 1286 A/C in the *MTHFR* gene, was associated with an increased risk of developing this cancer. However, this relationship has not been confirmed in other studies, in patients from the American population [12].

In this study we confirmed the association between the occurrence of some genotypes and the analysed polymorphisms in the *MTHFR* gene and the development of BCC. Based on the obtained results we found that the presence of the CC or CT genotype is associated with an increased risk of cancer development. Genotype 665 CT causes more than three-fold increased risk of BCC, while the presence of CC genotype in the polymorphism 1268 A/C augments the risk more than four times. Hence, these two genotypes might be considered as a factor predisposing to the development of BCC in the Polish population. The literature data suggest that the 665T allele is associated with reduced enzyme activity, which leads to elevated concentration of homocysteine and lower folate level in plasma, DNA repair abnormalities and consequently to carcinogenesis [9]. In the present study we also found a relationship between presence of the T allele and the development of BCC, which confirms the above hypothesis.

The presence of polymorphism 1286 A/C also inhibits the functional activity of the enzyme, although it does not directly affect the level of homocysteine and folic acid in the plasma. The study showed, however, that the presence of allele 1286C in patients with low folate concentration can cause impairment of their metabolic pathway [10]. In the present study we also found an association of CC genotype with risk of developing basal cell carcinoma, but this dependence was not analysed for folate concentration in serum of patients with BCC.

In the process of skin carcinogenesis, ultraviolet radiation plays a key role through the possibility of DNA dam-

age. In addition, UVR causes degradation of folate in the photolysis process, which causes a deficiency in the serum and consequently leads to abnormal repair processes damaged by UVR genomic DNA [26].

On the basis of these results, we conclude that the interaction between recognized pathogenetic environmental (excessive exposure to ultraviolet radiation) and genetic (polymorphisms in the gene *MTHFR*) factors predisposes to non-melanocytic skin cancers, including BCC.

Acknowledgements

The project was funded by Medical University Research Grant nr 503-1152-1 and Polish Scientifique Committee nr NN402474731.

References

1. Housman TS, Feldman SR, Williford PM, et al. Skin cancer is among the most costly of all cancers to treat for the Medicare population. J Am Acad Dermatol 2003; 48: 425-9.
2. Lesiak A, Slowik-Rylska M, Rogowski-Tylman M, et al. Risk factors in Central Poland for the development of superficial and nodular basal cell carcinoma. Arch Med Sci 2010; 6: 270-5.
3. Lacour JP. Carcinogenesis of basal cell carcinomas: genetics and molecular mechanisms. Br J Dermatol 2002; 146 (Suppl. 61): 17-9.
4. Sreevidya CS, Fukunaga A, Khaskhely NM, et al. Agents that reverse UV-Induced immune suppression and photocarcinogenesis affect DNA repair. J Invest Dermatol 2010; 130: 1428-37.
5. Saladi RN, Nektalova T, Fox JL. Induction of skin carcinogenicity by alcohol and ultraviolet light. Clin Exp Dermatol 2010; 35: 7-11.
6. Stern LL, Mason JB, Selhub J, Choi SW. Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. Cancer Epidemiol Biomarkers Prev 2000; 9: 849-53.
7. Festa F, Kumar R, Sanyal S, et al. Basal cell carcinoma and variants in genes coding for immune response, DNA repair, folate and iron metabolism. Mutat Res 2005; 574: 105-11.
8. Thoms KM, Kuschal C, Emmert S. Lessons learned from DNA repair defective syndromes. Exp Dermatol 2007; 16: 532-44.
9. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995; 10: 111-3.
10. van der Put NM, Gabreëls F, Stevens EM, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 1998; 62: 1044-51.
11. van der Molen EF, Arends GE, Nelen WL, et al. A common mutation in the 5,10 methylenetetrahydrofolate reductase gene as a new risk factor for placental vasculopathy. Am J Obstet Gynecol 2000; 182: 1258-63.
12. Han J, Colditz GA, Hunter DJ. Polymorphisms in the MTHFR and VDR genes and skin cancer risk. Carcinogenesis 2007; 28: 390-7.
13. Ericson U, Sonestedt E, Ivarsson MI, et al. Folate intake, methylenetetrahydrofolate reductase polymorphisms, and breast cancer risk in women from the Malmö Diet and Cancer cohort. Cancer Epidemiol Biomarkers Prev 2009; 18: 1101-10.
14. Iacopetta B, Heyworth J, Girschik J, et al. The MTHFR C677T and DeltaDNMT3B C-149T polymorphisms confer different risks for right- and left-sided colorectal cancer. Int J Cancer 2009; 125: 84-90.
15. Galván-Portillo MV, Cantoral A, Oñate-Ocaña LF, et al. Gastric cancer in relation to the intake of nutrients involved in one-carbon metabolism among MTHFR 677 TT carriers. Eur J Nutr 2009; 48: 269-76.
16. Prinz-Langenohl R, Fohr I, Pietrzik K. Beneficial role for folate in the prevention of colorectal and breast cancer. Eur J Nutr 2001; 40: 98-105.
17. Laing ME, Dicker P, Moloney FJ, et al. Association of methylenetetrahydrofolate reductase polymorphism and the risk of squamous cell carcinoma in renal transplant patients. Transplantation 2007; 84: 113-6.
18. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol 1988; 124: 869-71.
19. Larsson SC, Giovannucci E, Wolk A. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. Gastroenterology 2006; 131: 1271-83.
20. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. PNAS 1997; 94: 3290-5.
21. Choi SW, Kim YI, Weitzel JN, Mason JB. Folate depletion impairs DNA excision repair in the colon of the rat. Gut 1998; 43: 93-9.
22. Branda RF, Blickensderfer DB. Folate deficiency increases genetic damage caused by alkylating agents and gamma-irradiation in Chinese hamster ovary cells. Cancer Res 1993; 53: 5401-8.
23. Wei Q, Shen H, Wang LE, et al. Association between low dietary folate intake and suboptimal cellular DNA repair capacity. Cancer Epidemiol Biomarkers Prev 2003; 12: 963-9.
24. Das PM, Singal R. DNA methylation and cancer. J Clin Oncol 2004; 22: 4632-42.
25. Duthie SJ. Folic acid deficiency and cancer: mechanisms of DNA instability. Br Med Bull 1999; 55: 578-92.
26. Jablonski NG, Chaplin G. Colloquium paper: human skin pigmentation as an adaptation to UV radiation. PNAS 2010; 107 (Suppl. 2): 8962-8.