Single nucleotide polymorphisms of the *EVER2* gene in squamous cell carcinomas in patients with actinic keratosis

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ABSTRACT

Introduction. In recent years, two novel genes, *EVER1* and *EVER2*, have been detected, mutations in which play a role in development of epidermodysplasia verruciformis (EV), a genodermatosis associated with squamous skin cancers (SCC). Recently it was found that polymorphism of the *EVER2* gene is related to increased risk of SCC in the general population. The aim of this study was based on the hypothesis that *EVER* genes, mutations of which play a key role in skin cancers in EV, can also be impaired in SCC in patients with actinic keratosis (AK) in the general population.

Objective. To evaluate the association of polymorphisms rs7208422, rs35748721, rs62079073, rs112802399, rs12452890 of the *EVER2* gene in SCC in patients with actinic keratosis in the general population.

Material and methods. Hundred patients with actinic keratosis were analyzed due to presence of SSC. In the study 360 persons were also included as a control group. All the participants were genotyped using real-time polymerase chain reaction (RT-PCR) with reagents from Applied Biosystems.

Results. In the group of patients with AK and coexisting SCC there was no presence of genotypes TG, TT or allele T of polymorphism rs62079073 of the *EVER2* gene and the whole group had only genotype GG. Frequency of genotype GG in AK and coexisting SCC was statistically significant compared to the group of patients without coexisting SCC (100% vs. 82.9%; $p = 0.01$). Similarly, frequency of allele T was significantly higher in the group of patients without presence of SCC (0% vs. 10.5%; $p = 0.026$). Multivariate regression analysis confirmed that an independently associated factor connected with appearance of SSC in patients with actinic keratosis is genotype GG ($p = 0.034$).

Conclusions. Genotypes TT and TG as well as allele T of polymorphism rs62079073 of the *EVER2* gene have a protective influence on the appearance of SCC in patients with AK.

INTRODUCTION

Actinic keratosis (AK) is the most common premalignant condition of the skin. It is thought to be a result of both environmental and genetic factors. Actinic keratosis was first described as a separate medical condition over 100 years ago, and its former name *keratosis*

senilis draws attention to old age which is a factor predisposing to the development of the disease. The risk of AK definitely increases with age, which is mainly related to the accumulation of sun damage occurring in keratinocytes over the years. This is when irreversible mutations occur in the DNA, while the decline in immunity, which is typical for the elderly, accelerates the onset of the disease [1]. The current name of the condition – *keratosis actinica* – brings into focus sun exposure as the most important etiopathogenetic factor [2]. Some authors also claim that the name should reflect the potentially malignant nature of the disease or state plainly that it represents an early form of squamous cell carcinoma, i.e. *carcinoma in situ* [3, 4]. Actinic keratosis is known to be a potential starting point for squamous cell carcinoma (SCC), the second most common cancer of the skin after basal-cell carcinoma, belonging to the so-called nonmelanoma skin cancers (NMSC) [5]. Squamous cell carcinoma arises from actinic keratosis in nearly 60% of cases, whereas 97% of all squamous cell carcinomas have histopathological features of AK [6]. The majority of authors agree that squamous cell carcinomas derived from actinic keratosis are less malignant and associated with a lower metastatic risk [7]. There are, however, reports which contradict this theory claiming that up to 40% of cases carry the risk of metastasis [8]. Consequently, the question whether AK should be considered an early stage of squamous cell carcinoma remains open. Fu and Cockerell suggested that the process of development of actinic keratosis and its subsequent malignant transformation into squamous cell carcinoma should be considered by analogy to cervical intraepithetial neoplasia (CIN) and described with a similar term, i.e. keratotic intraepidermal neoplasia (KIN) [9]. The term appropriately describes the process of gradual progression of sparse atypical cells into invasive cancer. The grading system proposed by this authors combines clinical and histopathological features of skin lesions. KIN1 denotes flat skin lesions with focal atypia of keratinocytes confined to the lower one-third of the epidermis, KIN2 has clinically papular features with focal atypia in the lower two thirds of the epidermis, while KIN3 creates larger foci with diffuse atypia involving the full thickness of the epidermis [9]. In 2002, Berhane *et al*. developed a different classification dividing AK into three categories: asymptomatic AK, inflammatory AK and SCC [10]. The inflammatory form of the disease has a erythematous halo and may be painful. Inflammatory infiltrate accompanying actinic keratosis is thought to be a defence mechanism which, if effective, leads to the regression of lesions. Its ineffectiveness, however, results in progression to squamous cell carcinoma [10].

It is difficult to determine unambiguously which lesions of actinic keratosis has a potential to transform into squamous cell carcinoma. In addition to clinical features including greater palpability, skin hardening and bleeding or inflammation, some authors suggest that malignant transformation may have underlying genetic factors [10]. Genetic predisposition related to functional disorders of recently identified *EVER* genes may play a role here.

OBJECTIVE

The aim of the study was to identify relationships between selected polymorphisms of the *EVER2* gene, including rs7208422, rs35748721, rs62079073, rs112802399, rs12452890, and the development of squamous cell carcinomas in patients suffering from actinic keratosis.

MATERIAL AND METHODS

The analysis involved a total of 100 patients with actinic keratosis, aged between 48 and 91 years, including 50 women and 50 men. The diagnosis of actinic keratosis was based on the patients' medical history, clinical manifestations and histopathological findings. The mean age of the patients was 75.27 ±7.07, with the median age being 75 years (range: 48.0–91.0). The mean duration of actinic keratosis for the whole group was 7.38 ±5.88 years, while the median duration was 5 years (range: from 3 months to 30 years). The first lesions on the patients' skin appeared at the mean age of 67.93 ±8.51; the median age was 69 years (range: 43.0–89.0).

The control group (CG) consisted of 380 subjects including 190 women and 190 men. The control group comprised individuals who had undergone paternity tests in the Mazowieckie Province (courtesy of Prof. Rafał Płoski, PhD, MD, from the Department of Medical Genetics, Medical University of Warsaw). Blood samples of control group individuals were derived from the DNA bank and selected at random. All the subjects from the control group provided their written consent to the anonymous use of their DNA for research purposes.

DNA was isolated from full blood transferred into test tubes containing EDTA (ethylenediaminetetraacetic acid). DNA isolation was performed with a Macherey-Nagel kit using MB1, MB2, MB3, MB4, MB5 and MB6 buffers, magnetic beads and a magnetic separator. After isolation, samples were subjected to a spectrophotometric measurement of absorbance of the DNA solution at the wavelength of 260 nm and the optical path length of 1 mm. Measurements were carried out with a NanoDrop® ND-100 Spectrophotometer. After determining concentrations the samples were diluted (or thickened) to obtain the final concentration of 600 ng/µl. The genotyping of polymorphisms was based on RT-PCR (real-time polymerase chain reaction) using Taqman probes, i.e. oligonucleotides with a length of 20–30 bp.

The probes are labelled with fluorescent dyes: a reporter dye at the 5′ end and a quencher at the 3′ end. The reaction was performed with two types of reporter dyes including 6-carboxyfluorescein (FAM) and VIC. The quencher was 6-carboxytetramethylrhodamine (TAMRA). To avoid errors resulting from inaccurate pipetting or variable sample concentrations, the method additionally involves a passive dye – 6-carboxy-X-tetramethylrhodamine (ROX).

Statistical analysis

The distribution of genotypes in the groups under comparison was determined using χ^2 test. The analyses were performed assuming different inheritance models: recessive, codominant or dominant. Computations were conducted with the Web-Assotest programme (http://www.ekstroem.com/assotest/assotest.html) [11]. The frequency of alleles in the study groups was compared with the aid of an application available online (http://ihg.gsf.de/cgi-bin/hw/hwal.pl). Multiple factor analyses were based on multifactorial logistic regression using the SPSS package. The threshold of statistical significance was set at $p = 0.05$.

RESULTS

Table I presents the characteristics of all the polymorphisms under study.

In the group of patients with actinic keratosis and coexisting squamous cell carcinomas, genotypes TT and TG of polymorphism rs62079073 of the *EVER2* gene, and allele T, were absent. The only genotype represented in this group was GG. In the group of AK patients without coexisting SCCs, on the other hand, the distribution of polymorphism rs62079073 was the following: genotype GG was found in 63 patients (82.9%), TG – in 10 patients (13.2%) and TT – in 3 patients (3.9%). Analyses showed the frequency of genotype GG in the group of patients with SCCs to be higher – in a statistically significant manner – than among patients without coexisting SCC (in 20 out of 20 patients, 100%; *vs.* 63 out of 76 patients, 82.9%, $p = 0.01$) (table II). Allele T was also found to occur more frequently, in a statistically significant way, in the group of SCC-free patients than in the group of subjects with diagnosed squamous cell carcinomas (0% vs. 10.5%; $p = 0.026$). A comparative analysis was performed for patients with AK and SCC, and the control group in which the frequencies of genotypes and allele T of polymorphism rs62079073 exhibited no statistical variation ($p = 0.18$ for genotype GG; $p = 0.126$ for allele T) (table II). Due to the lack of confirmation of the result in relation to the control group, a multifactorial logistic regression analysis was performed for the following clinical parameters: sex of the patients, number of skin lesions, age at onset of actinic keratosis, duration of the disease, extent of AK lesions, coexistence of basal-cell carcinomas, skin phototype, history of child-

Table I. *EVER2* gene polymorphisms studied

Polymorphism	Location in c-DNA	Location in the *EVER2* gene	Location on chromosome	Amino acid substitutions
rs35748721	69 G>A	2 exon	76127738	Glu(E) with Glu(E)
rs7208422	917 A>T	8 exon	76130575	Asn(N) with Ile(I)
rs62079073	988-4 G>T	8 intron	76130947	–
rs112802399	1024 G>T	9 exon	76130987	Gly(G) with Trp(W)
rs12452890	1107 G>A	9 exon	76131070	Glu(E) with Glu(E)

Table II. Distribution of genotypes and analysis of associations between polymorphism rs62079073 and presence of squamous cell carcinomas in patients with actinic keratosis

AK vs. SCC	rs62079073 Genotypes			Frequency of allele T%		Comparison of alleles	Model		
	GG%	TG%	TT%				Recessive (TT vs. GG/TG)	Codominant (TT vs. TG TG vs. GG)	Codominant (TG/TT vs. GG)
					OR	0.1	NE	NE	NE
					(95% CI)	(0.006–1.74)	NE	NE	NE
AK/SCC	20 (100)	0 (0)	0 (0)	0 (0)	Value of *p*	0.026	NE	NE	0.01
AK	63 (82.9)	10 (13.2)	3 (3.9)	16 (10.5)	OR	0.16	NE	NE	NE
GK	87.4	10.9	1.7	7.2	(95% CI)	(0.01–2.61)	NE	NE	NE
					Value of *p*	0.126	NE	NE	0.18

NE – not evaluated, CG – control group

Table III. Multivariate evaluation of association of various factors with occurrence of squamous cell carcinomas in patients with actinic keratosis

Parameter	Value of *p*
Genotype GG	0.034
Men	0.528
Age	0.437
Number of AK lesions < 10	0.997
AK onset before 70 years of age	0.883
Duration of AK ↓ 10	0.158
Number of affected body areas < 3 areas	0.307
Coexistence with BCC	0.834
Phototypes I and I / II	0.712
Childhood burns	0.225
Prolonged UV exposure	0.079

hood sunburns and prolonged sun exposure. The analysis demonstrated genotype GG to be an independent factor implicated in the development of SCCs in AK patients ($p = 0.034$) (table III).

The analysis of occurrence of another polymorphism of the *EVER2* gene – rs7208422 – in patients with actinic keratosis depending on the coexistence of squamous cell carcinomas revealed no differences in the frequency of both genotypes and individual alleles in both patient groups. The distribution of genotypes and alleles was similar ($p = 0.811$ for genotype TT, $p = 0.459$ for genotype AA, $p = 0.76$ for allele T) (table IV). Similarly, no differences were shown for the frequency of occurrence of both genotypes and individual alleles in groups of patients with or without SCCs for the third polymorphism under study – rs12452890 – of the *EVER2* gene ($p = 0.96$ for genotype GG; $p = 0.83$ for genotype AA, $p = 0.86$ for allele G) (table V). In view of the very rare occurrence of two remaining polymorphisms (rs35748721 and rs112802399) in the study groups, no statistical analyses were performed.

DISCUSSION

Data on correlations between the *EVER* genes and precancerous skin conditions/skin cancers are derived from the rare genetically conditioned skin disorder epidermodysplasia verruciformis (EV). So far, studies have successfully identified two genes, *EVER1* and *EVER2*, whose mutations are responsible for the development of symptoms of the disease [12, 13]. Epidermodysplasia verruciformis is characterized by verrucous cutaneous lesions and spots resembling pityriasis versicolor which, after a period of varying duration, may progress to squamous cell carcinomas [14]. The basic factor underlying carcinogenesis in EV are oncogenic EV-type HPVs whose DNA is also identified in a considerable proportion of actinic keratosis and squamous cell carcinoma cases in the general population [15].

Actinic keratosis is known to be the most common premalignant condition which may be a starting point for squamous cell carcinoma. The process of transformation of actinic keratosis into SCC is prolonged, usually encompassing 10–20 years [16]. There are various estimates of the frequency of transformation, ranging from 0.025% to 16% of all AK cases [17, 18]. It must also be noted that over 25% of AK cases may resolve spontaneously if patients avoid sun exposure throughout the year [19]. In addition to excessive sun exposure, age, sex and immunosuppression, progression of actinic keratosis to squamous cell carcinoma seems likely to be influenced by genetic factors as well [20].

Assumptions underlying the present study were based on the hypothesis that *EVER* genes in actinic keratosis patients may be disturbed and contribute to the development of SCC in the general population. It is interesting to note that previous studies reported a higher incidence of actinic keratosis and squamous cell carcinomas in patients with chromosomal disorders of the LOH (loss of heterozygosity) type on chromosome 17 qter, where both *EVER* genes are located [21, 22]. The genes have been identified on the EV1 locus on chromosome 17q25 in a 1 cM region between the D17S939 and D17S802 markers. It needs to be stressed that there are, as yet, no literature reports on the detection of mutations in the *EVER* genes in any disorders other than epidermodysplasia verruciformis. Available publications assess polymorphisms of the *EVER* genes in cervical cancer based on experimental, clinical and epidemiological studies encom-

Table IV. Distribution of genotypes and analysis of associations between polymorphism rs7208422 and presence of squamous cell carcinomas in patients with actinic keratosis

AK vs. SCC	rs7208422 Genotypes			Frequency of allela T%		Comparison of alleles	Model		
	AA%	AT%	TT%				Recessive (TT vs. AA/AT)	Codominant (TT vs. AT AT vs. AA)	Dominant (AT/TT vs. GG)
AK/SCC	3 (21.4)	6 (42.9)	5 (35.7)	57.1	OR	1.14	0.86	1.1	1.68
AK	16 (31.4)	15 (29.4)	20 (39.2)	54	(95% CI)	(0.49–2.65)	(0.25–2.94)	(0.53–2.28)	(0.4–6.84)
					Value of *p*	0.76	0.811	0.794	0.459

Table V. Distribution of genotypes and analysis of associations between polymorphism rs12452890 and presence of squamous cell carcinomas in patients with actinic keratosis

AK vs. SCC	rs12452890 Genotypes			Frequen of allele G%		Comparison of alleles	Model		
	AA%	AG%	GG%				Recessive (GG vs. AA/AG)	Codominant (GG vs. AG AG vs. AA)	Dominant (AG/GG vs. AA)
AK → SCC	7 (35)	9 (45)	4 (20)	17 (42.5)	OR	1.07	1.03	1.06	1.12
SCC					(95% CI)	(0.53–2.16)	(0.3–3.54)	(0.54–2.07)	(0.4–3.14)
AK	29 (37.7)	33 (42.9)	15 (19.4)	6 (40.9)	Value of *p*	0.86	0.96	0.86	0.83

passing many years, which have demonstrated the role of oncogenic HPVs in its development [23, 24].

Our research into polymorphisms of the *EVER2* gene in skin carcinogenesis have been inspired by studies by Patel *et al*. who showed a link between genetic variation in the *EVER2* gene and elevated risk of squamous cell carcinoma [25]. The study, the only one on this topic published so far, showed that genotype TT of polymorphism rs7298422 is associated with a 70% increase in the risk of squamous cell carcinoma compared to the control group (OR = 1.7; 95% CI = 1.1–2.7; $p = 0.01$).

Genetic analyses performed for the present study also involved the *EVER2* gene because mutations in this gene has been found in the Polish population of *epidermodysplasia verruciformis* patients (Majewski *et al*., unpublished data).

Results of the analyses suggest a correlation between polymorphism rs62079073 of the *EVER2* gene and the coexistence of squamous cell carcinomas in the group of patients suffering from actinic keratosis. Polymorphism rs62079073 consists in the substitution of guanine with thymine in intron 8, in exon splice site, which may have pathogenetic relevance. There are no studies in literature assessing the nature of this particular polymorphism. It was selected for the present work because it is more common than other polymorphisms: the frequency of allele T among the European population is known to be 0.09. An analysis of AK patients showed a lack of genotypes TT and TG in the group of patients with coexisting SCCs compared to patients who only had AK-type skin lesions. Similarly, allele T was not identified in the group of patients with AK and SCC. The frequency of genotype GG among patients with AK and SCC was 100% compared to the AK-only group, where the frequency was 82.9%. The findings may point to the protective effect of genotype TT and allele T with regard to the development of SCCs in the group of actinic keratosis patients. Evidence for the hypothesis is the result of multiple regression analysis which was performed for all clinical parameters under study. Dominant genotype GG was demonstrated as an independent factor affecting the development of squamous cell carcinomas in the group of patients with AK.

No statistically significant evidence, however, was obtained for polymorphism rs7208422 of the *EVER2* gene which was linked to the risk of squamous cell carcinoma in the cited study by Patel *et al*. [25]. No statistically significant differences were identified for the remaining three polymorphisms, either.

Statistically significant results obtained in the present study may indicate the role of genetic variation in the *EVER2* gene for skin carcinogenesis. In order to elucidate in greater detail the role of polymorphisms of the *EVER1* and *EVER2* genes in cancers and precancerous conditions, further research is needed. Studies conducted to date to investigate the function of the *EVER* genes have shown that proteins coded by these genes have an ability to bind to the major zinc transporter ZnT1, forming ZnT-1/*EVER* complexes, and are implicated in maintaining cellular zinc balance. The mechanism is probably involved in the control of keratinocyte infection by HPV and/or affects immune response controlling the removal of keratinocytes infected by EV HPV [26]. The exact role of this process for skin carcinogenesis requires further clarification in the course of subsequent observations.

CONCLUSIONS

Varying expression of squamous cell carcinoma in patients with actinic keratosis may be a consequence not only of environmental factors but also genetic predisposition possibly related to abnormalities in the *EVER* genes. The studies reported above may indicate the implication of polymorphism of the *EVER2* gene in the process of progression of cancerous skin lesions. The nature of polymorphisms is known to be not only a potentially promoting factor but also, as demonstrated by our study, a protective factor influencing skin carcinogenesis determined by the location of polymorphisms within the gene. Further research is required to fully elucidate the roles of polymorphisms of the *EVER2* gene in skin carcinogenesis.

References

1. **Frost C.A., Green A.C.:** Epidemiology of solar keratoses. Br J Dermatol 1994, 131, 455-464.
2. **Marks R.:** Nonmelanotic skin cancer and solar keratoses. The quiet 20th century epidemic. Int J Dermatol 1987, 26, 201-205.
3. **Evans C., Cockerell C.J.:** Actinic keratosis: time to call a spade a spade. South Med J 2000, 93, 734-736.

4. **Lober B.A., Lober C.W.:** Actinic keratosis is squamous cell carcinoma. South Med J 2000, 93, 650-655.
5. **Madan V., Lear J.T.,** Szeimies R.M.: Non-melanoma skin cancer. Lancet 2010, 375, 673-685.
6. **Marks R., Rennie G., Selwood T.S.:** Malignant transformation of solar keratoses to squamous cell carcinoma. Lancet 1988, 1, 795-797.
7. **Bendl B.J., Graham J.H.:** New concepts on the origin of squamous cell carcinomas of the skin: solar (senile) keratosis with squamous cell carcinoma: a clinicopathologic and histochemical study. Proc Natl Cancer Conf 1970, 6, 471-488.
8. **Fukamizu H., Inoue K., Matsumoto K., Okayama H., Moriguchi T.:** Metastatic squamous-cell carcinomas derived from solar keratosis. J Dermatol Surg Oncol 1985, 11, 518-522.
9. **Fu W., Cockerell C.J.:** The actinic (solar) keratosis: a 21st-century perspective. Arch Dermatol 2003, 139, 66-70.
10. **Berhane T., Halliday G.M., Cooke B., Barnetson R.S.:** Inflammation is associated with progression of actinic keratoses to squamous cell carcinomas in humans. Br J Dermatol 2002, 146, 810-815.
11. **Hausen S.K., Gjesing A.P., Rasmussen S.K., Glumer C., Urhammer S.A., Andersen G. i inni:** Large-scale studies of the insulin gene variable-number-of tandem-repeats polymorphism in relation to type 2 diabetes mellitus and insulin release. Diabetologia 2004, 47, 1079-1087.
12. **Ramoz N., Rueda L.A., Bouadjar B., Favre M., Orth G.**: A susceptibility locus for epidermodysplasia verruciformis, an abnormal predisposition to infection with the oncogenic human papillomavirus type 5, maps to chromosome 17qter in a region containing a psoriasis locus. J Invest Dermatol 1999, 112, 259-263.
13. **Ramoz N., Taieb A., Rueda L.A., Montoya L.S., Bouadjar B., Favre M., et al.:** Evidence for a nonallelic heterogeneity of epidermodysplasia verruciformis with two susceptibility loci mapped to chromosome regions 2p21-p24 and 17q25. J Invest Dermatol 2000, 114, 1148-1153.
14. **Orth G.:** Genetics of epidermodysplasia verruciformis: insights into host defense against papillomaviruses. Semin Immunol 2006, 18, 362-374.
15. **Pfister H., Fuchs P.G., Majewski S., Jablonska S., Pniewska I., Malejczyk M.:** High prevalence of epidermodysplasia verruciformis-associated human papillomavirus DNA in actinic keratoses of the immunocompetent population. Arch Dermatol Res 2003, 295, 273-279.
16. **zur Hausen H.:** Papillomavirus infections: a major cause of human cancers. Biochim Biophys Acta 1996, 1288, F55-F78.
17. **Glogau R.G.:** The risk of progression to invasive disease. J Am Acad Dermatol 2000, 42, 23-24.
18. **Jeffes E.W., Tang E.H.:** Actinic keratosis. Current treatment options. Am J Clin Dermatol 2000, 1, 167-179.
19. **Marks R., Foley P., Goodman G., Hage B.H., Selwood T.S.:** Spontaneous remission of solar keratoses: the case for conservative management. Br J Dermatol 1986, 115, 649-655.
20. **Kalińska-Bienias A., Majewski S.:** Rola czynników genetycznych w skórnej kancerogenezie. Przegl Dermatol 2013, 100, 118-124.
21. **Quinn A.G., Sikkink S., Rees J.L.:** Basal cell carcinomas and squamous cell carcinomas of human skin show distinct patterns of chromosome loss. Cancer Res 1994, 54, 4756-4759.
22. **Rehman I., Takata M., Wu Y.Y., Rees J.L.:** Genetic change in actinic keratoses. Oncogene 1996, 12, 2483-2490.
23. **Castro F.A., Ivansson E.L., Schmitt M., Juko-Pecirep I., Kjellberg L., Hildesheim A., et al.:** Contribution of TMC6 and TMC8 (EVER1 and EVER2) variants to cervical cancer susceptibility. Int J Cancer 2012, 130, 349-355.
24. **Wang S.S., Gonzalez P., Yu K., Porras C., Li Q., Safaeian M., et al.:** Common genetic variants and risk for HPV persistence and progression to cervical cancer. PLoS One 2010, 5, e8667.
25. **Patel A.S., Karagas M.R., Pawlita M., Waterboer T., Nelson H.H.:** Cutaneous human papillomavirus infection, the EVER2 gene and incidence of squamous cell carcinoma: a case-control study. Int J Cancer 2008, 122, 2377-2379.
26. **Lazarczyk M., Pons C., Mendoza J.A., Cassonnet P., Jacob Y., Favre M.:** Regulation of cellular zinc balance as a potential mechanism of EVER-mediated protection against pathogenesis by cutneous oncogenic human papillomaviruses. J Exp Med 2008, 205, 35-42.

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