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 SCC. 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 SCC 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.

KEY WORDS: actinic keratosis, squamous cell carcinoma, gene EVER2/TMC8, epidermodysplasia verruciformis.

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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 keratosi
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. car cinoma 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 non-melanoma 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 intraepithelial 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.
sing single nucleotides polymorphism of EVER2 gene in squamous cell carcinomas in patients with actinic keratosis

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 χ² 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.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Location in c-DNA</th>
<th>Location in the EVER2 gene</th>
<th>Location on chromosome</th>
<th>Amino acid substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs35748721</td>
<td>69 G&gt;A</td>
<td>2 exon</td>
<td>76127738</td>
<td>Glu(E) with Glu(E)</td>
</tr>
<tr>
<td>rs7208422</td>
<td>917 A&gt;T</td>
<td>8 exon</td>
<td>76130575</td>
<td>Asn(N) with Ile(I)</td>
</tr>
<tr>
<td>rs62079073</td>
<td>988-4 G&gt;T</td>
<td>8 intron</td>
<td>76130947</td>
<td>–</td>
</tr>
<tr>
<td>rs112802399</td>
<td>1024 G&gt;T</td>
<td>9 exon</td>
<td>76130987</td>
<td>Glu(G) with Trp(W)</td>
</tr>
<tr>
<td>rs12452890</td>
<td>1107 G&gt;A</td>
<td>9 exon</td>
<td>76131070</td>
<td>Glu(E) with Glu(E)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>AK vs. SCC</th>
<th>rs62079073 Genotypes</th>
<th>Frequency of allele T%</th>
<th>Comparison of alleles</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG%</td>
<td>TG%</td>
<td>TT%</td>
<td></td>
</tr>
<tr>
<td>AK/SCC</td>
<td>20 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>AK SCC</td>
<td>63 (82.9)</td>
<td>10 (13.2)</td>
<td>3 (3.9)</td>
<td>16 (10.5)</td>
</tr>
<tr>
<td>AK</td>
<td>87.4</td>
<td>10.9</td>
<td>1.7</td>
<td>7.2</td>
</tr>
<tr>
<td>GK</td>
<td>(95% CI)</td>
<td>(0.01–2.61)</td>
<td>(0.01–2.61)</td>
<td>(0.01–2.61)</td>
</tr>
</tbody>
</table>

NE – not evaluated, CG – control group
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 17qter, 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 V. Distribution of genotypes and analysis of associations between polymorphism rs12452890 and presence of squamous cell carcinomas in patients with actinic keratosis

<table>
<thead>
<tr>
<th>AK vs. SCC</th>
<th>rs12452890 Genotypes</th>
<th>Frequen of allele G%</th>
<th>Comparison of alleles</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA%</td>
<td>AG%</td>
<td>GG%</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>AK → SCC</td>
<td>7 (35)</td>
<td>9 (45)</td>
<td>4 (20)</td>
<td>17 (42.5)</td>
</tr>
<tr>
<td>SCC</td>
<td>29 (37.7)</td>
<td>33 (42.9)</td>
<td>15 (19.4)</td>
<td>6 (40.9)</td>
</tr>
</tbody>
</table>

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


Received: 9 IX 2013
Accepted: 23 IX 2013