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Lack of Association Between the *ACE2* G8790A Gene Variation and Risk for Basal Cell Carcinoma

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Abstract. *Background/Aim: The G8790A (rs2285666) functional polymorphism of the angiotensin-converting enzyme 2 (ACE2) gene influences alternative mRNA splicing and quantitatively affects the enzyme's production. Specifically, the presence of the A allele has been associated with higher ACE2 plasma levels. In this study, we investigated the possible association of the functional polymorphism ACE2-G8790A with the pathogenesis of basal cell carcinoma (BCC). Patients and Methods: A total of 190 DNA samples were studied, including 91 BCC patients and 99 controls of Greek origin. Molecular genotyping for the ACE2 G8790A polymorphism was carried out by PCR amplification, followed by AluI enzyme digestion and agarose gel electrophoresis of the DNA fragments. Results: The allelic and genotypic frequencies presented no statistical difference between the patient and the control group. Conclusion: There is no association between the ACE2 G8790A polymorphism and pathogenesis of BCC.*

Basal cell carcinoma (BCC) is the most prevalent human cancer in the world, with an annual rising incidence of 10%, and at the same time, it represents about 80% of nonmelanoma skin cancers (1). Although most often BCC appears as a mild, slow-growing skin tumor that can be invasive at a local level, some of its subtypes are highly infiltrative, with a strong metastatic potential (2-5). Most cases occur in the ages between 40 and 79 years (6), with greater frequency in men compared to women (7, 8).

The cause of BCC is multifactorial and includes exposure to certain environmental factors, underlying physical characteristics and genetic predisposing variations. The predominant risk factor is exposure to solar ultraviolet rays, particularly during childhood and adolescence. Individuals with light-toned skin, who experience solar burns rather than tanning, are the most prone to develop skin carcinomas, including BCC. In addition, secondary risk factors include exposure to ionizing radiation as well as skin absorption or digestion of certain chemical compounds like arsenic (4, 7, 9, 10). Finally, BCC often occurs in people with a compromised immune system such as transplant recipients, and in individuals with genetic conditions such as the Gorlin, Bazex-Dupre-Christol and Rombo syndromes (4, 11). BCC patients most often, have *de novo* or inherited mutations in the highly studied tumor suppressor genes patched (*PTCH1*) and tumor protein 53 *(TP53)* (12), as well as variations in genes coding for proteins of the renin-angiotensin system (RAS) (13-17).

RAS plays a key role in the control of homeostasis by regulating blood pressure, extracellular volume, and electrolyte balance, while at the same time is involved in fibrosis and local inflammatory responses (18-20). In recent years, RAS has been prominently involved in several types of cancer, through its paramount effector angiotensin II (Ang II), which exerts oncogenic effects after binding to its main

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receptor AT1R (21-25). Our group has previously shown that the involvement of RAS in BCC pathogenesis is associated with certain polymorphic variants in the genes of angiotensinogen (*AGT*) and angiotensin-converting enzyme (*ACE*), which indirectly increase the levels of Ang II (13-15). Our findings were in accordance with previous observations of substantially reduced occurrence of BCC in individuals receiving medical treatment with either ACE inhibitors or angiotensin receptor blockers (16, 17).

Across the RAS cascade, the adverse effects of Ang II are endogenously controlled by the heptapeptide angiotensin 1-7 (Ang 1-7), which is the final product of the hydrolytic action of the ACE2, a transmembrane, two-domain, metallopeptidase of 805 amino acids (26-30). While ACE converts Ang I to Ang II, ACE2 degrades Ang II into Ang1-7 and also converts Ang I into Ang1–9, which is then hydrolysed into Ang1–7 by the ACE (27, 31-34).

The *ACE2* gene consists of 18 exons and is located on the X chromosome (Xp22), within a region that skips X-inactivation in females (35-38). One of the most studied functional polymorphisms of *ACE2* gene is the SNP G8790A (rs2285666), which affects mRNA splicing, influences gene expression and quantitatively affects the enzyme's production (34, 39-41). In fact, the presence of the A allele has been strongly associated with increased ACE2 circulating levels (42), but there are currently no available data on whether or not it affects the functionality of the protein products.

The present study investigated the possible association of the functional polymorphism *ACE2*-G8790A with the pathogenesis of BCC. Alternatively stated, the study aimed to determine whether another factor of RAS is involved in BCC pathogenesis, namely the quantity of ACE2.

Patients and Methods

Ethics statement. The protocol used was approved by the Ethics Committee of the University Department of Oral and Maxillofacial Surgery (27022019) in accordance with the standards of the 1964 Declaration of Helsinki. The individuals under study gave their informed consent to be included the study.

Subjects. A total of 190 individuals of Greek origin were enrolled in this study, including 91 BCC patients aged between 28 and 96 years, and 99 controls from the same age range. In terms of gender ratios, we included 41 male (45.1%) and 50 female (54.9%) patients with a mean age of 70.1 (±12.5) years and 72.2 (±11.2) years, respectively. The control group consisted of 45 male (45.5%) and 54 female (54.5%) healthy individuals with a mean age of 68.2 (±11.6) and 71.0 (±12.2) years, respectively. The diagnosis for all patients was made in the last 5 years and confirmed by clinical examination and biopsy findings.

Genotyping. The genotyping of *ACE2* G8790A polymorphism was carried out by PCR amplification based on the protocol developed by Pinheiro *et al*. (39), using the following primers: Forward: 5'- CAT GTG GTC AAA AGG ATA TCT-3' and Reverse: 5'-AAA GTA AGG TTG GCA GAC AT-3'. The initial denaturation step at 95˚C was followed by 34 cycles of 95˚C for 1 min, 55˚C for 1 min, 72˚C for 1 min, and finally an elongation step at 72˚C for 8 min, resulting in an amplified DNA product of 466 bp. Incubation with restriction endonuclease AluI in the presence of the A allele resulted in a 281 bp and a 185 bp fragment visualized after agarose gel electrophoresis (Figure 1).

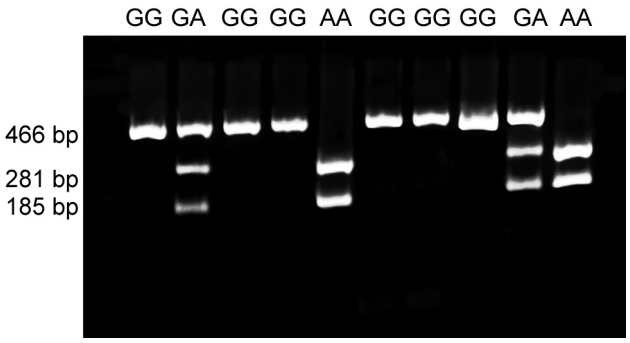

Figure 1. *Agarose gel electrophoresis results illustrating the G8790A polymorphism genotypes GG, GA, and AA. The intact fragment size of the normal allele G is 466 bp, while the two fragments of 281 and 185 bp correspond to the variant allele A that was recognized and cleaved by the restriction endonuclease AluI.*

Statistical analysis. Statistical analysis was performed using SPSS v.21.0 (IBM Corp., Armonk, NY, USA). The frequencies of the alleles and genotypes of the whole group of patients were compared to the respective frequencies of the control group using the Fisher's exact test. All the observed genotype and allele frequencies were prior tested for compliance with Hardy-Weinberg equilibrium. The Maentel-Haenzel method was used for the calculation of all odds ratios with a 95% confidence interval (CI). A *p*-value of less than 0.05 was considered statistically significant. As to the Hardy -Weinberg equilibrium (HWE), two different approaches were followed since the *ACE2* G8790A polymorphism is located on the X chromosome and skips X inactivation. in the initial approach, the whole population of the control group was included in the calculations and comparisons. In the second phase, healthy males were excluded and the HWE calculations corresponded only to the women of the control population.

Results

The detected genotype frequencies of the *ACE2* G8790A polymorphism in the group of BCC patients and in the group of healthy controls are shown in Table I. The genotyping results were comparable in females and males of the two studied groups and no statistically significant difference in the allelic and genotypic frequencies between the control and patient group was observed (Table I). Therefore, no association was found between the G8790A polymorphism of the *ACE2* gene and BCC pathogenesis or protection against it.

Interestingly, analysis for putative Hardy–Weinberg equilibrium showed that the observed and expected

Table I. *Comparison of genotype and allele frequencies concerning the ACE2-G8790A polymorphism between controls and patients.*

ACE2 - G8790A polymorphism genotypes	Controls (n=99)	Patients (n=91)	*p*-Value*	OR (95%CI)
Females (n=54)				
Heterozygotes	33 (33.3%)	38 (12.1%)	0.131	2.094 (0.885-4.942)
Normal homozygotes	20 (20.2%)	11 (41.8%)		reference
Mutant homozygotes	1 (1.0%)	1 (1.1%)	1.000	1.818 (0.173-19.160)
Males (n=45)				
Normal hemizygotes	42 (42.4%)	38 (41.8%)		reference
Mutant hemizygotes	3 (3.0%)	3 (3.3%)	1.000	1.105 (0.239-5.104)
Carrier frequency of mutant allele	37 (37.3%)	42 (46.2%)	0.501	1.240 (0.732-2.099)
Allele frequency				
Mutant allele	38 (24.8%)	43 (30.5%)	0.298	reference
Normal allele	115 (75.2%)	98 (69.5%)		1.328 (0.797-2.213)

*Two tailed Fisher's exact test.

Table II. *Hardy–Weinberg equilibrium comparison results for the entire control group and for the female control sub-population after excluding male control subjects.*

ACE2 - G8790A polymorphism genotype frequencies for all controls			
Genotype	Observed	Expected	*p*-Value*
Female heterozygotes	33 (33.3%)	20 (20.2%)	**0.0145**
Female normal homozygotes	20 (20.2%)	31 (31.3%)	
Female mutant homozygotes	1 (1.0%)	3 (3.0%)	
Male normal hemizygotes	42 (42.4%)	34 (34.3%)	
Male mutant hemizygotes	3 (3.0%)	11 (11.1%)	
ACE2 - G8790A polymorphism genotype frequencies for female controls			
Genotype	Observed	Expected	*p*-Value*
Heterozygotes	33 (61.1%)	12 (22.2%)	**0.001**
Normal homozygotes	20 (37.0%)	25 (46.3%)	
Mutant homozygotes	1 (1.9%)	6 (11.1%)	

*Two tailed Fisher's exact test. Bold values represent statistical significance.

frequencies differed significantly among all control subjects and among the female controls. Therefore, the selected group of controls deviates from Hardy-Weinberg equilibrium for the studied polymorphism (Table II).

Discussion

ACE2 is a crucial component of the renin-angiotensin system, which combats the vasoconstrictive and inflammatory effects of AngII, through its hydrolyzation product Ang1-7 (26-30). It is well established that the adverse effects of Ang II are endogenously counterbalanced by Ang1-7, which exerts vasodilative, antioxidant, and antiproliferative effects by activating the Mas receptors and consequently, several signal transduction pathways (26-30). Previous studies have shown that elevated AngII levels are associated with higher risk for basal cell carcinogenesis (Figure 2), and that relatively lower Ang II levels are associated with protection against it (13-17).

In this study, we investigated the role of the *ACE2* G8790A functional polymorphism in BCC pathogenesis. The polymorphic genotype affects ACE2 plasma levels through splicing of the *ACE2* gene transcript. A total of 190 Greek individuals were studied, comprising a group of BCC patients and a group of age- and gender-matched healthy controls. No significant difference was observed in the

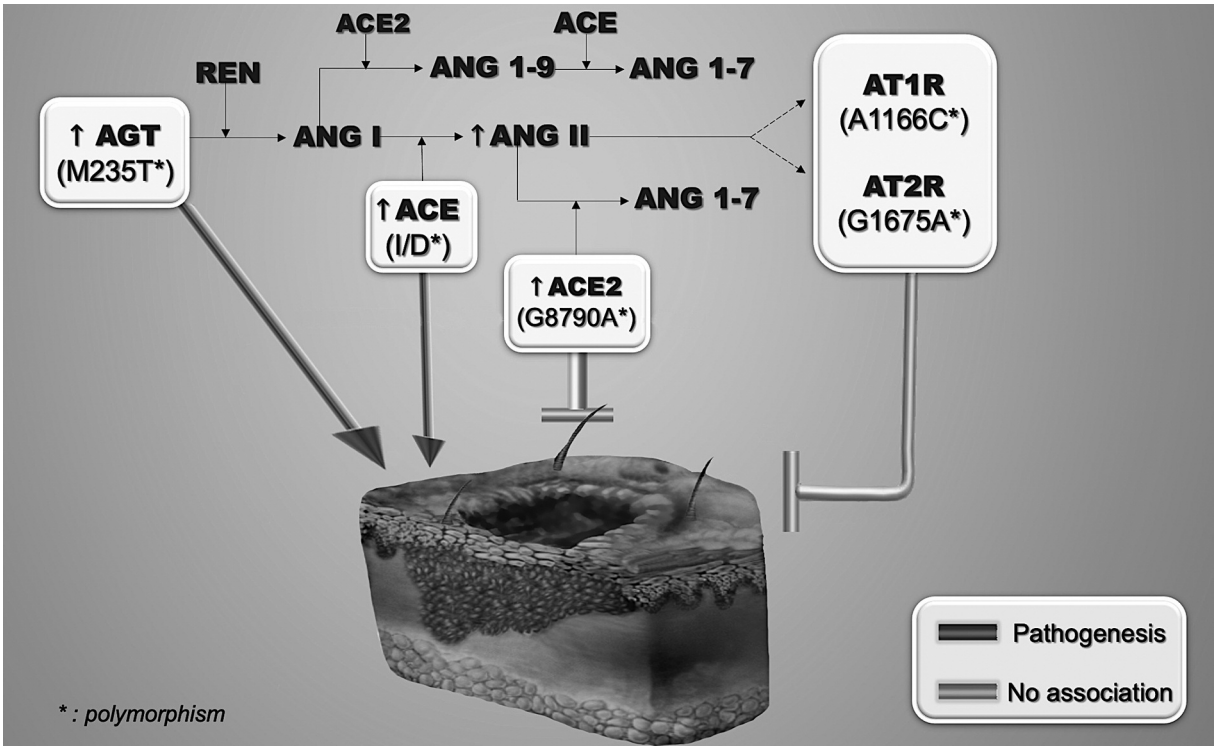

Figure 2. *Illustrative representation of the combined results of the studies that have investigated the association of functional polymorphisms of four genes, that encode major RAS compounds, with basal cell carcinoma (13, 14). The present study is the most recent addition to the above set and classifies the G8790A polymorphism of the ACE2 gene into the "no association" category.*

genotypic and allelic frequencies between the patients and controls, showing lack of association of the G8790A polymorphism of the *ACE2* gene and BCC pathogenesis or protection against this particular carcinoma.

It is important mentioning that this study has certain limitations. More specifically, the participants have been initially collected for the study of autosomal genetic traits and as a result, participants of both genders have been selected in almost equal numbers in both groups. This may have a limiting effect on a study of an X-linked trait due to male hemizygosity. In a possible future study, it would be useful for men to participate in the sample in almost twice the number of women, in order to balance the numbers of alleles from each sex. An additional limitation is the deviation of the control population from the Hardy-Weinberg equilibrium, although the same population satisfies the equilibrium with respect to at least three autosomal gene polymorphisms encoding RAS protein molecules (14). The deviation may possibly reflect the numerical distribution of the sample as to both sexes and in particular, the small size of the male subpopulation in the sample.

Further studies are needed in order to determine the role of ACE2 levels in BCC occurrence. Such studies may ultimately reveal possible genetic predisposition factors for BCC, so that high-risk individuals may have timely access to preventive medical care.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

Iphigenia Gintoni performed the molecular analysis, literature research, authored the first draft and drew the figure. Stavros Vassiliou collected patients and made corrections in the first draft. Dimitris Avgoustidis performed statistical analysis. Mary Adamopoulou assisted in the initial laboratory work. Nikos Zavras and Veronica Papakosta collected patients. Dimitris Vlachakis made critical corrections in the manuscript. Christos Yapijakis conceived the study, supervised the molecular analysis and authored the final draft of the manuscript.

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