**Association study of three single nucleotide polymorphisms in PTCH1 gene with basal cell carcinoma**

Maryam Abdollahzadeh¹, Maryam Beigom Mobasheri*¹,², Homayoun Moslehi³, Mohammad Hossein Modarressi¹

**ABSTRACT**

**Background:** Basal Cell Carcinoma (BCC) is the most common human malignant neoplasm which is more frequent in white people. PTCH and p53 are two major tumor suppressor genes which play important roles in pathogenesis of BCC. PTCH is a twelve-pass transmembrane protein. It is an essential component of the sonic hedgehog signaling pathway that plays as a receiving receptor for members of the Hedgehog family. PTCH signaling pathway is actively involved in regulation of main processes of growth differentiation, stem cell growth and etiology of cancer. There are three single nucleotide polymorphisms (SNPs) including rs17852533, rs200902126 and rs3811553 in the PTCH1 gene; however, their effects on PTCH protein have remained unknown. This study was aimed to analyse the possible association between these SNPs and risk of BCC.

**Methods:** One hundred fifty-three BCC patients in conjunction with 175 healthy controls were selected and matched with each other in terms of age and gender. DNA obtained from each of the groups was subjected to analysis through polymerization chain reaction-restriction fragment length polymorphism (PCR-RFLP).

**Results:** All the studied samples from both the groups were determined to be homozygous for the wild type genotype of the three studied SNPs. There was no significant association between those genetic variants and risk of BCC.

**Conclusion:** Our findings revealed no effect of rs147067171, rs78708791 and rs201125580 variants of the PTCH1 gene on BCC. This indicates that rs147067171, rs78708791 and rs201125580 are not considered as polymorphism among the studied subjects. Perhaps they are mutations associated with other diseases that carry PTCH1 defect such as esophageal squamous cell carcinoma, trichoepitheliomas, Holoprosencephaly and Medullablastoma.

**Keywords:** Association study, PTCH1, basal cell carcinoma, BCC, rs147067171, rs78708791, rs20112558, polymorphism
Introduction

Non-melanoma skin cancer (NMSC) is by far the most common cancer in Caucasians and numerous studies have shown that incidence rates, especially for basal cell carcinomas (BCC), are increasing worldwide\(^1\). Basal-cell carcinomas constitute approximately 80 percent of all non-melanoma skin cancers\(^4\). The burden of BCC is becoming an increasingly important public health issue, because of the rapidly increasing numbers of patients with a history of BCC, the total number of BCC patients and the costs of their treatment and follow-up\(^5\).

Basal cell carcinoma is a malignant skin tumor consisting of plugs and clusters of basal cells, with various clinical manifestations in accordance with the presence of various morphological features, related to their histological types\(^6\). BCC can be classified as nodular, infiltrative, superficial apparently multifocal and mixed in terms of the histological growth pattern\(^7\). BCCs are frequently found in the skin of elderly people, and have been associated with long term sun exposure\(^8\).

PTCH1 and p53 are two major tumor suppressor genes which play important roles in pathogenesis of BCC and effects of PTCH1 gene mutations have been reported frequently in this case\(^9\). PTCH is a human homolog of the Drosophila segment polarity gene, patched\(^8\) which contains 24 exons (23 coding) spanning approximately 70 kb, and encodes a 1447-amino acid transmembrane glycoprotein. It has a predicted structure of 12 transmembrane domains and two large extracellular loops, with a putative sterol-sensing domain (SSD) encompassing transmembrane domains 2–6\(^12,13\).

The PTCH gene is located on chromosome 9q22-31 and encodes for the transmembrane glycoprotein that acts as a membrane receptor for the SHH signal\(^14,15\). Under normal conditions, the PTCH protein binds to SMO (smoothened) so PTCH inhibits SMO and acts as a cell cycle regulator by blocking downstream events resulting in transcriptional activation of target genes. This patched-mediated inhibition of smoothened can be relieved when soluble SHH binds to PTCH. This results in activation of the Hedgehog pathway and affects downstream events, such as cell growth and differentiation. Inactivating PTCH or activating mutations of SMO lead to increased smoothened signaling and growth promotion with subsequent neoplasm development\(^12\). \(^14\)-\(^17\). Deregulations of the components in the HH signaling pathway may lead to cancer in different tissues\(^18\). For example, BCC and medulloblastoma are associated with loss-of-function mutations in the PTCH gene\(^8\). Disease-associated PTCH1 mutations are dispersed over much of the coding sequence and 75% are predicted to truncate the protein. The remaining 25% are amino acid changes and in-frame insertions and deletions\(^19,20\).

There are three single nucleotide polymorphisms (SNPs) including rs17852533, rs200902126 and rs3811553 in the PTCH1 gene, the effect of which on PTCH protein has remained unknown (the provided database is; http://www.ncbi.nlm.nih.gov/SNP/). This study was aimed to analyse the possible association between these SNPs and risk of BCC.

Methods

Sample selection

Patient and Control Samples: A case-control study was performed with 153 patients with BCC (male/female: 2.32, mean age 66.29 [± 12.44] years) the case group and 175 healthy age- and sex-matched individuals without BCC (male/female: 2.18, mean age 65.96 [± 7.40] years) as the control group. Pearson’s Chi-square and t-tests did not show any significant difference in gender and age distribution between the case and control groups (p>0.05, \(\chi^2= 0.2\). BCC in patients was confirmed through
histological assessments and were randomly collected from April 2011 to April 2014, at Imam Khomeini Hospital Complex in Tehran, Iran. The Medical Ethics Committee of the Tehran University of Medical Sciences (TUMS) approved the study. Written informed consent was obtained from individuals who participated in this study.

DNA isolation
Five ml of peripheral blood was obtained from the control group in canonical tubes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Genomic DNA was isolated from the blood samples using DNA extraction Blood Mini Kit (Qiagen, Chatsworth, CA). Then, 2-3 sections of paraffin-fixed skin tissue, each section with a thickness of 5-10 µm, was obtained from the case group in microtubes, and genomic DNA was isolated from formalin fixed paraffin embedded BCC tissues using QIAamp DNA FFPE Tissue Kit (Qiagen). The quality, purity and quantity of isolated DNA samples were determined using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and electrophoresis on a 1% agarose gel.

Genotyping Analysis
Genotyping was performed by polymerase chain reaction– restriction fragment length polymorphism (PCR-RFLP), as described as follows: The primer pairs for amplification of each DNA template were designed through the online primer 3 program as follows; for rs147067171, forward primer: 5'- GTGGTCCATCCCGAATCCAGG -3' and reverse primer: 5'- GTGGCACTCACCTCAGTTGGA -3'; for rs78708791 and rs201125580, forward primer: 5'- ACATTGTACCTCGGGAAACC -3' and reverse primer: 5'- AAGCCAGTCTCTGAAGTAGTGC -3'.

The PCR mixture included 10 pmol of each forward and reverse primers, 2.5 µl of 10x buffer including 1.5 mM MgCl2, 0.2 mM of dNTP mixture and 1U of Taq DNA polymerase (Cinnagen, Iran), along with 100 ng of each genomic DNA sample adjusted with ddH2O up to final volume of 25 µl. After an initial denaturation at 95˚C for 3 minutes, 33 cycles of PCR were performed according to the following program in a TC-512 Techne Thermal Cycler (Bibby Scientific Limited, Staffordshire, England): denaturation at 94˚C for 30 seconds, annealing at 58˚C for 30 seconds, extension at 72˚C for 45 seconds and final extension at 72˚C for 5 minutes. All the PCR products were resolved on agarose gel (1.5%). The size of the PCR product was 552 bps for rs147067171 (T/C) while it was 221 bps for rs78708791(C/T) and rs201125580 (C/T).

In the next step, digestion of the PCR products for the rs147067171 (T/C) using pstI (Invitrogen, 10 u /µl ) produced 2 bands for homozygous wildtypes (TT: 494, 58 bps), 4 bands for heterozygotes (CT: 58, 145, 349 and 494 bps) and 3 bands for homozygous mutants (CC: 58, 145, 349). Digestion of the PCR products for the rs201125580 (C/T) using MaeII (Tail) (fermentas, 10 u /µl ) produced 2 bands for homozygous wildtypes (CC: 148, 73 bp), 3 bands for heterozygotes (CT: 221, 148 and 73 bps) and 1 band for homozygous mutants (TT: 221bp ). Finally, digestion of the PCR products for the rs78708791(C/T) using MboII (fermentas, 5 u /µl ) produced 2 bands for homozygous wildtypes (CC: 172, 49 bps), 3 bands for heterozygotes (CT: 221,172 and 49 bps) and 1 band for homozygous mutants (TT: 221 bps).

Results
The frequency of the rs147067171 (T/C) variant was similar in both the case and control group, which was as follows: 100% wild type (TT), 0% heterozygous (CT) and 0% homozygous mutants (CC) (Figure 1).

Also, the frequency of the rs78708791(C/T) (Figure 2) and rs201125580 (C/T) (Figure 3) variants in
**Figure 1**: PCR-RFLP results for the rs147067171 (T/C); 1,2,3,…,10: homozygous TT, L:50bp DNA Ladder, Ud: un digested DNA

**Figure 2**: PCR-RFLP results for the rs78708791 (C/T); 1,2,3,…,10: homozygous CC, L:50bp DNA Ladder, Ud: un digested DNA
both the case and control group was the same: 100% wild type (CC), 0% heterozygous (CT) and 0% homozygous mutants (TT).

Discussion

There are more than 300 known mutations and 50 published polymorphisms scattered through the PTCH1 gene\textsuperscript{12}. Recent dbSNP database search (http://www.ncbi.nlm.nih.gov/SNP/) provided almost 200 missense variants, many of which have uncertain significance and unknown clinical effects on protein function. In this study, association of three of these missense variants with basal cell carcinoma was assessed. There was no significant association between these genetic variants and risk of BCC. Our findings revealed no effect of the rs147067171, rs78708791 and rs201125580 variants of the PTCH1 gene on BCC among the participants.

References